A multidisciplinary approach to investigate the osteobiography of the Roman Imperial population from Muracciola Torresina (Palestrina, Rome, Italy)

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ABBREVIATIONS: OM (Optic Microscopy); GC-MS (Gas-Chromatography Mass-Spectrometry); EPA (Eicosapentaenoic Acid); DHA (Docosahexaenoic Acid).

Abstract

The present research provides the osteobiographical reconstruction of the Roman Imperial population of the rural area of Muracciola Torresina (Palestrina, Rome, Italy) through an innovative multidisciplinary approach, combining evidence from skeletal biology, biomolecules and archaeobotany.

The excavation of the site, unearthed 76 individuals: 84.2% adults and 15.8% non-adults. Morphological examination showed a higher prevalence of females with respect to males (M:F=0.89). Musculoskeletal stress marker analysis highlighted a probable division of daily tasks between sexes; the observed modifications mainly affected the upper limbs with a particular involvement of shoulder and elbow joints. The population seems to have experienced physically strenuous life conditions, as suggested by the high frequency of degenerative and infectious diseases.

Carbon and nitrogen stable isotope data supported an omnivorous diet mainly based on C_3 plants and terrestrial animal protein. No statistically significant difference was found between sexes or age classes, even though a discrete variability of nitrogen isotopic values was observed which was hypothesized to reflect the consumption of pulses by certain individuals with the lowest values. Microscopic analysis of dental calculus detected Triticeae starch granules in the majority of the analysed individuals. Chromatographic profiles additionally revealed the presence of ephedrine derivatives in the calculus of two individuals, an alkaloid which might indicate the consumption of *Ephedra* species used as medicinal plant due to its bronchodilator, nasal decongestant and vasoconstrictor properties.

This use of multiple cutting-edge techniques has revealed a detailed snapshot of the diet and lifeways of the first Roman Imperial population to be recovered from the area of ancient *Praeneste.*

1. Introduction

The present research aims use a multidisciplinary approach to investigate the osteobiography of the Roman Imperial population of Muracciola Torresina in Palestrina, a small municipality in the territory of Rome (Italy) (Fig. 1). The archaeological surveys directed by *Sorprintendenza per i Beni Archeologici del Lazio* ("Archaeological Authority of Latium"), between 2007 and 2008, led to the recovery of the first Roman Imperial necropolis in the territory of ancient *Praeneste* (today known as Palestrina). The graveyard was set along a crossroads between Labicana and Prenestina, three Kilometers S-W from the city. The necropolis dates to the 1st-3rd centuries CE and consists of a wide area of six funeral enclosures from which a total of 79 burials (73 inhumations and 6 cremations) were recovered.

Most of the skeletons were found articulated and are classified as primary burials. Some of the cremations are *bustum,* where the funeral pyre was settled within the earthen grave in which the individuals were recovered (Duday, 2006). Only the cremations were equipped with devices for funeral libations, obtained with stacked terracotta tubules and arranged vertically above the tile tent (*cappuccina*) tomb. The majority of the tombs were E-W oriented and made of earthen graves.

Fifteen of them showed masonry coverage (e.g. shingles, stones both flat and sloping) with evidence of coffin nails (Fig. 2). Generally, burials were occupied by a single individual, but occasionally two or three individuals were present in the same grave. All individuals but one (T. 56 SU 769), were laid out supine.

Unfortunately, in some cases the skeletal remains were too poorly preserved for comprehensive anthropological analysis. Grave goods such as tankards and earthenware pots were found in forty graves and eight cremation burials and in several cases, glass unguentaria (T. 8, T. 67, T. 76, and T. 80) and oil lamps (T. 31, T. 43, T. 46, T. 47, T. 76, T. 88) were found.

The analysis of the recovered individuals was performed applying a multidisciplinary approach aiming to reconstruct their osteobiography In particular the morphological examination was necessary to determine the biological features (e.g. sex, age at death, stature) as well as to reconstruct lifeways and health status. Isotopic and archaeobotanical analysis, on the other hand, allowed investigating the dietary pattern of the population. The combined techniques were used to add useful data for the understanding of the hinterland of Rome during the Imperial period.

During the Imperial period (1st-5th centuries CE), the population of Rome was the largest in the world, with an estimated one million or more people living within the urban center and the suburban area (Wiseman, 1969; Champlin, 1982; Storey, 1997; Scheidel, 2001). The vast majority of people would have been among the lower socioeconomic strata, composed of the non-elite, slaves, and freed people (MacMullen, 1974; Alföldy, 1985; Bradley, 1994; Scheidel, 1997; 2004; Noy, 2000).

Much of our knowledge of ancient Roman diet derives from historical sources (e.g. Cato the Elder, Varro and Columella) that cannot be considered wholly representative of all Roman populations because it usually only refers to the dietary patterns of the upper classes (Garnsey, 1999; Prowse, 2001; Alcock, 2006; Cool, 2006). Nevertheless, documentary sources indicate that cereals made up approximately 70-75% of the caloric intake of ancient Romans (Foxhall and Forbes, 1982; Garnsey, 1983; Brothwell, 1988; White, 1988). Wheat was by far the most popular grain eaten and was often imported from Egypt and North Africa (Garnsey, 1999). Millets, despite being grown easily and cheaply, tended to be generally considered as a low status food (Evans, 1980; Spurr, 1983; 1986; Nenci, 1999), although its consumption is documented isotopically (Killgrove and Tykot, 2013). Most individuals also had access to vegetables, fruits, and nuts, either grown locally or purchased at market (Garnsey, 1988).

The protein component of the Roman diet is not completely understood. Despite the importance of the livestock trade to the Roman economy (Kron, 2002; MacKinnon, 2004), meat and other animal byproducts did not appear to be major components of the diet. Meat consumption varied according to socioeconomic status. Sources of meat included goat/sheep, poultry, pig, and fish (White, 1976; Brothwell, 1988; Brothwell and Brothwell, 1998; Garnsey, 1999; MacKinnon, 2004). Pig was part of the Mediterranean diet from the Neolithic period onwards (Brothwell, 1988) and sheep and goat were important for the diet but also for their secondary products, wool and milk (Brothwell, 1988; Garnsey, 1999). Cattle, on the other hand, were mainly used as draft animals so they played a minor role in the diet apart from that of the Roman soldiers (White, 1988).

Dietary evidence from Pliny also indicates the use of legumes as protein sources rural areas, consumed on their own or mixed with grains like millets (C_4 crops) and wheat (Faas, 1994; Garnsey, 1999; Evans, 1980; Spurr, 1983). The extent to which fish was consumed outside of the popular fish sauce known as *garum* is still not completely understood. Fish consumption would

have strictly depended on the individual's social status and occupation as well as availability due to environmental factors (Purcell, 1995; Craig et al., 2009; Beer, 2010).

In the last two decades, multiple isotopic studies have investigated the dietary patterns of the Roman period (Prowse, 2001; Prowse et al., 2004; 2005; 2008; Craig et al., 2009; 2013; Rutgers et al., 2009; Crowe et al., 2010; Killgrove, 2010; Scorrano et al., 2014; Ricci et al., 2016; Killgrove and Tykot, 2018; Tafuri et al., 2018), however most of these have focused on the immediate urban area of Rome. The investigation of the hinterland, still limited, could offer a new perspective to investigate dietary habits during the Roman Empire (Killgrove and Tykot, 2018).

Dental calculus (tartar, or calcified plaque) has been investigated as a valuable deposit for studying past diet, culture and environment, providing intriguing and novel data on palaeodiet. Calculus deposits are composed of inorganic salts deriving from saliva that has entrapped dietary and non-dietary micro-debris during formation. Poor oral hygiene, genetic predisposition and foods contribute to the development of this matrix, especially in areas of the mouth near the salivary glands. Archaeobotanical investigations on plant micro-remains (e.g. starch granules, pollens, secondary metabolites) embedded in calculus flakes have provided new information about ancient human lifestyles (Henry and Piperno, 2008; Radini et al., 2017; Baldoni et al., 2018; Cristiani et al., 2018; Gismondi et al., 2018).

The analysis of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in bone protein represents a robust and informative quantitative method to obtain evidence of long-term dietary patterns to the level of the individual. The isotopic composition of bone proteins, reflects an average of the diet during adulthood (Hedges et al., 2007). Carbon isotope values can be used to distinguish between terrestrial and marine foodwebs and between C_3 and C_4 pathway plants. Nitrogen values give information on the trophic level an organism is feeding at despite being highly affected by metabolic processes (DeNiro and Epstein, 1981; Schoeninger and DeNiro, 1984; Hedges and Reynard, 2007). Nitrogen isotope ratios may be used to identify aquatic protein intake, as in both freshwater and marine ecosystems the food chains tend to be longer, leading to higher $\delta^{15}N$ values in these systems (Schoeninger and DeNiro 1984; Eriksson et al., 2008).

The multi-proxy approach applied in the present research, combining skeletal biology, molecular anthropology and archaeobotany, aims at reconstructing not only the diet but also the osteobiography of the first Roman Imperial population recovered in the area of the ancient *Praeneste.* In detail, the osteological analysis enables us to reconstruct the biological features of these Roman individuals, while molecular and archeobotanical analyses are used to shed light on dietary patterns and medicinal habits of the population of Muracciola Torresina.

2. Materials and methods

2.1. Skeletal biology

The analyzed series consists of 76 individuals. For the analyses only inhumations were taken into account. The preservation index was calculated following the method proposed by Walker et al. (1988). For each individual, some bone fragments were sampled for biomolecular isotopic analyses (further described below). Age at death was estimated on the whole sample. The estimation for adult individuals (from ca. 18 year old) primarily followed methods based on morphological changes to the auricular surface of the ilium (Lovejoy et al. 1985), the pubic symphysis (Todd, 1920a; 1920b; Brooks and Suchey, 1990), and in the sternal end of the fourth ribs (İşcan et al. 1984;

1985). Dental wear (Brothwell, 1981; Lovejoy, 1985) and obliteration of the cranial sutures (Meindl and Lovejoy, 1985) were also observed.
In non-adult skeletal remains (until ca. 18 years old), diaphyseal length of bones (Fazekas and Kósa, 1978; Stloukal and Hanáková, 1978; Scheuer and Black, 2000), tooth eruption (Ubelaker, 1989) and secondary ossification centers (Scheuer and Black, 2004) were taken into account. Sex determination was performed only on adult samples, following the methods proposed by Acsàdi and Nemeskèri (1970) and revised by Ferembach et al. (1979) in addition to those of Phenice (1969). Metric analysis complemented the morphological data, where the state of preservation of the sample allowed the measurement of sexually dimorphic bones, utilizing univariate and multivariate techniques (Di Bennardo and Taylor, 1979; Bass, 1987; Berrizbeitia, 1989; Safont et al. 2000; Cowal and Pastor, 2008).
Osteometrics were applied following methods and standards proposed by Martin and Saller (1957) and Borrini (2007; 2011). Cranial and post-cranial indices were calculated following the guidelines proposed by different researchers (Bass, 1987; Borgognini Tarli and Pacciani, 1993; Dal Poz et al. 2002; Minozzi and Canci, 2015). Stature was estimated using several methods (Pearson, 1899; Telkkä, 1950; De Mendonça, 2000; Radoinova et al. 2002; Belmonte Expósito, 2012). For fragmented skeletal remains, Steele's formulae (1970) were applied to estimate long bone length. Musculoskeletal stress markers were also analyzed as proposed by Mariotti et al. (2004; 2007) and by Borgognini Tarli and Reale (1997). The paleopathological survey was performed through morphological observation of the skeletal remains.

2.2. Stable isotope analyses from bone proteins

Fifty-two human skeletal remains were processed for carbon and nitrogen stable isotope analysis. Protein extraction was performed on rib fragments as reported in Longin (1971) with modifications (Baldoni et al., 2018; Scorrano et al., 2019). Firstly, a sterile surgical blade was used on the outer surface of the bone samples to remove potential contaminants and ≈ 0.5 g of cleaned bone was pulverized using a mortar and pestle. Bone samples were demineralized in 8 mL of HCl 0.6 M at 4°C for two days, changing the acid after 24 hours. Once the mineral component of the bone was removed, samples were rinsed to neutral pH with bi-distilled water. The resultant pellet was gelatinized at 75 °C for 24–48 hours with pH 3.0 (0.001 M) HCl. The sample was then frozen at -80°C for four hours before being freeze-dried for 48 hours. A simultaneous extraction on modern bovine bone was performed and used as reference control. Approximately 0.8–1.2 mg were weighed out in duplicate in tin capsules and analysed by EA-IRMS on a Sercon GSL analyser coupled to a Sercon 20±22 Mass Spectrometer at the University of York (UK) and on a Delta V Advantage (Thermo Scientific) coupled with a Flash EA 1112 Series (Thermo Scientific) at the "Dipartimento di Scienze e Tecnologie Ambientali Biologiche e Farmaceutiche" of the Università degli Studi della Campania Luigi Vanvitelli (Caserta, Italy). Isotope data were normalized to V-PDB (Vienna Pee Dee Belemnite) and AIR (atmospheric air) scales using International Atomic Energy Agency (IAEA) standards (N-1, N-2, USGS25 and CH-6, CH-7, USGS24) for carbon and nitrogen, respectively at Università degli Studi della Campania Luigi Vanvitelli and IAEA 600 (caffeine) $\delta^{13}C_{true}$ = -27.8 ± 0.04‰, $\delta^{15}N_{true}$ =1.0 ± 0.2‰; IAEA N2 $\delta^{15}N_{true}$ = 20.3 ± 0.2‰; IA-R006 (Cane), $\delta^{13}C_{true}$ = -11.6 ± 0.03‰ and an internal laboratory reference standard of cold water fish gelatin (Sigma-Aldrich) $\delta^{13}C_{true}$ = -15.3, $\delta^{15}N_{true}$ = 15.2‰ at York University. Collagen samples analysed at different laboratories have been found to result in slight variations in $\delta^{13}C$ and $\delta^{15}N$

values (0.2‰ and 0.4‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively, Pestle et al. 2014) and any differences at this level are not interpreted here.
Carbon content (%C), nitrogen content (%N), protein yield, and C/N ratios were used to determine bone protein quality for paleodietary reconstruction, according to DeNiro (1985) and van Klinken (1999). In order to compare the isotopic data to other Roman sites, comparisons were performed with coeval sites from literature for which the isotope value data were available for each single individual. Statistical tests (Mann-Whitney) were performed by PAST v. 3.08 (Hammer et al., 2001).

2.3. Dental calculus analysis

Dental calculus investigation was performed on all six individuals that possessed a suitable calculus deposit. According to Brothwell (1981), deposits of calculus could be defined as slight on all dentition. The decontamination procedures, meticulously based on Gismondi et al. (2016) and Crowther et al. (2014), were employed to clean tools and working surfaces of the different laboratories used for the analyses. The sampling was conducted under a sterile vertical laminar flow hood (Heraeus HERAsafe HS12 Type) in the Laboratory of Botany of the Department of Biology at University of Rome "Tor Vergata".
Any external particle was removed from calculus flakes, with validated sterilization protocols (Gismondi et al., 2018; Baldoni et al., 2018). Both light microscopy (LM) and gas-chromatography mass-spectrometry (GC-MS) were carried out on ancient dental plaque, in qualitative and non-quantitative terms. Ten mg of calculus for each individual were dissolved in 1 M HCl and observed at OM (Nikon Eclipse E100), under white and polarized light. Each micro-fossil was photographed, using a software for capturing images (ProgRes CapturePro 2.9.0.1), and measured by the SuperAmpelo 2.0 program. Taxonomic identification of starch granules was conducted by comparison with an experimental reference collection (Gismondi et al., 2019) and data from the literature. To perform the chromatographic investigation, once solubilized in 6% HCl, dental calculus (10 mg) were resuspended in hexane and derivatized with Methyl-8-Reagent (Thermo Scientific). Two µL of extract were injected in GC-MS (QP2010, Shimadzu, Japan, DB-5 column Phenomenex). Temperature gradient was: 60 °C for 5 min, and according to a rate 6 °C/min, 156 °C for 5 min, 256 °C for 5 min and 336 °C for 30 min. Mass spectrum was obtained by electron impact at 70 eV, scanning from 100 to 600 m/z. Ion source was set at 230 °C and interface temperature at 320 °C. Each sample was analysed in triplicate and no significant difference among replicates was observed. The mass spectrum of each identified molecule was compared with those registered in the NIST Library 14. Similarity values higher than 85% were considered acceptable for analytic identification. The detected compounds in each specimen were associated on the basis of literature and scientific food databases (FooDB 2013; TGSC 2015).

3. Results

3.1. Skeletal biology

The skeletal sample consists of 76 individuals: 64 (84.2%) adults and 12 (15.8%) non-adults. The preservation index (Walker et al., 1988) of the analyzed series ranged from 0 to 78% (mean 14%). The individuals were sorted into 8 age classes: four non-adult groups (1–6 years, 7–12 years, 13–18 years and generic non-adults) and four adult groups (19–30 years, 31–40 years, 41–50 years and

generic adults). The composition by age and sex of the studied skeletal remains is summarized in Table 1.

The sex ratio (M:F) for the analyzed series was 0.89 showing a slightly higher percentage of females with respect to males. Unfortunately, due to the poor preservation status of the skeletal remains, 39.4% of the sample sex could not be assessed. For adult individuals, the highest mortality of females falls between the young (44.4%) and adult (38.8%) classes, whereas for males it falls in the mature adult class (37.5%). None of the individuals reached senile (>50 years) adulthood. Stature was estimated for 22 individuals of both sexes (10 males and 12 females) selecting those with a good state of preservation for the skeletal elements required to correctly perform the analysis. The mean height of males was 167.3 cm (±5.7 cm) and that of females 157.3 cm (±4.9 cm), and the difference in stature between the sexes was statistically significant (p-value=0.001). The complete list of stature estimates is provided in Appendix Table A1.

Musculoskeletal stress markers were recorded on the adult individuals (N=64). No attempt was made for non-adults because of their skeletal immaturity and the consequent high plasticity of the bone tissue, which could invalidate the results. It was possible to detect an intense use of the upper limbs, with a particular involvement of the shoulder and the elbow joints as well as the forearm. Statistically significant differences were observed between sexes (Student's t-test $p\text{-value}_{(left\ shoulder)}=0.028$; $p\text{-value}_{(left\ elbow)}=0.002$). This result is in agreement to the one obtained by the laterality index highlighting a higher use of the right upper limb in females (ILM=3.02) with respect to males (ILM=0.78), thus it is possible to hypothesize that males' daily activities would involve both the left and the right side. The difference between sexes was confirmed by the post-cranial indices revealing a greater robusticity in male individuals with respect to females as well as a marked flattening of ulna diaphysis (olenic index <79.9) and a highly developed interosseous crest in radius (diaphyseal index <73.9). Statistically significant differences were also observed between young and mature adults (Student's t-test $p\text{-value}_{(right\ elbow)}=0.002$; $p\text{-value}_{(right\ forearm)}=0.019$; $p\text{-value}_{(left\ elbow)}=0.008$).

Non-metric traits were also recorded (Table 2). The most represented features are the acetabular sulcus as well as Charles' and squatting facets on the distal epiphysis of femur and tibia respectively. In one single male individual (T. 72 SU 864) the exostosis of the acoustic meatus was detected. Investigation of palaeopathology was hampered by the high fragmentation of the skeletal remains which meant it was hard to attribute bone alterations to pathological lesions. The results are provided in Table 3. The most common pathological lesions were attributable to degenerative diseases (34.4%), Schmörl nodes (28.1%), and non-specific infectious diseases (periostitis, 12.5%). As regards degenerative disease, arthrosis was mainly represented by slight or moderate modifications. The proliferative arthropathy observed in one of the analyzed individuals (T. 37 Aa, SU 602) were compatible with diffuse idiopathic skeletal hyperostosis (DISH) (Fig. 3a). The only case of neoplasia was represented by a benign lesion on the left tibia of the individual T. 9 Aa SU 526 (Fig. 3b). The overgrowth is relatively small and localized, due to its location it is possible to suppose that it did not represent a serious threat to health (Sjøvold et al., 1974; Mays, 2010).

3.2. Stable isotope analyses from bone protein

Bone protein extraction was performed on a total of 41 samples. The results of isotopic investigation along with the quality control indicators are shown in Table 4. Preservation was

variable, fifteen samples did not have satisfactory quality indicators (specimens highlighted in red in Table 4) according to DeNiro (1985) and van Klinken (1999) and are therefore removed from the subsequent analysis and discussion. Figure 4a shows the plot of $\delta^{13}C$ *versus* $\delta^{15}N$ values for the analyzed human specimens. Due to the absence of faunal remains in the investigated area, published data from the sites of Isola Sacra (Prowse, 2001; Prowse et al., 2004), Lucus Feroniae (Tafuri et al., 2018), Velia (Craig et al., 2009), Herculaneum (Craig et al., 2013), and Pompei (Craig et al., 2013) were used. The analyzed samples exhibited $\delta^{13}C$ values ranging from −20.2‰ to −18.8‰ (mean −19.7‰ ±0.4‰) and a wide range in $\delta^{15}N$ values (5.5‰) from 6.7‰ to 12.2‰ (mean 9.1‰ ±1.4‰) with an average human-herbivore offset of 4.4‰.

The $\delta^{13}C$ values are compatible to a diet mainly based on the consumption of C_3 resources. Despite the high variability of nitrogen isotope values for the population, no statistically significant differences were identified between sexes (males *vs* females $\delta^{13}C$ Mann Whitney p=0.7698; $\delta^{15}N$ Mann-Whitney p=0.2607). The four non-adult individuals (T. 3 SU 69; T. 26 SU 772; T. 33Sb SU 738; T. 53 SU 759) possess $\delta^{13}C$ values ranging from -18.8‰ and -20.0‰ and $\delta^{15}N$ values ranging from 9.4‰ to 12.2‰ (mean $\delta^{13}C$ -19.4‰ ±0.6‰; mean $\delta^{15}N$ 10.6‰ ±1.3‰). These are within the overall range of the adult population and there is no statistical difference between adults and juveniles (adults *vs* juveniles $\delta^{13}C$ Mann Whitney p=0.2825; $\delta^{15}N$ Mann-Whitney p=0.0753) (Fig. 4b).

The one burial who was found to be buried prone, Individual T. 56 SU 769 has $\delta^{13}C$ and $\delta^{15}N$ values of -18.9 and 11.3 respectively, which in the case of nitrogen at least places them among the highest in the population. They cannot, however, be considered an outlier in this population in terms of their isotope values.

3.3. Archaeobotanical evidence

Overall, optic microscopy (OM) analysis revealed the presence of 76 starches and 4 Poaceae phytoliths in ancient calculus (Table 5 and Figure 5). Starch granules were classified in 4 morphotypes (or types) described using the international nomenclature codes (ICSN, 2011).

Only one granule, reported as Type I, was likely to derive from Fabaceae seeds, due to its morphological and morphometric characteristics (e.g. irregular ovoid shape; 30 µm in length and 14 µm in width; concentric *lamellae*; presence of the typical fissure). Thirty-nine starches (Type II) showed traits (polyhedral units; size range: 5-9 µm in length and width; indistinct *hilum* and *lamellae*) consistent with grains of Poeae caryopses, such as *Avena* sp. L. (oat). Type III included sixteen polyhedral granules (with pentagonal or hexagonal faces; rounded off edges; size range: 6-7 µm in length and 5-7 in width; radial fissures) coherent with those of Paniceae species, such as *Panicum* sp. L. and *Setaria* sp. P. Beauv. In the samples, eighteen discoidal granules (Type IV) were also detected; they were ascribable to Triticeae cereals, such as wheat (*Triticum* sp. L.) or barley (*Hordeum* sp. L.), according to their phenotype (e.g. size range: 5-39 µm in length and 4-40 µm in width; indistinct *hilum*; concentric *lamellae*). Only two starch granules were not determined, as their diagnostic features were probably altered by exposure to alfa-amylase activity or/and food cooking procedures (Copeland and Hardy, 2018).

The molecules detected by GC-MS in each sample are indicated in Appendix table A2. Most of the compounds observed in all chromatograms were saturated (e.g. dodecanoic acid) and unsaturated fatty acids (e.g. 9-octadecenoic acid), n-alkanes and n-alkenes (C_4 to C_{35}). In the chromatographic profiles, the presence of polyunsaturated omega-3 fatty acids (EPA, DHA and their derivatives)

indicated the intake of aquatic sources (e.g. molluscs, fishes, algae) and/or dried fruits (e.g. nuts) (Swanson et al., 2012). Lactose and 11-octadecenoic acid were attributable to ruminant milk and/or dairy products (Destaillats et al., 2005). Only one dental calculus sample (T. 56 SU 769) presented the typical molecular markers of Brassicaceae (e.g. isothiocyanatoacetaldehyde and isothiocyanic acid) (Bell and Wagstaff, 2017). In two specimens (T. 56 SU 769 and T. 58 SU 817), GC–MS analysis also detected ephedrine derivatives, an alkaloid which might indicate the consumption of *Ephedra* species (Laccourreye et al., 2015).

4. Discussion

Archaeological evidence suggests that Muracciola Torresina represents the first Roman Imperial graveyard recovered in the *Praeneste* area. Osteological examination revealed the presence of a higher percentage of females with respect to males in the burial population, this is in agreement with the values observed for some other coeval Italian populations (Catalano et al., 2001; Pantano and De Angelis, 2009; De Angelis et al., 2015). The result, however, should be taken as an approximation due to the high percentage of "not-recordable" individuals showing a poor state of preservation of their skeletal remains. The highest mortality of females in the younger adult classes could be related to the high risk of pregnancy and childbirth complications such as infections, and hemorrhages generally common in past populations (Heligman, 1983; Roberts, 2009). The close-range (multiple successive) pregnancies and the demands of breast-feeding took a significant toll on women's health and, if combined with nutritional and/or pathological stresses that were also common in past populations, this could have led to untimely death. It is interesting to note that in both sexes, however, none of the individuals reach old age. The relatively low lifespan could be due to particularly strenuous activities as well as to the precarious sanitary conditions in Roman Muracciola Torresina. The latter in particular could cause gastrointestinal infections that could have been the cause of death of many individuals. This can thus explain the highest mortality for infant I age class (1-6 years).

It is known that the transition from breastfeeding to a more mature subsistence causes a loss of maternal immunological defense and can expose children to gastrointestinal infections that could weaken their health leading, in some cases, to death (Hühne-Osterloh and Grupe, 1989; Dittmann and Grupe, 2000). The isotope data, indicates that three (T. 3 SU 69, T. 33Sb SU 738, and T. 53 SU 759) out of the four infants analysed here were probably already weaned at time of death. Children who are still breastfed at the time of death are expected to have about 1‰ ^{13}C enrichment and $\delta^{15}N$ values of 2-3‰ higher than female individuals. ^{15}N enrichment is only observed in the individual T. 26 SU 772 included in the 1 and 6 year age class (the morphological analysis allowed estimating the individual was aged between 1 and 2 years at the time of death) who possesses $\delta^{15}N$ value of 12.2‰ suggesting the individual was probably still nursed at time of death.

Adult skeletal morphological and metric variations could be determined by different factors, including health status and nutritional intake during life, as well as genetics, sex, environment, and social conditions. Stature estimation is one of the commonly used indicators for reconstructing the living body mass, physical constitution and activity levels of past populations (Ruff, 2000; Holt, 2003; Ruff et al., 2005). Moreover, it can also provide information on the adaptation conditions and on health status because it can be considered a non-specific indicator of metabolic stress (Pietrusewsky et al., 1997; Pietrusewsky and Tsang, 2003; Maat, 2005).

Stature has, in fact, a genetic base although the final phenotype is significantly influenced by socioeconomic and environmental factors. In the analyzed sample, data should be taken as an approximation because of the small sample size available due to the poor state of preservation of many skeletal remains, but they are consistent with those observed for coeval Italian populations (Facchini and Guerra, 1969; Belcastro and Giusberti, 1997; Catalano et al., 2001; De Angelis et al., 2015). The difference in stature observed between sexes could only be related to sexual dimorphism, the lack of statistically significant differences in diets between sexes, in fact, could led to exclude a different access to nutritional sources within this Roman Imperial population.

Males and females also demonstrated differing levels of robustness, reflected in the different biomechanical stress these individuals were subjected to. Morphological changes at enthesal sites depend on repeated daily exercises that stimulate bone remodeling and increase blood flow as a consequence (Hawkey and Merbs, 1995; Ruff et al., 2006). However, although the macroscopic analysis of bone morphological modifications can be related to muscles subjected to a high biomechanical stress, and thus be used to reconstruct movements, often the absence of archaeological data or documents confirming the validity of these assumptions does not allow the determination of the exact activity carried out in lifetime (Dutour, 1986).

Many of the studies that emphasize the correlation between the degree of development of muscular insertion sites and the exact employment are performed on samples with known sexes, occupations and ages at death (Lopreno et al., 2013), but even in these cases, sometimes, it is not possible to identify the activity carried out as claimed by earlier studies. It has been impossible, for instance, to determine the exact occupation for a female sample (Villotte, 2006), or to establish activities with low biomechanical impact (Cardoso, 2008). It is obviously necessary to consider the temporal and the geographical location of the analyzed sample, which permits us to assume that the most part of activities were manual, probably related to agriculture and livestock keeping.

The detected biomechanical stress is in agreement with the paleopathological data highlighting a high prevalence of degenerative diseases and Schmörl nodes. The frequency observed in the individuals buried in Muracciola Torresina is higher than those observed at Quarto Cappello del Prete (Rome, Italy; 34.4 *vs* 30.3; Caldarini et al., 2015) but lower than in Via Padre Semeria (Rome, Italy; 40.3%; Caldarini et al., 2015) Castel Malnome (Rome, Italy; 50.9%; Caldarini et al., 2015), and Casal Bertone (Rome, Italy; 61.5%; Caldarini et al., 2015). At Muracciola Torresina, osteoarthrosis mainly affected the shoulder, elbow and hip joints as well as the vertebral column.

As regards the latter, one female individual in particular aged between 41 and 50 years (T 37 Aa SU 602) showed pathological lesions attributable to diffuse idiopathic skeletal hyperostosis (DISH) (Fig. 3). This is a proliferative arthropathy properly described in medical literature in the 19th century (Smythe and Littlejohn, 1998). It is characterized by an excessive bone formation at joint margin and it is particularly evident in the vertebral column in which the ossification of the spinal ligaments may cause the fusion of several vertebral bodies with large flowing osteophytes (Ortner, 2003). The etiology is still unclear, it can be associated with diabetes and obesity (Julkunen et al., 1971) although it seems that in some cases individuals show a tendency toward bone formation in ligaments and other soft tissues (Rogers and Waldron, 1995). Generally, it is about twice as common in males than in females and the prevalence increases with age (Smythe and Littlejohn, 1998). It is worth notice that nitrogen isotopic values of the individuals are not among the highest of the population despite ^{15}N enrichment has been observed in some cases of DISH (Müldner and Richards, 2007; Quintelier et al., 2014)

Data from musculoskeletal stress markers and the distribution of degenerative alterations also fit well with the high frequency of Charles' and squatting facets on femur and tibia respectively. As for using these traits to determine kinship among individuals, however, the fragmentary nature of the skeletal remains and the infrequency of findings within the analyzed population did not allow for the identification of possible relationships between individuals nor a different spatial distribution of the burials.

It is worth mentioning the presence of acoustic meatus exostosis in one individual (T. 72 SU 864). This is a bony production that can develop inside the acoustic meatus causing, in some instances, the complete closure of the auditory canal (Steinbock, 1976; Capasso, 1988). The aetiology is far from fully understood, mechanical stress due to mastication (Burton, 1923), genetic background (Turner, 1879; Blake, 1880; Hartmann, 1893; Stewart, 1933; Aufderheide and Rodriguez-Martin, 1998) as well as epigenetics (Berry and Berry, 1967; Berry, 1975) have been considered as possible explanations. It is generally agreed, however, that this condition is related to environmental factors (Crowe et al., 2010). In particular, a positive correlation has been found between the exostosis of the acoustic meatus and the prolonged and habitual exposure to cold water (Kroon et al., 2002; Sheard, 2002; Altuna Mariezkurrena et al., 2004; Skoulakis et al., 2007; Cooper et al., 2008; Mlynski et al., 2008; Sheard and Doherty, 2008). There is experimental evidence that cold water can stimulate osteoblastic activity, and therefore bone production of the ear (Van Gilse, 1938; Fowler and Osmun, 1942). The presence of these traits has been detected in archaeological series since prehistory (Velasco-Vázquez, 2000; Agelerakis and Serpanos, 2002; Okumura et al., 2005–2006; 2007). In particular, it was particularly common in several coastal or riverine populations (Frayer, 1988; Manzi et al., 1991; Sakalinskas and Jankauskas, 1993; Tommaseo Ponzetta et al., 1997; Gregg, 2000; Okumura et al., 2005–2006; 2007). In Imperial Rome the presence of this condition was associated with baths in *thermae* (Van Gilse, 1938; Ascenzi and Balistreri, 1975; Manzi et al., 1991). However, the results obtained by Crowe et al. (2010) strongly suggest the existence of a relationship with aquatic activities related to the economy of the Roman coastal societies of Isola Sacra and Velia. The presence of this feature in one individual of Muracciola Torresina seems more probably related to this latter case due to the proximity to the Aniene river (ca. 30 Km).

The morphological examination of the population represented the first step for reconstructing an osteobiography of these individuals. Molecular and archaeobotanical analyses were further applied and integrated with the morphological evidence to present a fuller picture of the lifeways of these individuals.

Stable isotope data suggested a diet mainly based on terrestrial proteins. The $\delta^{13}C$ values indicated an almost exclusive consumption of C_3 plants. However, starch granules identified in dental calculus deposits also included some C_4 crops, namely broomcorn millet (*Panicum* sp. L) alongside C_3 cereals and legumes. It may be that although these C_4 crops were consumed, they were eaten in amounts too small to be detected in bone collagen. Wheat and barley were widely employed by the Romans in porridge, semolina and breads (Purcell, 2003; Tricase et al., 2018), while millet was used especially for gruels (Murphy, 2016). Oats, although generally considered crops of minor importance in the economy of the Roman Empire (Brombacher and Hecker, 2015), were also detected in calculus deposits.

The lower nitrogen values observed for some individuals of the analysed population (around 7‰) may be indicative of the consumption of small amounts of animal protein but a greater consumption of legumes (Szpak et al., 2014). Pulses represented a cheap sources of protein (Murphy, 2017) and,

together with milk and dairy products, they were commonly consumed by Roman agricultural populations (Borstad et al. 2018). Their presence in the dental calculus of the individuals recorded here is also in keeping with our knowledge of Roman diet.

The isotope values reported here would seem to exclude any major consumption of fish in the Muracciola Torresina population. As previously noted, the real extent of fish in human diets during the Roman Empire is not fully understood, even for the well-known *garum*. This sauce, generally used for both cooking and medicinal recipes (Curtis, 1991), was mainly exported from North Africa (Alcock, 2006). Five *garum* samples from Italy (Prowse, 2001; Prowse et al., 2004) possessed a mean $\delta^{15}N$ value of 6.5±1.7‰ suggesting it was probably made from lower trophic level fish species (e.g. sprats, smelt and shellfishes, Prowse et al., 2004). The consumption of this kind of *garum* would not cause significant enrichment in ^{15}N that would be detectable in $\delta^{15}N$ values for human bone collagen as *garum* and terrestrial animals have similar $\delta^{15}N$ values. It should also be considered that even if higher trophic level marine fish were consumed, they would not have been eaten in sufficient amounts to shift human $\delta^{13}C$ and $\delta^{15}N$ values towards the values typical for fish consumption. It has to be acknowledged however that the Mediterranean is problematic in the regard (Craig et al. 2013).

Individual T56 SU 769 was particularly interesting for being the only one buried prone. This position is quite uncommon, Amicucci and Carboni, (2015) described a frequency of 1.2% (mainly females) on a total of 3500 analyzed individuals from several graveyards in Rome. Prone burials were generally considered a means of ensuring that undesirable members of society didn't disturb the living (Hirst, 1985) and this hypothesis has been put forward for some necropolis in Rome (Amicucci and Carboni, 2015) and in Southern France (Castella, 1999). In the case of Muracciola Torresina, the archaeological context contrasted with the dudgeon hypothesis. This individual possesses nitrogen values among the highest detected in the analyzed sample although she cannot be considered an outlier. Prone burials were also recovered in a Roman Imperial skeletal sample analysed from Valencia (Spain; Cerdá, 2016). Two of the three prone individuals that were studied showed lower nitrogen isotopic values than those possessed by individuals buried in simple earthen graves, whereas one individual exhibited the highest nitrogen and carbon values among the sampled population, suggesting the consumption of larger quantities of marine protein than the rest of the human sample (Cerdá, 2016). In the case of Muracciola Torresina, although fish consumption cannot be excluded, it is not possible to assume its high contribution to her subsistence.

Isotopic data from Muracciola Torresina is compared with that of published coeval sites from Italy in Figures 6a and 6b and statistical comparisons are reported in Table 6. Herbivore baselines showed isotopic ratios varying between -20.7‰ and -21.9‰ for $\delta^{13}C$ and between 3.5‰ to 7.8‰ for $\delta^{15}N$. Comparison reveals a general dietary heterogeneity among Roman sites. The pattern of carbon isotope data suggest that the individuals from Muracciola Torresina were significantly different than all other populations with the exception of the catacombs of St. Callixtus in Rome (Rutgers et al., 2009), Lucus Feroniae, a rural center 30 km northeast of Rome (Tafuri et al., 2018), and Paestum in Southern Italy (Ricci et al., 2016). Nitrogen values also varied widely with the Muracciola Torresina specimens showing similar animal protein intake to the inland sites of ANAS (Prowse 2001; Prowse et al., 2004), and Castellaccio Europarco (Killgrove and Tykot, 2012). All the other sites but one (Paestum, Ricci et al., 2016) exhibit higher $\delta^{15}N$ values than those observed at Muracciola Torresina. At those sites individuals consumed a greater amount of animal proteins, both terrestrial (Casal Bertone, Killgrove and Tykot, 2012) and aquatic (Isola Sacra, Prowse et al., 2004; Crowe et al., 2010; Velia, Craig et al., 2009; St. Callixtus, Rutgers et al., 2009; Herculaneum,

Craig et al., 2013; Gabii, Killgrove and Tykot, 2018). With regards to Lucus Feroniae, the authors suggest that some of the individuals could have different social origin and thus the opportunity to consume marine and anadromous fish (Tafuri et al., 2018). The archaeological site of Paestum, on the contrary, shows lower nitrogen values than Muracciola Torresina, the authors also suggested in that case that marine foods were not consumed in any appreciable amount, although a minor contribution of low trophic marine resources could not be excluded (Ricci et al., 2016). The obtained data confirmed the high variability of the diet of lower social classes during the Imperial period. Fish intake is particularly variable and generally dependent on geographical location.

Chronology could be a factor in this dietary variation. Although all of these sample populations date to the same general Imperial period, historical information surrounding famines, wars, and socioeconomic and political crises should also be taken into account. For example, the Crisis of the 3rd century CE caused major problems with the economic and transportation infrastructure leading to a compromised food distribution and market system throughout Italy. Therefore, it could not be excluded that such a crisis and its aftermath, if experienced by any of the populations here, could have affected dietary variation and abnormality.

GC-MS analysis of calculus showed a great number of analytes which, reasonably, could derive from several edible species (e.g. plant oils, seed oils, animal fats) or represent degradation forms of foods and oral bacteria (Eglinton et al., 1962; Evershed et al., 1992; Buckley et al., 1999; Kanthilatha et al., 2014). This investigation highlighted the consumption of Brassicaceae, which represented one of the main plant foods for the Romans (Jashemski et al., 2002). Moreover, the use of *Ephedra* species was documented in two individuals (T. 56 SU 769 and T. 58 SU 817) which were affected by Schmörl nodules and, only for the latter, an aspecific infection in the form of periostitis. It is noteworthy that both Dioscorides and Pliny the Elder knew the therapeutic purposes of this medicinal plant (Laccourreye et al., 2015), nowadays scientifically demonstrated to possess bronchodilator and vasoconstrictor properties (Limberger et al., 2013).

5. Conclusions

This research represented the first study of a Roman Imperial necropolis recovered in the area of the ancient *Praeneste* (currently Palestrina). The multidisciplinary approach we have used has provided a holistic reconstruction of the analyzed population. Osteological examination provided information on paleodemography and paleopathology, the pattern of which is in keeping with that published for other coeval Italian sites. The combined results are indicative of sufficiently strenuous life conditions. Dietary pattern reconstruction from isotopic analysis indicates that the diet was mainly based on C_3 plants and terrestrial animal protein, although a minor contribution of low trophic level aquatic resources cannot be excluded. Starch granules entrapped in dental calculus, however revealed the consumption of C_4 plants that were not detected in isotope analysis of bone proteins and also indicated that oat was consumed, which was thought of as a minor crop during the Roman period. This highlights the utility of a combined approach to dietary analysis. In addition, the use of GC-MS potentially identified the presence of plants in dental calculus that could have been used for medicinal purposes. Altogether the applied multidisciplinary approach allowed reconstructing the osteobiography of this Roman population from the hinterland of Rome.

Conflict of Interest

The authors declare no conflict of interest

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Author Contribution

A.G., and C.M.L. designed the research; E.C. directed the excavation at Muracciola Torresina archaeological site; M.B., performed the anthropological analyses of the skeletal remains; M.A., and D.T., performed the isotope analyses; A.G., A.D.A. and G.D.M. analysed dental calculus and performed the archaeobotanical analyses; M.B., A.G., and C.M.L. wrote the paper; A.C. and O.R. provided financial support; all authors edited, revised and provided comments to the manuscript.

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FIGURE CAPTIONS

Figure 1. Geographical location of Palestrina (Rome, Italy) and blow up of the Roman Imperial necropolis.

Figure 2. a) Example of the coverage recovered during the excavation of the Roman Imperial cemetery of Muracciola Torresina; **b)** individual within the earthen grave after the removal of the coverage.

Figure 3. a) Proliferative arthropathy attributable to Diffuse Idiopathic Skeletal Hyperostosis (DISH) detected in the individual T. 37 Aa SU 602; **b)** Benign neoplasia (osteoma) on the left tibia of the individual T. 9 Aa SU 526.

Figure 4. a) Plot of carbon *vs* nitrogen isotope values for human skeletal samples from Muracciola Torresina and faunal remains from Isola Sacra (Prowse, 2001; Prowse et al., 2004), Lucus Feronaie (Tafuri et al., 2018), Velia (Craig et al., 2009), and Pompei (Craig et al., 2013). Human specimens are plotted individually, for faunal remains median values are reported. Horizontal and vertical lines end at 25th and 75th percentile; **b)** Plot of carbon *vs* nitrogen isotope values for human samples plotted individually and divided by sex (males and females) and age at death (adults and juveniles).

Figure 5. Micro-remains found in dental calculus. Representative images of plant micro-debris were shown: aggregate of Triticeae starch granules and relative polarized image (A); Poaceae phytolith (B); aggregate of Poeae starches and relative polarized image (C). The scale bar indicates 30 µm.

Figure 6. a) Plots of carbon and nitrogen isotopic values for human (ANAS, Prowse, 2001; Prowse et al., 2004; Casal Bertone, Castellaccio Europarco, Killgrove and Tykot, 2012; Isola Sacra, Prowse et al., 2004; Crowe et al., 2010; St. Callixtus, Rutgers et al., 2009; Gabii, Killgrove and Tykot, 2018; Lucus Feroniae, Tafuri et al., 2018; Velia, Craig et al., 2009; Herculaneum, Pompei, Craig et al., 2013; Paestum, Ricci et al., 2016) and faunal samples (Isola Sacra, Prowse, 2001; Prowse et al., 2004; Lucus Feroniae, Tafuri et al., 2018; Velia, Craig et al., 2009; Herculaneum, Craig et al., 2013; Pompei, Craig et al., 2013) from Muracciola Torresina and coeval Italian Roman sites: symbols describe median values of the sites, horizontal and vertical lines end at 25th and 75th percentile of the site sample. **b)** Focus on human samples from Muracciola Torresina and coeval Italian Roman sites (ANAS, Prowse, 2001; Prowse et al., 2004; Casal Bertone, Castellaccio Europarco, Killgrove and Tykot, 2012; Isola Sacra, Prowse et al., 2004; Crowe et al., 2010; St. Callixtus, Rutgers et al., 2009; Gabii, Killgrove and Tykot, 2018; Lucus Feroniae, Tafuri et al., 2018; Velia, Craig et al., 2009; Herculaneum, Pompei, Craig et al., 2013; Paestum, Ricci et al., 2016), horizontal and vertical lines end at 25th and 75th percentile of the site sample

APPENDIX

Table A1. Mean stature estimates for the analyzed sample. For each individual data on sex and age at death are provided.

Table A2. GC-MS profiles. The list of molecular markers detected by GC-MS analysis per each sample (excluding n-alkanes and n-alkenes) was shown. Chemical compounds were clustered in biochemical classes.

Table 1. Age at death and sex distribution in the analyzed sample from Muracciola Torresina (Palestrina, Rome Italy). Not-determinable was used for adult individuals foe whom sex determination could not be performed due to the poor state of preservation of their skeletal remains. Indeterminate was used for non-adult individuals for whom no assumption of sex was made.

AGE CLASS	Male	Female	Not-Determinable (ND)	Indeterminate	Total
< 1 year	0	0	0	0	0
Infant I 1-6 years	0	0	0	6	6
Infant II 7-12 years	0	0	0	2	2
Juvenile 13-18 years	0	0	0	2	2
Generic Non-adult (GNA) x-18 years	0	0	0	2	2
Young Adult 19-30 years	1	8	0	0	9
Adult 31-40 years	3	7	0	0	10
Mature 41-50 years	6	2	0	0	8
Generic Adult (GA) 19-x years	6	1	30	0	37
Total	**16**	**18**	**30**	**12**	**76**
Percentage (%)	**21.1**	**23.7**	**39.4**	**15.8**	

Table 2. Occurrence of the non-metric traits in the Roman Imperial population of Muracciola Torresina (Palestrina, Rome, Italy). For each individual (SU: stratigraphic unit) sex and age at death estimate were provided. M indicates males, F females, ND indicates not-determinable (it was used for those individual for whom no assumption of sex could be made), GA indicates Generic Adult (it was used for those individuals for whom no age at death estimate could be performed).

Individual	SU	Sex	Age at death	Exostosis acoustic meatus	Transverse foramen bipartite	Atlas facet double	Posterior bridge (atlas)	Septal aperture	Suprascapular foramen	Accessory acromial facet	Circumflex sulcus	Cervical rib	Sternal foramen	Sacroiliac accessory facets	Acetabular sulcus	Third trocanter	Femoral neck plaque	Allen's fossa	Exostosis in throcanteric fossa	Martin's facet	Charles' facet	Vastus notch	Emarginate patella	Parson's third intercondilar tubercle	Squatting facets	Anterior calcaneal facet double	Peroneal tubercle
T. 1	4	F	GA					x								x									x	x	
T. 6	620	F	19-30												x				x		x						
T. 8	718	F	31-40																								
T. 9 Aa	526	F	31-40		x										x												
T. 9 Ab	526	ND	GA																								
T. 10	530	F	41-50			x										x											
T. 11	538	M	41-50																								
T. 12	533	M	41-50											x	x								x				
T. 14	546	F	31-40						x		x	x			x	x		x									
T. 16	547	M	GA																								
T. 17	567	F	19-30						x							x	x		x	x	x	x			x		
T. 19	638	ND	GA																					x			
T. 24	655	M	19-30			x			x				x		x												
T. 29	675	ND	GA																								
T. 31	644	ND	GA																								
T. 32	697	F	31-40												x		x		x								
T. 34 Aa	729	ND	GA																						x	x	
T. 34 Ac	730	M	GA																						x	x	

T. 35	**735**	**ND**	**GA**																							
T. 36 Aa	**585**	**M**	**GA**																							
T. 36 Ab	**586**	**F**	**31-40**																							
T. 36 Ac	**587**	**M**	**41-50**																					x		
T. 37 Aa	**602**	**F**	**41-50**								x					x	x		x		x	x			x	x	
T. 37 Ab	**602**	**ND**	**GA**																						x	x
T. 37 Ac	**602**	**ND**	**GA**																							
T. 37 Aa	**603**	**ND**	**GA**																						x	
T. 37 Ab	**603**	**ND**	**GA**																							
T. 38	**731**	**F**	**19-30**																		x					
T. 39	**742**	**M**	**31-40**																							
T. 41 Aa	**820**	**ND**	**GA**																							
T. 41 Ab	**820**	**ND**	**GA**																							
T. 43	**672**	**F**	**31-40**																	x						
T. 44	**760**	**M**	**31-40**											x				x						x		
T. 48	**626**	**M**	**41-50**																							
T. 50	**702**	**ND**	**GA**																							
T. 51	**704**	**ND**	**GA**																							
T. 55	**677**	**ND**	**GA**																	x		x				
T. 56	**769**	**F**	**19-30**							x		x						x		x	x			x		x
T. 58	**817**	**F**	**19-30**											x	x			x		x	x					
T. 59	**818**	**ND**	**GA**																							
T. 61	**788**	**ND**	**GA**																							
T. 64	**810**	**ND**	**GA**																							
T. 65	**801**	**F**	**31-40**																							
T. 66 Aa	**824**	**M**	**GA**																							
T. 66 Ab	**824**	**M**	**GA**																x	x				x		
T. 66 Ac	**824**	**M**	**41-50**											x	x	x		x		x						
T. 66 Ad	**824**	**ND**	**GA**																							
T. 66 Ae	**824**	**ND**	**GA**																	x						
T. 70	**839**	**F**	**19-30**																							
T. 72	**864**	**M**	**31-40**	x														x								

T. 74	**857**	**F**	**19-30**																							
T. 76	**871**	**F**	**19-30**											x		x		x			x					
T. 77	**869**	**ND**	**GA**																							
T. 78	**854**	**ND**	**GA**																							
T. 80	**883**	**ND**	**GA**																							
T. 82	**888**	**M**	**41-50**				x	x			x				x	x					x		x		x	x	
T. 83	**892**	**ND**	**GA**																							
T. 84	**900**	**M**	**GA**																							
T. 85	**910**	**ND**	**GA**																							
T. 86	**115**	**ND**	**GA**																							
T. 87	**902**	**ND**	**GA**																							
/	**743 Aa**	**ND**	**GA**																							
/	**743 Ab**	**ND**	**GA**																							
/	**743 Ac**	**ND**	**GA**																							
Total (N)				**1**	**1**	**2**	**1**	**2**	**3**	**1**	**4**	**2**	**1**	**1**	**11**	**8**	**5**	**3**	**8**	**2**	**11**	**6**	**3**	**1**	**10**	**7**	**2**
Percentage (%)				**1.6**	**1.6**	**3.1**	**1.6**	**3.1**	**4.7**	**1.6**	**6.3**	**3.1**	**1.6**	**1.6**	**17.2**	**12.5**	**7.8**	**4.7**	**12.5**	**3.1**	**17.2**	**9.4**	**4.7**	**1.6**	**15.6**	**10.9**	**3.1**

Table 3. Prevalence of pathologies and stress markers for each individual from the Muracciola Torresina cemetery area (SU: stratigraphic unit). Neoplasia (NP), Infectious diseases (periostitis, PO; osteomyelitis, OM; infections, IN), trauma and stress markers (fractures, FR; grasping GR), degenerative pathologies (degenerative diseases, DG; and axial degereative diseases (SchmoÈrl's nodes, SN)) and congenital disorders (CO), and inflammation (IF). For each individual sex and age at death estimate were provided. M indicates males, F females, ND indicates not-determinable (it was used for those individual for whom no assumption of sex could be made), GA indicates Generic Adult (it was used for those individuals for whom no age at death estimate could be performed).

Individual	SU	Sex	Age at death	Neoplasia	Infectious diseases		Degerative pathologies		Inflammation	Trauma and stress markers	
				NP	PO	OM	DG	SN	IF	FR	GR
T. 1	4	F	GA		x						x
T. 6	620	F	19-30				x	x			x
T. 8	718	F	31-40								
T. 9 Aa	526	F	31-40	x	x		x	x			
T. 9 Ab	526	ND	GA								
T. 10	530	F	41-50				x				x
T. 11	538	M	41-50								
T. 12	533	M	41-50				x	x			
T. 14	546	F	31-40				x	x			
T. 16	547	M	GA								
T. 17	567	F	19-30		x		x	x			x
T. 19	638	ND	GA				x				
T. 24	655	M	19-30				x	x			
T. 29	675	ND	GA								
T. 31	644	ND	GA								
T. 32	697	F	31-40				x	x			
T. 34 Aa	729	ND	GA				x				
T. 34 Ac	730	M	GA								
T. 35	735	ND	GA				x				
T. 36 Aa	585	M	GA								
T. 36 Ab	586	F	31-40								
T. 36 Ac	587	M	41-50				x	x			
T. 37 Aa	602	F	41-50				x	x		x	

T. 37 Ab	**602**	**ND**	**GA**								
T. 37 Ac	**602**	**ND**	**GA**								
T. 37 Aa	**603**	**ND**	**GA**		x						
T. 37 Ab	**603**	**ND**	**GA**								
T. 38	**731**	**F**	**19-30**				x				
T. 39	**742**	**M**	**31-40**				x	x			
T. 41 Aa	**820**	**ND**	**GA**								
T. 41 Ab	**820**	**ND**	**GA**								
T. 43	**672**	**F**	**31-40**					x		x	
T. 44	**760**	**M**	**31-40**		x		x	x			
T. 48	**626**	**M**	**41-50**								
T. 50	**702**	**ND**	**GA**								
T. 51	**704**	**ND**	**GA**								
T. 55	**677**	**ND**	**GA**				x				
T. 56	**769**	**F**	**19-30**				x	x			
T. 58	**817**	**F**	**19-30**		x			x	x		
T. 59	**818**	**ND**	**GA**								
T. 61	**788**	**ND**	**GA**								
T. 64	**810**	**ND**	**GA**					x			
T. 65	**801**	**F**	**31-40**								
T. 66 Aa	**824**	**M**	**GA**								
T. 66 Ab	**824**	**M**	**GA**		x		x				
T. 66 Ac	**824**	**M**	**41-50**				x				
T. 66 Ad	**824**	**ND**	**GA**								
T. 66 Ae	**824**	**ND**	**GA**				x				
T. 70	**839**	**F**	**19-30**								
T. 72	**864**	**M**	**31-40**					x			
T. 74	**857**	**F**	**19-30**								
T. 76	**871**	**F**	**19-30**					x			
T. 77	**869**	**ND**	**GA**								
T. 78	**854**	**ND**	**GA**								
T. 80	**883**	**ND**	**GA**								

T. 82	**888**	**M**	**41-50**		x	x	x	x		x	
T. 83	**892**	**ND**	**GA**								
T. 84	**900**	**M**	**GA**								
T. 85	**910**	**ND**	**GA**								
T. 86	**115**	**ND**	**GA**								
T. 87	**902**	**ND**	**GA**								
/	**743 Aa**	**ND**	**GA**								
/	**743 Ab**	**ND**	**GA**								
/	**743 Ac**	**ND**	**GA**								
Total (N)				**1**	**8**	**1**	**22**	**18**	**1**	**3**	**4**
Percentage (%)				**1.6**	**12.5**	**1.6**	**34.4**	**28.1**	**1.6**	**4.7**	**6.3**

Table 4. Carbon and nitrogen stable isotope values and protein quality indicators of human and specimens from Muracciola Torresina (SU: Stratigraphic Unit). For each sample data on sex and age death were provided. M indicates male, F female, IND indeterminate (for juvenile individuals for who no attempt of sex determination was made), ND not-determinable, GA generic adult (> 18 years) and GNA generic non-adult (< 18 years). Specimens highlighted in red were excluded from the present research.

Individual	SU	Sex	Age at death (years)	$\delta^{13}C$	$\delta^{15}N$	%N	%C	C/N
T. 3*	69	IND	1-6	-18.8	9.4	14.0	39.0	3.2
T. 9 Aa	526	F	31-40	-19.9	8.4	15.5	42.6	3.2
T. 10	530	F	41-50	-19.9	8.5	15.1	42.2	3.3
T. 12*	533	M	41-50	-19.9	8.1	12.4	34.9	3.3
T. 14	546	F	31-40	-20.0	7.7	15.2	42.2	3.2
T. 16*	547	M	GA	-19.0	12.2	14.0	39.2	3.3
T. 17	567	F	19-30	-19.8	9.0	16.3	45.3	3.2
T. 19	638	ND	GA	-19.5	9.5	15.4	42.7	3.2
T. 23*	764	IND	7-12	-19.8	10.4	8.1	23.9	3.4
T. 24	655	M	19-30	-19.8	8.6	15.5	42.6	3.2
T. 26*	772	IND	1-6	-19.0	12.2	14.0	39.2	3.3
T. 27*	666	IND	13-18	-19.7	9.7	9.2	26.0	3.3
T. 31*	644	ND	GA	-21.1	6.5	2.7	8.2	3.6
T. 32	697	F	31-40	-19.8	9.1	17.0	45.4	3.1
T. 33 Sa*	737	IND	7-12	-20.2	9.5	8.5	24.4	3.4
T. 33 Sb*	738	IND	GNA	-20.0	9.7	11.8	33.3	3.3
T. 34 Aa*	729	ND	GA	-19.6	8.3	7.6	21.5	3.3
T. 34 Ab*	730	M	GA	-19.5	8.5	12.8	36.0	3.3
T. 35*	735	ND	GA	-19.6	10.1	8.5	24.3	3.3
T. 36 Ab	586	F	GA	-20.2	8.2	15.2	41.8	3.2
T. 37 Aa	602	F	41-50	-19.5	8.5	15.7	42.9	3.2
T. 38	731	F	19-30	-20.0	7.4	15.8	43.2	3.2
T. 39	742	M	31-40	-20.2	6.9	15.4	42.7	3.2
T. 40*	750	IND	GNA	-20.2	7.7	9.8	27.7	3.3
T. 43*	672	F	31-40	-19.7	10.5	6.5	19.1	3.4
T. 44	760	M	31-40	-20.3	6.7	14.9	42.5	3.3
T. 50*	702	ND	GA	-20.6	9.6	4.3	12.5	3.4
T. 53*	759	IND	1-6	-19.8	10.9	11.5	32.4	3.3
T. 55*	677	ND	GA	-20.5	8.6	1.5	4.1	3.2
T. 56*	769	F	19-30	-18.9	11.3	16.6	44.8	3.2
T. 57*	789	IND	1-6	-20.0	11.2	8.8	25.2	3.4
T. 58	817	F	19-30	-19.7	9.9	14.4	40.3	3.3
T. 65*	801	F	31-40	-19.9	9.9	11.5	32.6	3.3
T. 66 Ac	824	M	41-50	-20.0	8.1	15.4	42.8	3.3
T. 70	839	F	19-30	-19.9	10.1	13.9	40.5	3.4
T. 72*	864	M	31-40	-21.6	9.2	1.1	2.9	3.1
T. 74	857	F	19-30	-19.4	11.1	16.0	44.2	3.3
T. 78*	854	ND	GA	-19.8	9.7	9.6	27.3	3.3
T. 82	888	M	41-50	-19.7	9.8	15.0	41.6	3.3

T. 84*	900	M	GA	-20.4	8.5	3.6	9.9	3.2
T. 85*	910	ND	GA	-22.5	9.5	4.2	12.2	3.4

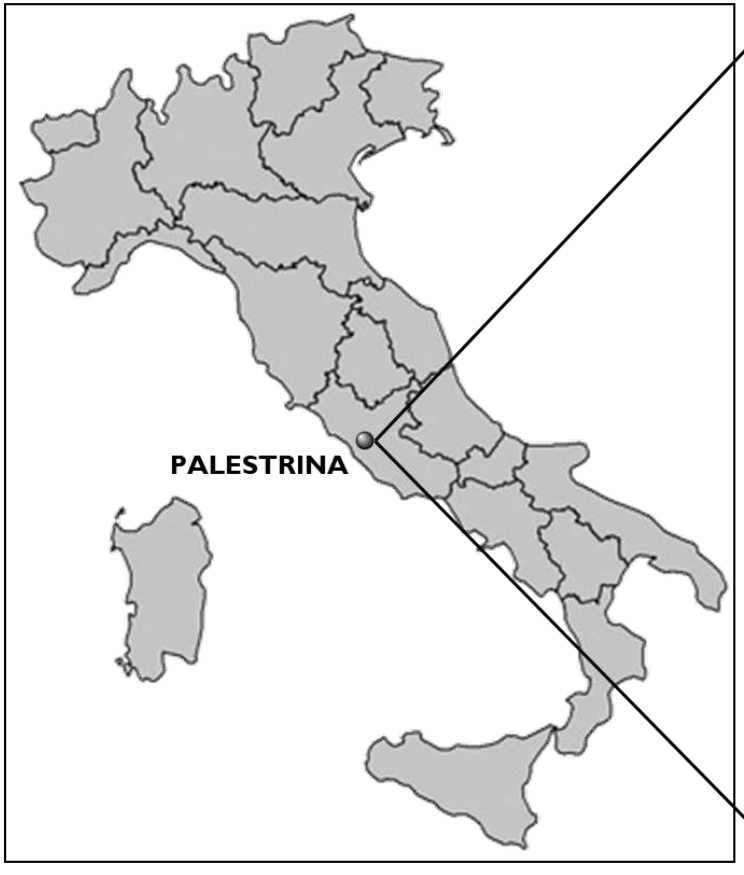
*Samples analysed at the "Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche" of the Università della Campania L. Vanvitelli.

Table 5. Types of starch granules and phytoliths identified by optic microscopy. The amount of plant micro-remains counted in each type and individual were reported. Type I: Fabaceae; Type II: Poeae; Type III: Paniceae; Type IV: Triticeae.

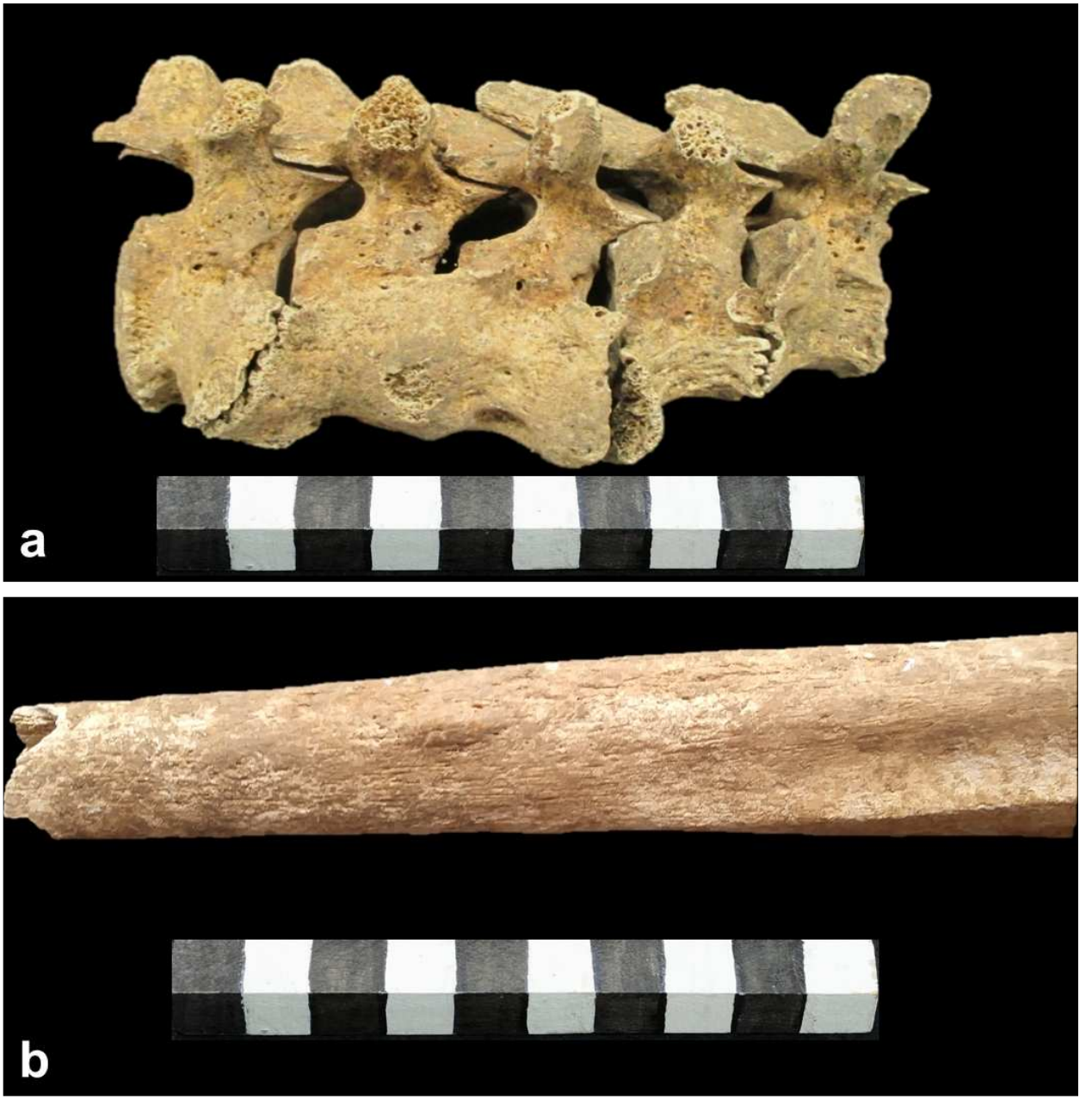
SU/BURIAL	Type I	Type II	Type III	Type IV	Not determined	Total starches per sample	Phytoliths
620/6		3		1		**4**	
655/28		36		4	1	**41**	
769/56	1		16	9	1	**27**	
817/58						**0**	
857/79				3		**3**	
869/72				1		**1**	4
Total	**1**	**39**	**16**	**18**	**2**	**76**	**4**

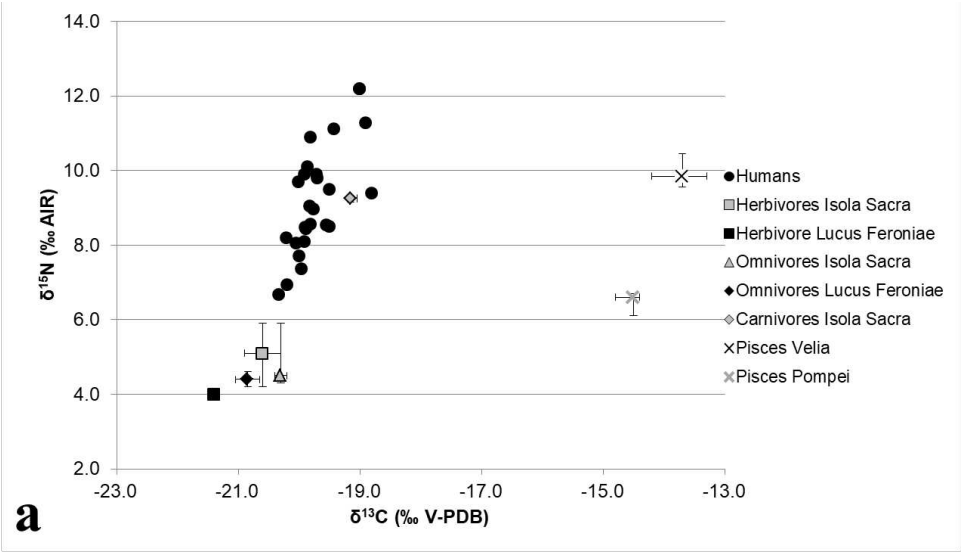
Table 6. Results of Mann-Whitney test of comparisons of Muracciola Torresina with Roman Italian coeval sites.

Archaeological sites	$\delta^{13}C$		$\delta^{15}N$	
	Mann-Whitney (Z)	**p-value**	**Mann Whitney (Z)**	**p-value**
Muracciola Torresina/ANAS	-2.125	0.030	-0.582	0.561
Muracciola Torresina/Casal Bertone	-6.007	1.887×10^{-9}	-2.084	0.037
Muracciola Torresina/Castellaccio Europarco	-3.516	4.000×10^{-4}	-0.397	0.691
Muracciola Torresina/Isola Sacra	-7.527	5.193×10^{-14}	-5.447	4.327×10^{-8}
Muracciola Torresina/St. Callixtus	-0.438	0.662	-3.821	1.331×10^{-4}
Muracciola Torresina/Gabii	-4.851	1.229×10^{-6}	-3.609	3.070×10^{-4}
Muracciola Torresina/Lucus Feroniae	-0.588	0.556	-2.153	0.031
Muracciola Torresina/Velia	-3.954	7.686×10^{-5}	-2.150	0.032
Muracciola Torresina/Herculaneum	-4.466	7.989×10^{-6}	-3.390	6.998×10^{-4}
Muracciola Torresina/Paestum	-1.145	0.252	-3.039	0.002


PALESTRINA


PALESTRINA
MURACCIOLA
TORRESINA
TB. 6 4.0308
a
PALESTRINA
MURACCIOLA
TORRESINA
TB. 6
b


a
b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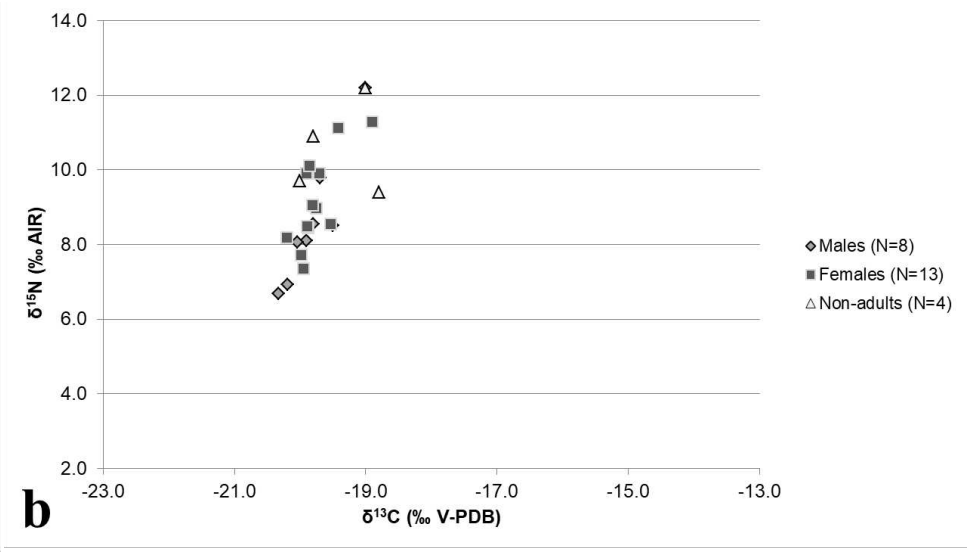
12.0
10.0
8.0
6.0
4.0
2.0
-23.0
-21.0
-19.0
-17.0
-15.0
-13.0
δ15N (‰ AIR)
δ13C (‰ V-PDB)
Humans
Herbivores Isola Sacra
Herbivore Lucus Feroniae
Omnivores Isola Sacra
Omnivores Lucus Feroniae
Carnivores Isola Sacra
Pisces Velia
Pisces Pompei
a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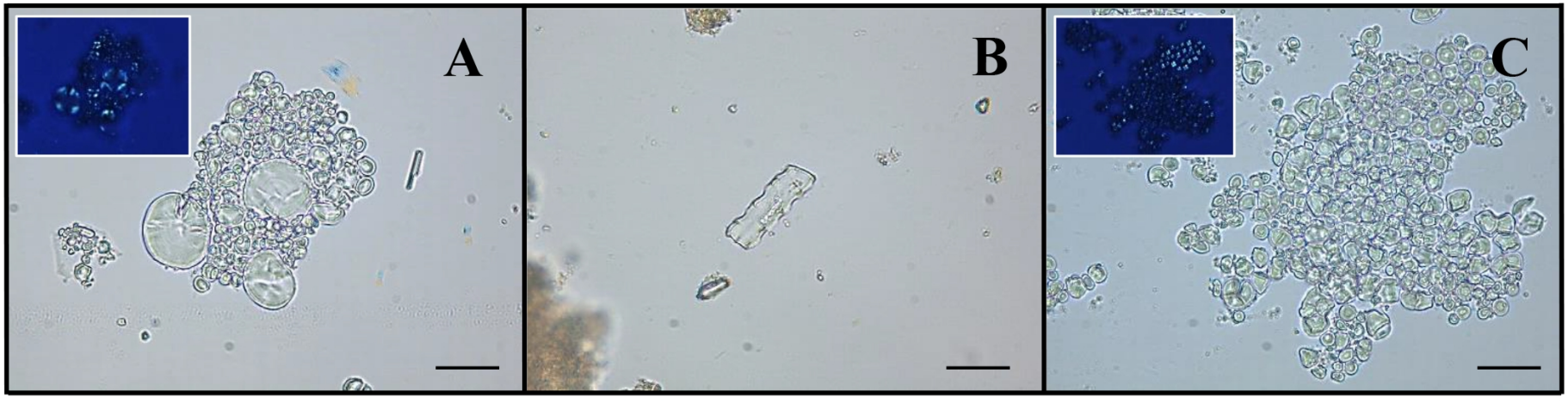
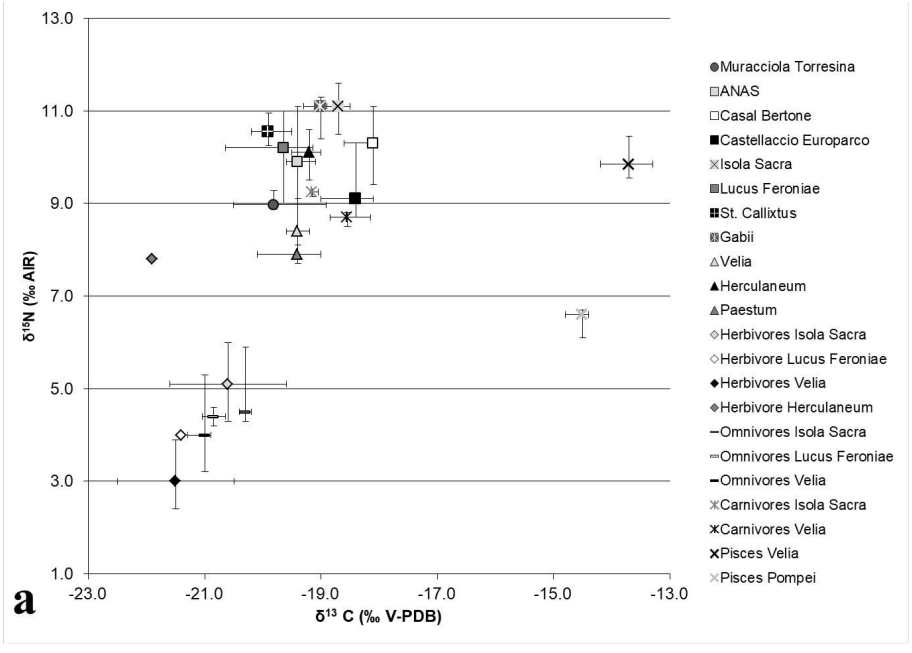
12.0
10.0
8.0
6.0
4.0
2.0
-23.0
-21.0
-19.0
-17.0
-15.0
-13.0
δ15N (‰ AIR)
δ13C (‰ V-PDB)
Males (N=8)
Females (N=13)
Non-adults (N=4)
b


A
B
C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7.0
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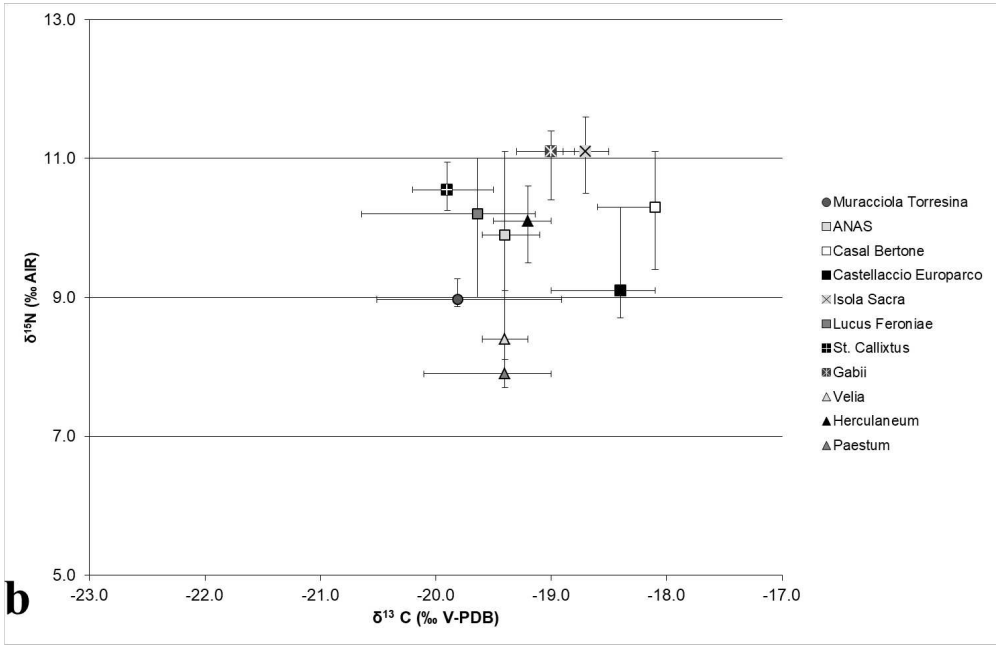
3.0
1.0
δ15N (‰ AIR)
-23.0
-21.0
-19.0
-17.0
-15.0
-13.0
δ13 C (‰ V-PDB)
Muracciola Torresina
ANAS
Casal Bertone
Castellaccio Europarco
Isola Sacra
Lucus Feroniae
St. Callixtus
Gabii
Velia
Herculaneum
Paestum
Herbivores Isola Sacra
Herbivore Lucus Feroniae
Herbivores Velia
Herbivore Herculaneum
Omnivores Isola Sacra
Omnivores Lucus Feroniae
Omnivores Velia
Carnivores Isola Sacra
Carnivores Velia
Pisces Velia
Pisces Pompei

a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11.0
9.0
7.0
5.0
δ15N (‰ AIR)
-23.0
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-21.0
-20.0
-19.0
-18.0
-17.0
δ13 C (‰ V-PDB)
Muracciola Torresina
ANAS
Casal Bertone
Castellaccio Europarco
Isola Sacra
Lucus Feroniae
St. Callixtus
Gabii
Velia
Herculaneum
Paestum

b

Table A1. Mean stature estimates for the analyzed sample. For each individuals data on sex and age at death are provided.

Individual	Sex	Age at death	Mean Stature Estimates	sd Stature Estimates
T. 1 SU 4	F	GA	168.1	4.4
T. 6 SU 620	F	19-30	149.0	5.0
T. 9 Aa SU 526	F	31-40	153.8	4.0
T. 10 SU 530	F	41-50	160.5	4.5
T. 11 SU 538	M	41-50	173.3	4.2
T. 12 SU 533	M	41-50	167.7	5.0
T. 14 SU 546	F	31-40	155.9	5.9
T. 17 SU 567	F	19-30	161.9	4.6
T. 24 SU 655	M	19-30	170.6	5.2
T. 32 SU 697	F	31-40	154.6	4.8
T. 36 Ac SU 587	M	41-50	179.9	5.2
T. 37 Aa SU 602	F	41-50	158.8	5.5
T. 43 SU 672	F	31-40	162.1	4.6
T. 44 SU 760	M	31-40	160.3	5.9
T. 56 SU 769	F	19-30	162.3	5.5
T. 58 SU 817	F	19-30	150.0	5.4
T. 66 Aa SU 824	M	GA	162.7	5.5
T. 66 Ab SU 824	M	GA	159.1	6.5
T. 66 Ac SU 824	M	41-50	167.7	6.8
T. 72 SU 864	M	31-40	169.2	6.3
T. 76 SU 871	F	19-30	150.2	5.0
T. 82 SU 888	M	41-50	162.5	6.3

Table A2. GC-MS profiles. The list of molecular markers detected by GC-MS analysis per each sample (excluding n-alkanes and n-alkenes) was shown. Chemical compounds were clustered in biochemical classes.

SU 620 BURIAL 6		
Sugars		Lactose
Fatty acids	Saturated	Dodecanoic acid Tetradecanoic acid Hexadecanoic acid Octadecanoic acid Heneicosanoic acid Docosanoic acid Tricosanoic acid Hexadecanedioic acid
	Unsaturated	Docosa-2,6,10,14,18-pentaen-22-al, 2,6,10,15,18-pentamethyl- 9,12-Octadecadienoic acid 7-Hexadecenoic acid 11-Eicosenoic acid 9-Octadecenoic acid Trans-11-Octadecenoic acid
Alcohols		1-Decanol 6-Tridecanol
Other markers		Isothiocyanatoacetaldehyde

SU 655 BURIAL 28		
Fatty acids	Saturated	Hexadecanoic acid Octadecanoic acid Docosanoic acid Tetracosanoic acid Octacosanoic acid
	Unsaturated	Docosahexaenoic acid 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene 9,12-Octadecadienoic acid 9-Octadecenoic acid
Alcohols		n-Tetracosanol-1
Terpens and terpenoids	Sesquiterpenes and derivatives	Pacifigorgiol

SU 769 BURIAL 56		
Sugars		Lactose
Fatty acids	Saturated	Dodecanoic acid Hexadecanoic acid Octadecanoic acid Heneicosanoic acid Docosanoic acid Triacontanoic acid
	Unsaturated	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene 9-Octadecenoic acid

		Trans-11-Octadecenoic acid
Alcohols		1-Decanol 1-Hexadecanol
Alkaloids and derivatives		Methylephedrine
Other markers		Isothiocyanatoacetaldehyde Isothiocyanic acid

SU 817 BURIAL 58		
Fatty acids	Saturated	Dodecanoic acid Hexadecanoic acid Octadecanoic acid Heneicosanoic acid Triacontanoic acid
	Unsaturated	9-Octadecenoic acid
Alcohols		2-Octadecen-1-ol
Alkaloids and derivatives		Methylephedrine
Other markers		7-Hexadecenal

SU 857 BURIAL 79		
Fatty acids	Saturated	Dodecanoic acid Hexadecanoic acid Octadecanoic acid Octacosanoic acid
	Unsaturated	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene 9,12-Octadecadienoic acid 9-Octadecenoic acid 6-Octadecenoic acid
Alcohols		1-Decanol
Phenolic compounds and derivatives		6-Methyloctahydrocoumarin
Other markers		Veratric acid

SU 869 BURIAL 72		
Amino acids		Alanine
Fatty acids	Saturated	Dodecanoic acid Hexadecanoic acid Octadecanoic acid Triacontanoic acid
	Unsaturated	7-Hexadecenoic acid 9-Octadecenoic acid
Alcohols		1-Heptanol
Terpens and terpenoids		CitronellolSqualene