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Abstract:	Human osteological samples (n = 23) taken from different anatomical parts of 11 individuals from the post-medieval (16th to 18th century AD) site of Roccapelago (Modena, Italy) were systematically analysed for δ13C, δ15N and trace elements to investigate their diet. δ13C and δ15N correlate and show a high variability between individuals, attesting for the dietary contribution of C4-plants. This is supported by pollen analysis of the burial site samples, which reveal the presence of maize. δ15N correlates with Sr/Ca suggesting that the main protein source could have been milk and dairy. We therefore interpret the strong correlation between δ13C and δ15N as the evidence of C4-plant foraging practice and exploitation of livestock for meat and milk combined with possible direct intake of C4-plants. The Roccapelago site represents an important case study to track post-medieval diet evolution and maize cultivation introduction in southern Europe as attested also by historical sources.

C_4-plant foraging in Northern Italy: stable isotopes, Sr/Ca and Ba/Ca data of human osteological samples from Roccapelago (16th–18th century AD)

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ABSTRACT

Human osteological samples (*n* = 23) taken from different anatomical parts of 11 individuals from the post-medieval (16th to 18th century AD) site of Roccapelago (Modena, Italy) were systematically analysed for $\delta^{13}C$, $\delta^{15}N$ and trace elements to investigate their diet.

$\delta^{13}C$ and $\delta^{15}N$ correlate and show a high variability between individuals, attesting for the dietary contribution of C_4-plants. This is supported by pollen analysis of the burial site samples, which reveal the presence of maize. $\delta^{15}N$ correlates with Sr/Ca suggesting that the main protein source could have been milk and dairy. We therefore interpret the strong correlation between $\delta^{13}C$ and $\delta^{15}N$ as the evidence of C_4-plant foraging practice and exploitation of livestock for meat and milk combined with possible direct intake of C_4-plants. The Roccapelago site represents an important case study to track post-medieval diet evolution and maize cultivation introduction in southern Europe as attested also by historical sources.

KEYWORDS

stable isotope analysis; strontium; barium; trace elements; diet; bone; Roccapelago; Italy; Early Modern; C4 plants

INTRODUCTION

Roccapelago was a secluded community (16th to 18th century AD) located in the Northern Apennines (Fig. 1) that practised animal husbandry and farming, but also exploited anthropic woods of chestnut as highlighted in historical documents. In 2011, excavation under the St. Paul's Conversion church in Roccapelago (Pievepelago district, Modena) brought to light a forgotten crypt used as a cemetery by the community between the 16th and the 18th centuries, where human bodies were massed in a pyramidal cumulus, and included infants, subadults and adults (Gruppioni et al. 2011). Some of these bodies are naturally (totally or partially) mummified due to the peculiar microclimate of the cryptal environment characterized by low humidity and intense aeration (Gruppioni et al. 2011), excluding external contamination and ensuring negligible diagenetic effects. In Northern Italy, the Roccapelago site is a unique case of natural mummification and a rare opportunity to reconstruct three centuries of the life, customs and traditions of a farmer community, which has lived during the arrival of maize in Europe (Rebourg et al. 2003). Several studies have followed this discovery including DNA (Cilli et al. 2015a), gut microbiota (Cilli et al. 2015b), clothing and artefacts (Schoenholzer Nichols 2016; Vernia 2016), palaeopathology (Traversari et al. 2016) and palaeoradiology (Petrella et al. 2016). However, little is known regarding the eating habits and detailed practices related to the diet of the inhabitants of Roccapelago (Pelù 2006; Traversari et al. 2016).

Stable isotope analyses of bone collagen are among the currently most widely used techniques applied to palaeodiet studies to unravel the type of diet and food sources of human and animals (e.g. DeNiro and Epstein 1981; DeNiro and Schoeniger 1983; Katzenberg et al. 1993; Ambrose et al. 1997). The isotope composition of bone collagen quantitatively reflects the isotope composition of the food ingested during the lifetime of the individual/animal (Richards et al. 2006). The most exploited isotopes for this purpose are $^{13}C/^{12}C$ and $^{15}N/^{14}N$, expressed as δ^{13}C and δ^{15}N per mil variation relative to two international standards (vPDB and AIR respectively).

δ^{13}C is used to distinguish C_3 from C_4 protein in the diet, because of the different carbon fixation of C_3 (such as wheat, rice, barley, rye and potato; δ^{13}C from –20 to –35‰) and C_4-plants (such as maize, sorghum and millet; δ^{13}C from –9 to –14‰) during photosynthesis. Because carbon reservoirs (dissolved carbonate) in marine environments show a $\delta^{13}C \approx 0$‰ (atmospheric $\delta^{13}C \approx -7$‰), δ^{13}C values can also be used to discriminate marine vs. terrestrial resources in the diet, with the more negative values for terrestrial provenance (Katzenberg 2008). In terrestrial ecosystems, an offset of *c.a.* 5‰ in δ^{13}C is observed between the herbivore and the plants they consume; an offset of *c.a.* 1‰ occurs between a carnivore and its herbivore prey.

δ^{15}N values reflect the protein portion of the diet, given the lack of nitrogen in carbohydrates and lipids. Thus, δ^{15}N depends on the position of the individual in the trophic chain, with an enrichment of +3-5‰ for each step because of the preferential fixation of heavier molecules in living tissues. The length of aquatic trophic chains is the cause of the high δ^{15}N values in these environments, especially for the top predators (Hedges and Reynard 2007; Katzenberg 2008). Likewise, δ^{13}C shows a stepwise enrichment along the trophic chain of *c.a.* 1‰, but this is generally too low to be detected.

Stable isotope variations of human collagen are generally explained as the result of two food sources mixed together modelled using simple linear mixing models with two end-members, chosen between the major food sources offered by the local environment. Thus, it is important to investigate the isotopic composition not only of human remains but also of the local fauna and flora. In more complicated contexts, where more than two food sources are part of the human diet it might be appropriate to use non-linear or bayesian mixing models (Phillips et al. 2014).

Trace elements have been used for decades to study the diet of individuals with mixed results due to diagenetic effects on their abundances and to the fact that the behaviour of many trace elements does not reflect the eating habits of the individual (Elias 1982; Pate and Hutton 1988; Pate et al. 1989; Tuross et al. 1989; Klepinger 1990; Pate 1994; Burton and Wright 1995; Ezzo 1994; Burton et al. 1999; Schutkowski et al. 1999; Balter et al. 2002; Hedges 2002; Mays 2003; Dolphin et al. 2005; Burton 2008; Arnay-De-La-Rosa et al. 2011; Austin et al. 2013; Lösch et al. 2014). This is especially true for archaeological samples that sit in the ground for years to thousands of years interacting with the chemicals dispersed in the soil and with those transported by rain and ground water. In this regard, Roccapelago represents a unique situation, where the dry and ventilated environmental conditions of the closed burial site (crypt) led to the natural mummification of the individuals and kept them away from the interaction with soil and water. In general, the exchange of Sr^{2+} (and other trace elements) ions between soil and bone occurs in low-pH environment, where the hydroxyapatite (more soluble at low-pH) interacts with soil solutions and underground waters. Hence, any precipitation of secondary calcium phosphate may lead to the incorporation of trace elements from both the soil and the bone tissue (Pate et al. 1989). Nevertheless, several works have shown the reliability of Sr/Ca and Ba/Ca ratios as dietary proxies (e.g. Burton and Wright 1995; Burton et al. 1999; Balter et al. 2002). Strontium physiology and behaviour along the food chain are well constrained (e.g. Burton and Wright 1995; Burton et al. 1999; Mays 2003; Bentley 2006; Lösch et al. 2014). Divalent Sr^{2+} cations introduced in the body with food tend to replace calcium (Ca^{2+}) in the hydroxyapatite of bones because of the close chemical and physical behaviour of the two elements. Sr has no metabolic functions within the body and, therefore, its concentration in bones is not regulated by homeostasis. However, Ca is preferentially assimilated with respect to Sr during biological activities, a process known as *biopurification* (Burton et al. 1999). This is because, the shift of Sr from the digestive system to the bloodstream is less efficient than that of Ca, hence, only *ca.* 20% of the strontium ingested is absorbed and fixed in bones. Therefore, the Sr/Ca ratio tends to decrease from low to high trophic positions, reflecting this preferential assimilation of Ca. Furthermore, Burton and Wright (1995) demonstrated that, in a multi-component diet, Sr/Ca does not show a linear relationship with the abundance of consumed meat/plant. For this reason, they suggest that the Sr/Ca ratio better reflects the highest Ca source rather than the proportion of meat vs. plants in a multicomponent diet (Burton and Wright 1995; Lösch et al. 2014).

Barium behaves similarly to strontium (Burton 2008). However, because of its larger ionic radius Ba is less efficiently fixed in bones than Sr and, as a consequence, its biopurification degree is higher, resulting in lower Ba concentrations in bones compared to Sr (Burton 2008). Yet, because of their comparable metabolic behaviour, Ba can also be used as a marker of a Ca-source in a multi-component diet (Burton and Wright 1995). Since both indices (Ba/Ca and Sr/Ca) carry the same information about the diet, a strong correlation between the two is expected in a biological integer sample (Burton et al. 1999). On the contrary, when the seafood is the main dietary component, the Ba behaviour is quite different from Sr, causing a lack of correlation between the Ba/Ca and Sr/Ca ratios (Burton and Price 1991).

In this work, we analyzed the trace element composition (Sr/Ca and Ba/Ca), the $\delta^{13}C$ and the $\delta^{15}N$ of bones and teeth of 11 individuals from the Roccapelago site. The main aim of this study was to detect the food components and changes with time of their diet comparing collagen data from bone and dentine with the idea of possibly identifying the introduction of C_4 plants (likely maize) into the diet, given that this community lived during the arrival of maize from America to Europe and the spread of the same to Italy (Cazzola 1991; Rebourg et al. 2003). While bone collagen reflects the latest years of life because of constant bone remodelling, primary dentine, once formed, does not physiologically remodel and, therefore, records the childhood eating habits (Lee-Thorp 2008). Unfortunately, no animal remains were found in the Roccapelago site because of the strictly mortuary function of the context and it has not been possible to compare the trace element and isotopic ratios of human and local animals. However, the high variability of the diet found in studied individuals, gave us the opportunity to infer at least two principal food sources and to reconstruct the probable local foraging practices.

Archaeological and historical background of the Roccapelago site

Roccapelago is a village located near the small town of Pievepelago (Frignano area, Modena Apennines) on the bank of the Scoltenna river, in the centre of the Pelago Valley (Fig. 1). The most important and relevant building in Roccapelago is the Church of the Conversion of St. Paul. The building was erected at the end of the 14th century, as a military fortification by Obizzo di Montegarullo. Founded on the bare rock, the structure is located on a high peak dominating the surrounding area (Gruppioni et al. 2011). In 1585, it lost all of its military functions and became a proper Christian church. Between October 2009 and March 2011, parallel to building restoration, the excavation of the site under the church by the Archaeological Superintendence of Emilia Romagna brought a hidden funerary crypt to light. This funeral context, dated through archaeological artefacts from the late 16th century to the 18th century, consists of bodies massed in a pyramidal cumulus, probably dropped through a manhole from the church floor (Gruppioni et al. 2011). The presence of two small windows in the crypt and the pyramidal arrangement of the bodies allowed drainage of decomposition fluids and, consequently, a partial to total mummification of some individuals. The crypt bears no traces of exogenous fluid flows suggesting no modification of the environment occurred since the burial. The bodies belong to a wide age range of individuals (from infants to elders) of both genders (Gruppioni et al. 2011), and from their clothing it has been inferred that they were all common people from a rural community mostly dedicated to farming (Schoenholzer Nichols 2016). Therefore, the mummies of Roccapelago represent a significant discovery that allows studying the peasant practices and rural life in a post-medieval era (16th to 18th century AD).

MATERIALS AND METHODS

A total of 23 human samples (n = 7 tooth samples; n = 16 bone samples) were recovered from different anatomical areas of 11 individuals, chosen from the best preserved within the 281 (minimum number of individuals) of the site (Table 1). Given some restrictions in accessing the site, we could only sample a limited number of individuals and have chosen only among those individuals articulated in anatomical connection, so we can exclude an oversampling of the same individual. We stress also that these remains were retrieved from a crypt, with no interaction with soil and/or water.

Teeth were extracted with tongs from the maxilla or the mandible. Bone samples were separated from humerus and femur or tibia (when femur was absent) with a diamond wheel saw. Samples were split in two parts: one for trace element analyses and the other for stable isotope analyses. Both splits were cleaned mechanically with brushes. Bones samples for trace element analysis were cut with a diamond low-speed wheel saw in a 2 mm slice in order to expose the inner parts. Each slice was polished with alumina powder and abrasive papers at decreasing sizes: 800, 500, 5 and 1 μm, then rinsed with MilliQ® water and washed with 2 cycles of ultrasonic bath (15 min) with MilliQ® water. Samples were then dried for 48h at room temperature (protocol modified from Cucina et al. 2007).

Samples for stable isotope analysis were treated for collagen extraction. First, the samples were mechanically cleaned with a power dentist drill and crushed to obtain 150-200 mg of powder. Bone and dentin chunks were left in a 0.5 M HCl solution for 72h at 4°C (Longin 1971; Ambrose 1990; Bocherens et al. 1991; Iacumin et al. 2014). After 48h, HCl was replaced with new HCl. Samples were then washed with deionized water and left in a 0.125 M NaOH solution for 20h at room temperature. Residuals were washed again with deionized water, to reach neutrality, and put in a drying oven for 17h at 100°C. At last, the centrifuged supernatant was frozen and lyophilized. Stable isotope analysis of δ^{13}C and δ^{15}N were run and duplicated with a Carlo Erba EA1110 CHN analyzer, coupled to a Finnigan Delta S mass spectrometer. Bone and dentine samples were analyzed in order to obtain information about any possible variation in the diet of the studied individuals, from juvenile to adult age.

In situ Sr, Ba, Pb, Mg, Zn, Fe and Cu concentration of bone samples were measured at the Centro Interdipartimentale Grandi Strumenti (CIGS) of the University of Modena e Reggio Emilia using a 213 nm Nd:YAG laser ablation system (NewWave Research) coupled to a quadrupole ICP-MS (Thermo Fisher Scientific X-Series^{II}). NIST 1486 Bone Meal (powder) was prepared as a pellet under a manual press and used as reference material. Data were acquired for ^{88}Sr and ^{138}Ba and data reduction was obtained with the Plasma Lab software. Calcium concentrations, obtained at CIGS by an ESEM Quanta 200 in low vacuum, were used as internal standard for the correction of matrix-related effects in samples (Hanć et al. 2013). Cortical tissue in bone samples was investigated with 5 random ablation points per sample. Before analysis, samples were carefully pre-ablated to clean further the external surface, reducing any possible contamination. In order to ensure the accuracy of the method, a two-tailed *t*-test was performed on two certified elements of the standard 1486 Bone Meal: Zn and Sr. Statistical analysis showed no significant difference between expected and measured values ($p < 0.01$; mean of 3 analyses). The Ba concentration is not certified for NIST 1486, we therefore used data reported by Porte et al. (1997). The typical RSD error for laser ablation trace element analyses is *c.a.* 10%.

Pollen analysis was performed in two ground samples collected from the crevices of a buried wall of the crypt contemporaneous with the latter mummies present in the site (18th century). The two soil assays where sampled, after manual removing of the exposed surface to avoid modern pollen contamination, from the most external part of the crevice, i.e. the closest to the room, and from the deeper part of the crevice between two stone blocks. Samples were treated according to the routine method in use at the Laboratory of Palynology and Palaeobotany of Modena (Florenzano et al. 2012). Cerealia pollen was identified under 1000x plain light microscope based on Beug (2004) and Faegri et al. (1989), with correction factor for glycerol jelly. About 500 pollen grains per samples were counted.

RESULTS

Stable isotopes

Data for bones and dentine for each individual are presented in Table 1 and Table 2.

$\delta^{13}C$ of bone samples ranges from –20.1 to –12.2‰. $\delta^{15}N$ values are also highly variable, ranging from 5.5 to 9.5‰ (Table 1). For each individual, both $\delta^{13}C$ and $\delta^{15}N$ from dentine show values quite similar to their respective bone samples (from –19.6 to –13.7‰ for $\delta^{13}C$ and from 5.5 to 8.9‰ for $\delta^{15}N$; Table 2). The quality of the data depends on the conservation state of collagen, that is evaluated based on the collagen yield and in modern bones is about

22 wt %. The mean of collagen yield for individuals from Roccapelago is 21.0 wt % ± 2.9 (1σ), very close to modern bones. Carbon and nitrogen can also be expressed as wt % of the combusted extracted collagen. Intact collagen is about 35 wt % for C and ranges between 11 wt % and 16 wt % for N. For the individuals here studied, C wt % ranges from 34 to 42 wt %, while N from 13 to 16 wt %. No C:N ratio falls outside the 2.9–3.6 range reported in the literature (Ambrose 1990; Van Klinken 1999). Routinely 1σ-uncertainties of standards are ± 0.2‰ for δ^{13}C and ± 0.1‰ for δ^{15}N.

Sr/Ca and Ba/Ca

Significant trace element ratios for diet studies of the Roccapelago individuals are presented in Table 1. However, for comparative purposes, we present in the Supplementary information other trace element data collected simultaneously during laser ablation ICP–MS analysis that, however, are less reliable and more problematic for palaeodiet reconstructions (Supplementary table 1).

The Sr/Ca [μg/mg] ratio in bone samples ranges from 0.45 to 0.79 and from 0.47 to 0.79 considering individual averages. The Ba/Ca [μg/mg] ratio ranges from 0.004 to 0.071 and from 0.004 to 0.065 considering individual averages (Table 1).

Pollen

About 40% of the recognized taxa are represented by pollens pertaining to entomophilous plants (e.g. roses, cornflowers, poppies among others). These plants are characterized by beautiful flowers, thus representing possible memories of offerings during burial rites. The pollen spectra also reflect the image of the neighbouring oak woods (with chestnut, deciduous Quercus, hazel), along with beech and conifers (including silver fir). In addition, our data show the presence of pastures (as suggested by high percentages of Cichorioideae and Poaceae wild grass group) and cereal fields (Avena/Triticum group, Hordeum group, *Secale cereale* and *Zea mays*). Cultivated fields might have been very close to the site, in accordance with geomorphological features observed on the western side of the site suitable for crop cultivations (Bosi et al. *in press*)

DISCUSSION

History and botany

The economic profile that characterized the population of Roccapelago was strongly influenced by the particular geographical situation, at least in the last 400 years. Located about 1200 m.a.s.l. between forests, Roccapelago has always used the sylvan environment for subsistence, using the communication routes to move goods, animals and people (Pelù 2006). Within the Frignano area, husbandry of cattle for milk and workforce and ovine (mainly sheep) for milk and wool was a common practice; moreover, many pigs were bred for meat and poultry for meat, eggs and feathers (Bellettini, 2012). One of the most important plant species for the inhabitants of Roccapelago was the chestnut, extending to about 1000 m.a.s.l., on top of which were usually built shelters for shepherds and charcoal burners, including species such as beech and oak. The cultivation of cereals (mainly barley and rye) was moderately present with corn appearing in the historical cadastral records (also called *estimi*) only during the 19th century (Cazzola 1997).

The archaeobotanical analysis of two ground samples from the crypt shows the presence of maize pollen (Fig. 1). In Europe, maize is a relevant chronological marker (16th cent.); moreover, it is one of the few outcrossing plants among the major cereals (Rebourg et al. 2003). In spite of the high pollen production of Zea, particularly significant in the oldest varieties (estimated range pollen quantum per tassel; 14-50 million vs. 2-5 million in modern hybrids), the atmospheric dispersion of maize pollen is quite narrow even in presence of strong winds (Vogler et al. 2009). In fact, the large size of Zea pollen grains and their rapid settling rate influence the airborne pollen dispersal. It has been demonstrated that at a distance of 60 m from the source plant, maize pollen concentrations average about 1% of those at 1 m and the quantity of pollen remaining airborne at 60 m is 5% of that one at 1 m (Raynor et al. 1972). The presence of maize in the two studied samples can be explained by the arrival of its pollen through the two windows of the crypt (aerial transport), also favoured by the high pollen production of the oldest landraces and suggesting cultivations close to the crypt itself. We cannot exclude, however, that maize pollen could have arrived by unintentional anthropic transport, perhaps through shrouds and clothes of the deceased.

Stable isotopes, Sr/Ca and Ba/Ca ratios

The high variability of the individual $\delta^{13}C$ values (–20.1 to –12.2‰; Fig. 2) suggests that at least two different sources have contributed to the Roccapelago inhabitant's diet: one with low $\delta^{13}C$ close to the range of C_3 plants (–23 to –32‰) and one with higher $\delta^{13}C$ in the range of C_4 plants (–10 to –16‰).

The variability of $\delta^{15}N$ (5.5 to 9.5‰) is smaller than $\delta^{13}C$ but reflects the combination of two different sources in the diet with different protein content (Fig. 2). The difference between the higher (9.5‰) and the lower (5.5‰) $\delta^{15}N$ is *c.a.* 4‰, corresponding to an entire trophic step.

The high positive correlation between $\delta^{13}C$ and $\delta^{15}N$ ($R^2 = 0.89$; $p < 0.01$) is usually used as a marker for marine fish consumption (e.g. Richards and Hedges 1999; Fornaciari 2008). However, within the Roccapelago community we exclude the consumption of aquatic resources. $\delta^{15}N$ is quite low when compared with the commonly high values typical of aquatic protein consumers (Richards and Hedges 1999; Hedges and Reynard 2007; Fornaciari 2008; Fuller et al. 2012) and none of the individuals cross the terrestrial–marine cut-off of 12.5‰ reported by Salamon et al. (2008) for an Italian medieval sample (Reitsema and Vercellotti 2012). Moreover, strong historical–archaeological evidences of fish consumption or presence are yet to be found. Fornaciari (2008) describes the diet of two Renaissance courts (Medici Gran Duke in Florence and Aragonese Princes in Naples) where individuals consumed fish and high protein foods (Fig. 3). Their $\delta^{15}N$ values are more positive (from *c.a.* 10 to 14‰) than those of Roccapelago, suggesting a much higher protein intake. $\delta^{13}C$ values are on average more negative (from *c.a.* –19 to –16.5‰) and this shift of $\delta^{13}C$ values from the classical C_3 environment range is interpreted as the effect of marine resources consumption

(Fornaciari 2008). The more negative $\delta^{15}N$ and more positive $\delta^{13}C$ values of Roccapelago inhabitants with respect to Medici and Aragonese lead us to a different interpretation of the correlation between the two variables. We suggest that the strong correlation in Roccapelago results from the intake of two protein sources from different trophic levels with different C_3-C_4 proportion. In particular, the high $\delta^{15}N$ and $\delta^{13}C$ food source is identifiable with the greater C_4 protein portion. One of the possible explanations for this enrichment in both C_4 portion and protein intake could be the practice of C_4-plant foraging and the exploitation of meat and dairy from C_4-fed livestock (bovines or caprovines; Galli 1980) that leads to an increase of $\delta^{13}C$ in human collagen.

Few cereal plants with C_4 photosynthetic pathways are known in Emilia-Romagna during the 18th century: maize (*Zea mays*), sorghum (*Sorghum bicolor*) and millet (*Panicum miliaceum* and *Setaria italica*). Maize, in particular, is the most important from a historical point of view. Introduced in Emilia-Romagna and in the Po delta territories between the end of 16th century and the first half of 17th century, this cereal supplanted all the other local crops in northern Italy at the beginning of 17th century and had its vastest diffusion during the first half of the 18th century (Cazzola 1991). The recovery of *Zea* pollen in the layer contemporaneous to the mummies indicates that the individuals cannot be prior to the last decades of the 16th century AD. Historical sources testify for a spread of corn in the Este Dukedom since the 16th century. During the first half of the 17th century, maize became an important element of the rural economy (Cazzola 1991). In Italy and central Europe, both millet and maize were used as main food (Cortonesi 2002), especially in poor community during time of famine, and as animal forage. Parmentieri (1791) states that “cows eat maize forage greedily, and it makes them yield a lot of milk” (Barrière et al. 2006). Young (1792) says that farms can be categorized in “good” and “bad”, in relation to the presence or absence of maize in animal feeding (Barrière et al. 2006). Both historical observations are in support of the C_4-plant feeding hypothesis.

From a geochemical point of view, we inferred the possible collagen stable isotope values of the Roccapelago livestock (Fig. 2) using our most enriched individuals (more positive $\delta^{13}C$ and $\delta^{15}N$ of human collagen) as proxy of a nearly total animal protein diet. Taking into account a fractionation of 1‰ for the carbon and of 4‰ for the nitrogen, we obtain a livestock $\delta^{13}C$ of c.a. –13.2‰ and a $\delta^{15}N$ of c.a. 5.5‰. We then extrapolate the possible livestock fodder composition in terms of C_3 and C_4 proportion, using a simple linear interpolation with two end-members (Tykot 2006). The $\delta^{13}C$ values of the two end-members are taken from Iacumin et al. (2014), with a $\delta^{13}C$ of –24.6‰ for the C_3 plants and a $\delta^{13}C$ of –10.4‰ for the C_4 plants. To correct for the diet-collagen offset, we consider an enrichment of +5‰ (Tykot 2006). Thus, our Roccapelago individuals fit in a model with a livestock fodder likely composed by 55% of C_3 plants and 45% of C_4 plants.

Individual depleted $\delta^{13}C$ and $\delta^{15}N$ values reflect an almost total vegetarian diet, with very little intake of high-protein foods. Their primary vegetal food source can be reliably identified in local cultivated C_3-cereals and forest products.

Stable isotopes of collagen primarily reflect the protein portion of the diet, for this reason we can not exclude the direct use of C_4 plants in the Roccapelago diet. This signal can be masked by the highest protein source that also consists of a high C_4 portion. In fact, high protein animal-derived food is the highest contributor to the collagen isotopic composition (Tykot 2006).

We do not observe any particular cluster in relation to sex or age, although the sample of our analyses is too small to provide a significant statistic. The differences between $\delta^{13}C$ and $\delta^{15}N$ of bone and dentine are in general very low. Taken together, all bone and dentine data do not show any specific trend in the protein intake or in the C_3-C_4 proportion. This highlights an almost unchanged diet during the growth of the individual. Considering the values individually, however, we suspect a small variation in the eating habits for four individuals. Roc11 shows a $\Delta^{13}C_{den\text{-}bone}$ of –1.2‰ and a $\Delta^{15}N_{den\text{-}bone}$ of –0.6‰, suggesting a lower intake of protein during childhood. Roc10 shows an unchanged $\Delta^{13}C_{\text{den-bone}}$ (0.1‰) but a $\Delta^{15}N_{\text{den-bone}}$ of –0.9‰, probably related to a very small decrease in protein intake not detected by the $\delta^{13}C$ value or possibly to a little variation in the protein source mixing. Conversely, both Roc5 and Roc2 show an increase in $\delta^{15}N$ of *c.a.* 1‰ and a correspondent increase in $\delta^{13}C$ (*c.a.* 0.5‰), testifying a likely small increase in the intake of protein foods (Table 2).

Strontium and barium in bones derive from food ingestion and drinking water, as well as from possible diagenetic contamination, particularly in bone samples. However, the peculiarity of the archaeological context of Roccapelago, where bones were not directly buried in the ground and away from direct contact with ground and meteoric water and the natural mummification of the corpses with muscles still in place in some cases, let us assume that the Sr and Ba variabilities can be related to in vivo processes rather than post-burial diagenesis (cf. Pate and Hutton 1988; Pate et al. 1989). Moreover, the strong correlation ($R^2 = 0.83$; $p < 0.01$) between Sr/Ca and Ba/Ca (Fig. 4c), suggests that the samples are biologically integer, as demonstrated also in studies of Burton and Price (1991) and Burton et al (1999).

Sr/Ca individual values were compared with $\delta^{15}N$ to observe any possible correlation between the two variables. If we hypothetically assume an almost mono-component diet for the studied individuals, the correlation between the Sr/Ca ratio and the $\delta^{15}N$ can be interpreted as a mere trophic effect, recorded in both variables (Fig. 4a). However, in the case of a multi-component diet, the strong negative correlation ($R^2 = 0.61$; $p < 0.01$; 1 outlier removed) between individual Sr/Ca ratio and $\delta^{15}N$ may suggest that the protein intake and the major Ca-source were linked. Individuals with a lower Sr/Ca ratio, and a consequent highly biopurified-Ca diet, show also a higher protein diet (high $\delta^{15}N$; Fig. 4a). The diet of these individuals was probably composed by Ca-enriched or Sr-depleted foods, such as milk and dairy, in agreement with the livestock exploitation hypothesis. A similar correlation can be observed between Ba/Ca and $\delta^{15}N$ ($R^2 = 0.68$; $p < 0.01$; 1 outlier removed; Fig. 4b). Individuals presenting the highest Sr/Ca ratio probably used to eat low-Ca and/or high-Sr foods like cereals and legumes

(Schutkowski et al. 1999). One outlier value, removed from the previous regressions (solid black diamond in Fig. 4), is represented by a single individual with Sr/Ca ratio of 0.79, Ba/Ca ratio of 0.063 and δ^{15}N value of 9.0‰. This individual shows a higher Sr/Ca ratio than the other individuals with a similar δ^{15}N. This may indicate that the main protein portion of the diet of this individual was represented by a Ca-depleted but high-protein food, like meat. However, no differences in clothes or ornaments was found in this individual to indicate that he was from a different rank in the society with a possibly different high-protein diet.

Given that Ba behaves quite differently from Sr in case of a seafood-based diet (Burton et al. 1999), the observed strong correlation between Ba/Ca and Sr/Ca ($R^2 = 0.83$; $p < 0.01$; Fig. 4c) confirms the lack of seafood in the diet of Roccapelago studied inhabitants as already showed by the δ^{15}N and δ^{13}C data. The only individual that seems to not perfectly fit the regression is, again, the outlier mentioned before (Roc8); in fact, removing this individual from the fit, we observe an even stronger correlation ($R^2 = 0.92$; $p < 0.01$; 1 outlier removed). Such evidence corroborates the hypothesis of a different main protein-source for Roc8.

A closer look at the correlation of δ^{15}N with δ^{13}C (Fig. 2) shows the presence of sub-clusters of individuals. In particular, individuals Roc1, 2, 3, 4, 5, and 7 (group 1) plot together at low δ^{15}N and δ^{13}C values; whereas individuals Roc6, 9 and 10 (group 2) and individuals Roc8 and 11 (group 3) cluster at higher δ^{15}N and δ^{13}C. Group 1 and group 3 define the two end-members of the correlation with group 2 lying in the middle between the two extremes. In the correlations of δ^{15}N with Sr/Ca and Ba/Ca and of Sr/Ca with Ba/Ca (Fig. 4), these clusters are fairly maintained. In fact, group 1 is still well defined in all plots while group 2 and 3 plot together at higher δ^{15}N and lower Sr/Ca and Ba/Ca values. Roc9 departs from group 2 and plots now in the middle of the correlations, while Roc8 becomes a total outlier following the group with the Ca-depleted diet (Group 1). These clustering of individuals can be interpreted as an ulterior proof of the diet-related significance of these ratios for the Roccapelago case study and, in general, an indication that the combined use of Sr, Ba and stable isotopes can push the archaeological community further in understanding past eating habits.

CONCLUSIONS

Stable isotopes and trace element ratios can be a powerful tool to examine not only the diet of ancient population but also diet-related practices as the foraging of livestock. We interpret the isotopic trends shown in this case study (high correlation between $\delta^{13}C$ and $\delta^{15}N$) as the mixing of at least two main food sources in the human diet, represented by C_3-plants (lowest protein source) and C_3-C_4-fed livestock (highest protein source). In Roccapelago, C_4-plants were probably used as forage for the livestock, but we cannot exclude a direct C_4-plants intake, quite common in this kind of rural community. Sr/Ca and Ba/Ca ratios reveal that the main source of protein was likely represented by milk and dairy, in agreement with the exploitation of livestock, and no evidence of seafood.

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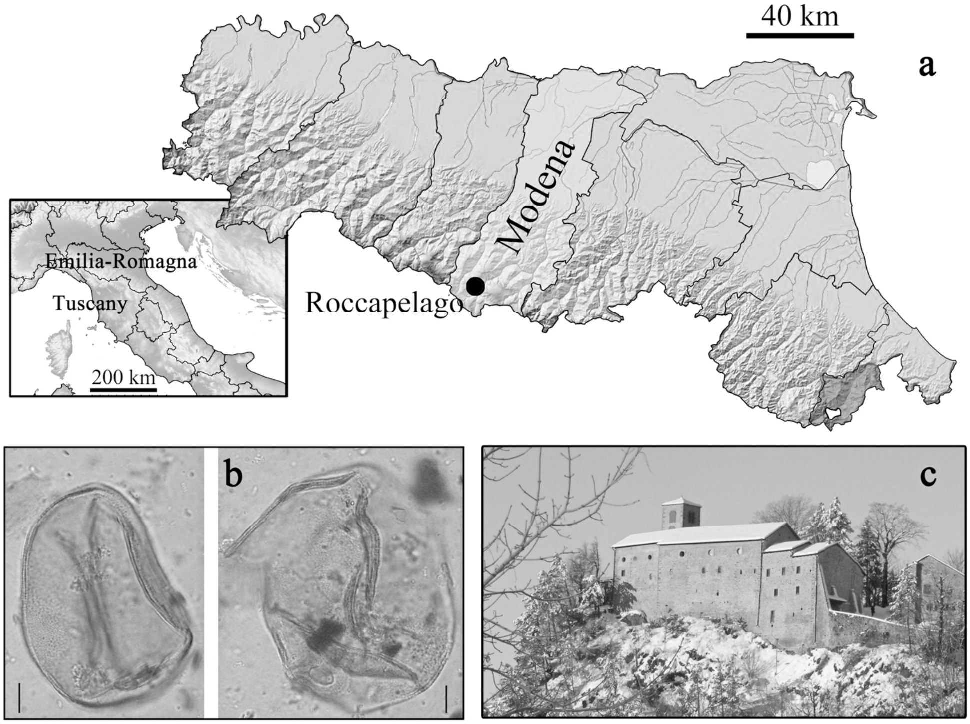
Figure 1. (a) Location of the village of Roccapelago (Modena) in Emilia-Romagna, Italy. (b) *Zea mays* pollen grain observed in the soil samples from Roccapelago crypt; scale: 10 µm – photos with Leica MC 120 HD at 1000X magnifications (by P. Torri). (c) Photo of the Conversion of St. Paul Church.

Figure 2. (a) Collagen stable isotopes of the Roccapelago individuals (black diamond) and fields of food end-members. C3 and C4 plant $\delta^{13}C$ values are from Iacumin et al. (2014) where $\delta^{15}N$ values are inferred from human data taking into account the trophic position. Plausible mixed C3-C4 fed livestock (bovines or caprovines) position is deduced from human and plant data. α (± standard error) is the intercept and β (± standard error) is the slope of the regression line. Circles outline clusters of individuals as explained in the text. (b) In the inset, the $\delta^{13}C$ value of a mammal with a pure C3-diet, a pure C4-diet and the modelled Roccapelago livestock mixed diet. Based on our data we extrapolate the $\delta^{13}C$ value of the Roccapelago livestock to be composed of a 45% C4 diet and 55% C3 diet.

Figure 3. $\delta^{13}C$ and $\delta^{15}N$ comparison between Roccapelago, Medici and Aragonese (Fornaciari 2008) and Mainizza (Iacumin et al. 2014) diet. In dark gray the field of Roccapelago individuals. In light gray the field of the Mainizza settlement. Dashed field represents Medici and Aragonese families. In bold, two of the possible diet end-members for each site. Medici (16th–17th AD) and Aragonese (15th–17th AD) were italian noble families, Mainizza (10th–11th AD) is a rural settlement in Friuli V.G., NE Italy.

Figure 4. Correlations of $\delta^{15}N$ with Sr/Ca and Ba/Ca ratios and of Sr/Ca with Ba/Ca ratios in bones of Roccapelago individuals. Black diamond is the outlier Roc8 not considered in the regressions (see text for details). Labels represent the number of individuals (IDs from Table 1), wording “Roc” is omitted to improve the readability of the graph. Circles outline clusters

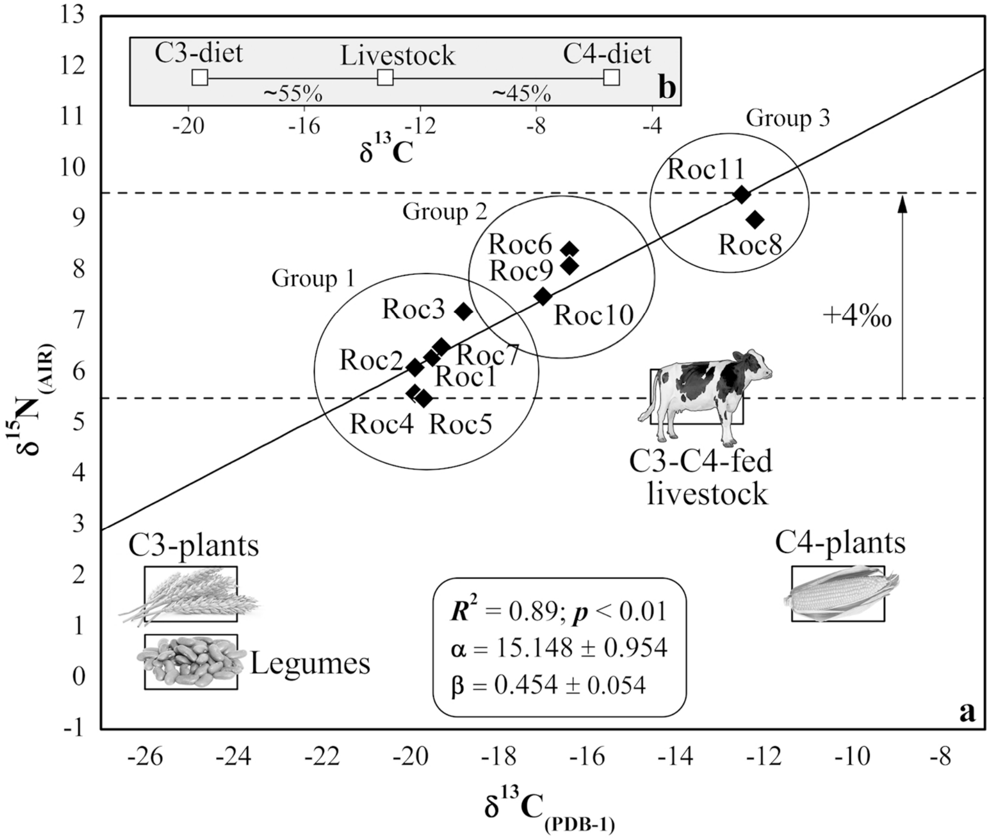
of individuals as explained in the text.



(a) Location of the village of Roccapelago (Modena) in Emilia-Romagna, Italy. (b) Zea mays pollen grain observed in the soil samples from Roccapelago crypt; scale: 10 μm – photos with Leica MC 120 HD at 1000X magnifications (by P. Torri). (c) Photo of the Conversion of St. Paul Church.

Fig. 1

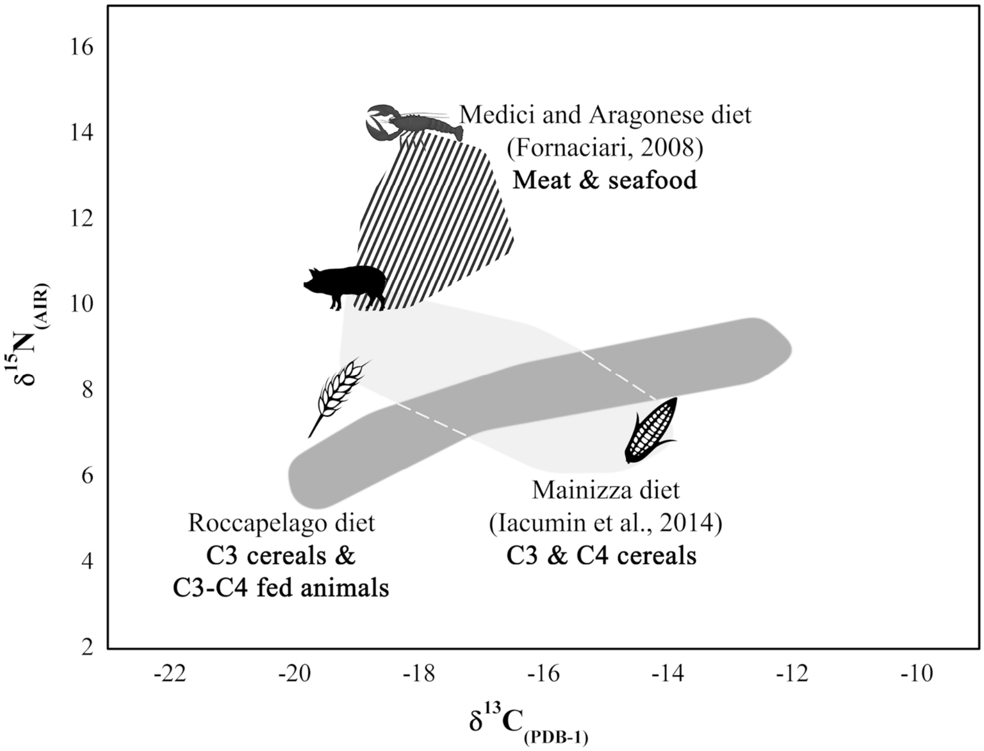
149x111mm (300 x 300 DPI)



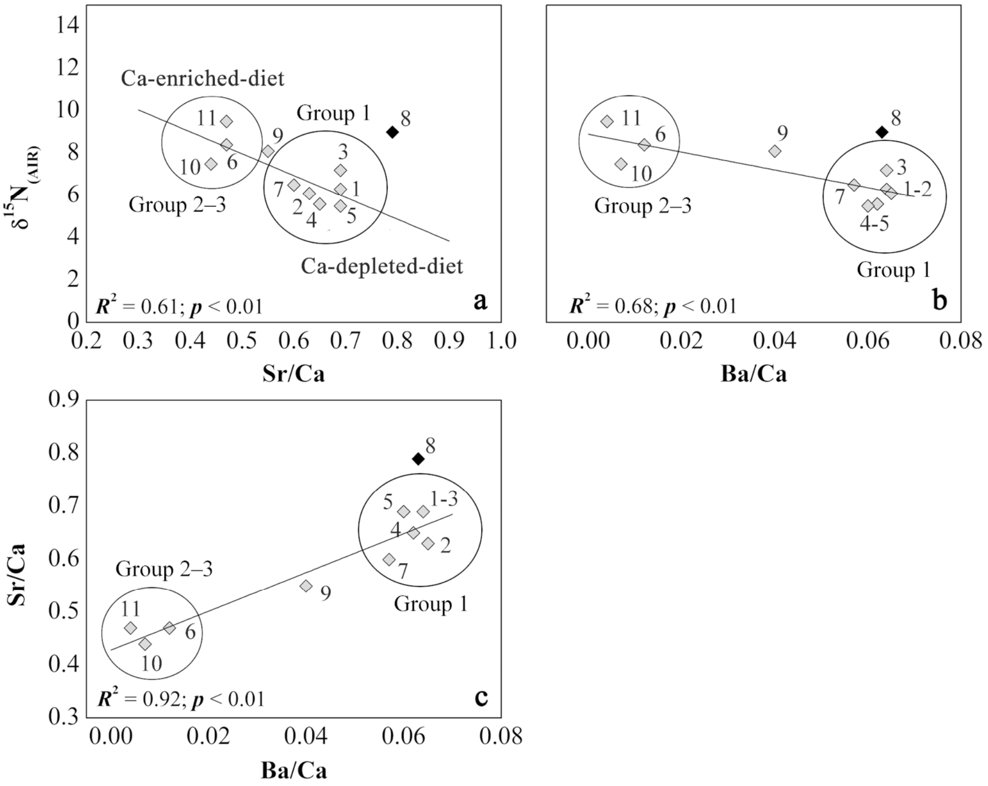
(a) Collagen stable isotopes of the Roccapelago individuals (black diamond) and fields of food end-members. C3 and C4 plant δ13C values are from Iacumin et al. (2014) where δ15N values are inferred from human data taking into account the trophic position. Plausible mixed C3-C4 fed livestock (bovines or caprovines) position is deduced from human and plant data. α (± standard error) is the intercept and β (± standard error) is the slope of the regression line. Circles outline clusters of individuals as explained in the text. (b) In the inset, the δ13C value of a mammal with a pure C3-diet, a pure C4-diet and the modelled Roccapelago livestock mixed diet. Based on our data we extrapolate the δ13C value of the Roccapelago livestock to be composed of a 45% C4 diet and 55% C3 diet.

Fig. 2

135x116mm (300 x 300 DPI)



δ13C and δ15N comparison between Roccapelago, Medici and Aragonese (Fornaciari 2008) and Mainizza (Iacumin et al. 2014) diet. In dark gray the field of Roccapelago individuals. In light gray the field of the Mainizza settlement. Dashed field represents Medici and Aragonese families. In bold, two of the possible diet end-members for each site. Medici (16th–17th AD) and Aragonese (15th–17th AD) were italian noble families, Mainizza (10th–11th AD) is a rural settlement in Friuli V.G., NE Italy.
Fig.3
154x116mm (300 x 300 DPI)



Correlations of δ15N with Sr/Ca and Ba/Ca ratios and of Sr/Ca with Ba/Ca ratios in bones of Roccapelago individuals. Black diamond is the outlier Roc8 not considered in the regressions (see text for details). Labels represent the number of individuals (IDs from Table 1), wording "Roc" is omitted to improve the readability of the graph. Circles outline clusters of individuals as explained in the text.

Fig. 4

153x121mm (300 x 300 DPI)

Table 1. Results of stable isotopes, Sr/Ca and Ba/Ca of human bone samples from Roccapelago.

ID	**Sex**	**Age**	**Body element**	**Bone sample data**					**Individual mean**			
				$\delta^{13}C_{(PDB-1)}$	**$\delta^{15}N_{(AIR)}$**	**C:N**	**Sr/Ca (µg/mg)**	**Ba/Ca (µg/mg)**	**$\delta^{13}C_{(PDB-1)}$**	**$\delta^{15}N_{(AIR)}$**	**Sr/Ca**	**Ba/Ca**
Roc1	M	Adult	Femur	–19.2	6.2	2.9	0.71	0.070	–19.5	6.3	0.69	0.064
			Humerus	–19.8	6.5	3.0	0.68	0.058				
Roc2	M	Adult	Femur	–20.1	6.0	3.1	0.65	0.065	–19.9	6.1	0.63	0.065
			Humerus	–19.7	6.2	3.0	0.62	0.066				
Roc3	F	Adult	Humerus	–18.8	7.2	3.2	0.69	0.064	–18.8	7.2	0.69	0.064
Roc4	I	Adult	Femur	–19.9	5.6	2.9	0.65	0.062	–19.9	5.6	0.65	0.062
Roc5	I	Adult	Humerus	–19.7	5.5	3.0	0.69	0.060	–19.7	5.5	0.69	0.060
Roc6	F	Adult	Tibia	–16.4	8.4	3.2	0.47	0.012	–16.4	8.4	0.47	0.012
Roc7	M	Adult	Tibia	–19.3	6.6	3.0	0.58	0.043	–19.3	6.5	0.60	0.057
			Humerus	–19.3	6.4	2.9	0.63	0.071				
Roc8	F	Adult	Humerus	–12.2	9.0	3.1	0.79	0.063	–12.2	9.0	0.79	0.063
Roc9	F	Adult	Humerus	–16.4	8.1	2.9	0.55	0.040	–16.4	8.1	0.55	0.040
Roc10	M	Adult	Femur	–17.1	7.2	2.9	0.56	0.009	–17.0	7.5	0.44	0.007
			Humerus	–16.9	7.7	3.0	0.33	0.006				
Roc11	M	Adult	Femur	–12.6	9.7	3.1	0.45	0.004	–12.5	9.5	0.47	0.004
			Humerus	–12.5	9.3	3.1	0.48	0.004				

Table 2. Stable isotopes of human tooth samples from Roccapelago.

ID	Tooth	Dentine $\delta^{13}C_{(PDB-1)}$	Dentine $\delta^{15}N_{(AIR)}$	C:N	$\Delta C_{dentine-bone}$	$\Delta N_{dentine-bone}$
Roc1	Canine	–19.2	6.8	3.1	0.3	0.5
Roc2	Canine	–19.5	7.2	3.0	0.5	1.1
Roc4	Premolar	–19.3	5.5	3.4	0.6	–0.1
Roc5	Premolar	–19.1	6.5	3.0	0.6	0.9
Roc7	Premolar	–19.6	6.8	3.0	–0.3	0.3
Roc10	Molar (1st)	–16.8	6.6	3.1	0.1	–0.9
Roc11	Premolar	–13.7	8.9	2.9	–1.2	–0.6