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Isotopic paleodiet studies of skeletons from the Imperial Roman-age cemetery of Isola Sacra, Rome, Italy

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Abstract

This is an isotopic study of collagen and bone apatite samples from individuals buried in the 1st–3rd centuries AD cemetery of Isola Sacra on the Mediterranean coast near Rome, Italy. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios of collagen and $^{13}C/^{12}C$ in carbonate of apatite are used to evaluate the dietary history of people ranging in age from 5 to 45+ years. The collagen data are also compared to a smaller skeletal sample from a nearby inland site (ANAS). Sources in Roman literature describe a typical diet of that period characterized by plant-derived foods; typically cereals, legumes, fruits, and vegetables. While individuals from the ANAS site display isotopic compositions consistent with a terrestrial-based diet, many of the skeletons from Isola Sacra are more enriched in ^{15}N and, to a lesser extent, in ^{13}C. We infer that their diet included a significant component of marine foods. Apatite $\delta^{13}C$ values show that total dietary carbon intake was dominated by terrestrial foods. The distribution pattern of the $\delta^{13}C$ and $\delta^{15}N$ data suggest that, while the Isola Sacra people obtained their nitrogen from a mixture of marine and terrestrial proteins, the carbon atoms used to construct non-essential amino acids were derived from the total diet (i.e., proteins, carbohydrates, and lipids).

Keywords: Carbon; Nitrogen; Palaeodiet; Collagen; Apatite; Roman; Archaeology; Italy; Fish

1. Introduction

Stable isotopes have been widely used to investigate dietary patterns in prehistoric human populations since the 1980s (see reviews in Refs [29,30,53]), but there are few stable isotope studies on samples from historic populations such as those of the Roman period in the Mediterranean region. Textual and archaeological evidence has traditionally been used to reconstruct the diet of the ancient Romans, and while these sources tell us a great deal about the range of food choices available, the isotopic data are an invaluable addition to the existing information as they provide an indication of what was actually consumed.

Historical accounts of Roman diet repeatedly refer to grain as the base of the Roman diet. Some scholars, while not denying the essential role played by cereals, have argued for a significant role in the diet played by dry legumes and "wild" foods [19,21,25]. In addition, maritime resources must have figured in the diet of inhabitants of the Graeco-Roman world, particularly those with access to the sea. The people buried in the necropolis of Isola Sacra (1st–3rd centuries AD) were inhabitants of *Portus Romae*. The location of *Portus Romae* on the western coast of Italy and its role as a key maritime trading center for the capital city makes it likely that marine foods were an important component of this population's diet, but how important? This study uses isotopic data to shed some light on the relative importance of maritime and terrestrial resources in this particular population. The major goal of this study is to

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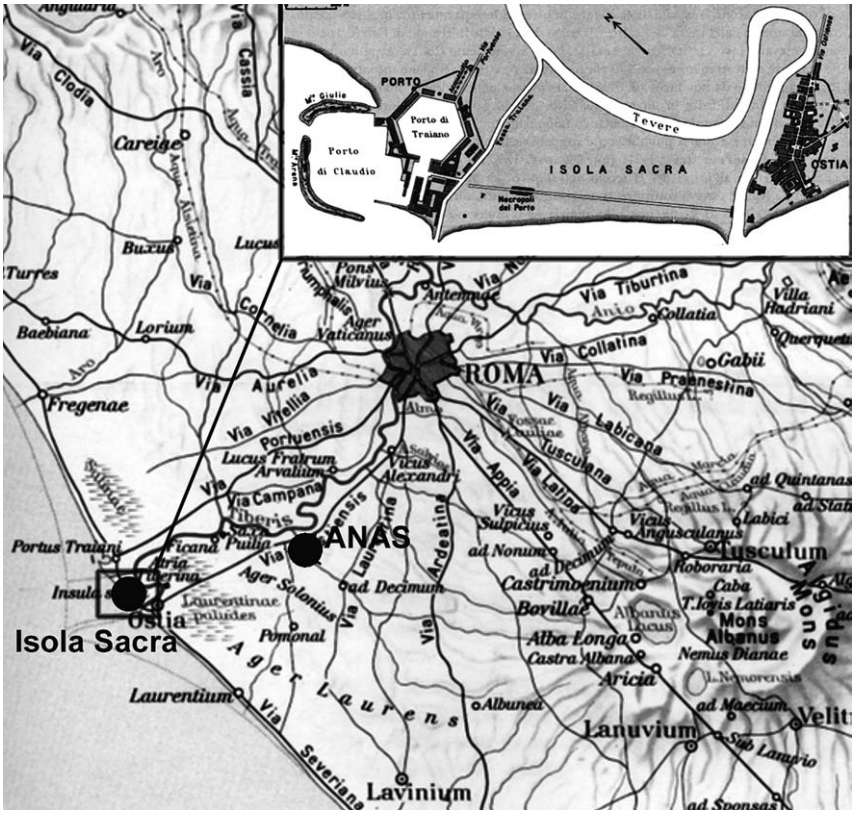

Fig. 1. Map showing locations of the Isola Sacra and ANAS cemetery sites. Inset shows the necropolis of Isola Sacra in relation to the site of *Portus Romae* on the left.

investigate the general diet of the Isola Sacra sample, and a secondary aim is to explore the social and historical context within which people gained access to food.

2. Portus Romae

The site of *Portus Romae* is located approximately 23 km southwest of Rome (Fig. 1). Claudius ordered the construction of this new port, built between AD 42 and 64, and Trajan ultimately completed an additional inner harbor in AD 112.

Trajan's port (*Portus*) was a key trading center for the Roman Empire and was directly linked to Rome through a series of docks and quays along the Tiber River. The political and economic stability of the Mediterranean region at the beginning of the Roman Imperial period contributed to the wealth of the city of Rome and, as the main port and warehouse to Rome, the lives of the inhabitants at *Portus* were intimately linked with the prosperity and political domination of the Roman Empire. Middle-class administrators, traders, and merchants were well represented among the inhabitants of *Portus* [37], but there is no reference to a local aristocracy in the inscriptional evidence unlike other Roman towns from the Imperial period [24].

The necropolis of Isola Sacra is situated on an artificial island that was created during the dredging of a canal, the *Fossa Traiana*, in AD 103 that connected the Tiber River with the coast (Fig. 1) [7]. The necropolis extends approximately 1.5 km along the road between the port city of Ostia and *Portus Romae* and was used by the inhabitants of *Portus* from the 1st to 3rd centuries AD. The cemetery eventually fell into disuse and was gradually covered over by encroaching sand [37].

3. Roman diet

Literary texts on agriculture by Roman writers such as Cato (2nd century BC), Varro (1st century BC), and Columella (1st century AD), provide evidence on the diet and subsistence practices of the Romans, in addition to many other texts that provide additional literary evidence of varying reliability and extent. Greek and Roman medical writers are also sources of dietary information, as are descriptions of food and dining practices in works of fiction; however, much of the literary evidence is biased towards descriptions of the Roman elite and their food preferences [27,60]. Depictions in art have sometimes been used to infer diet, although they are not considered as reliable as the ancient historians and agronomists [4].

The traditional diet of the Mediterranean region is the triad of cereals, wine, and olives, to which Garnsey [25] also added dry legumes. Most grain used in Rome was supplied from North Africa and Egypt [47]. It has been estimated that cereals made up approximately 70–75% of the caloric intake of the Roman diet [8,20,23,61]. Meat and other animal byproducts were not major components of the diet, but it is also likely that there was a great deal of variety in the typical diet [25]. Both literary and archaeological evidence indicate that pork was the most popular meat consumed by the ancient Romans; it was a standard component of the Mediterranean diet from the Neolithic period onwards [8]. Excavations at Ostia recovered a wide variety of animal remains from successive phases of occupation at this site, the overwhelming majority of which were pigs, corroborating much of the literary evidence on the importance of pork in the Roman economy and diet [11].

Archaeological evidence also suggests that sheep and goats made up $\frac{1}{4}$ to $\frac{1}{3}$ of the meat component of the Roman diet [8]. Goats and sheep were raised primarily for their wool and for milk, much of which was used for the production of cheese [25]. There are varied opinions about the popularity of milk in the Roman diet, although it is generally agreed that butter was not widely consumed probably because of problems with preservation [4,18]. Beef was probably a relatively minor component of the Roman diet because cattle were used primarily as draft animals, although it was reportedly a major component of the Roman military diet [61].

If Romans, or at least the very poor, ate an almost exclusively cereal-based diet, then this could have resulted in chronic protein–calorie malnutrition [52,60]. Evans [19] and Frayn [21] have therefore questioned the assumption that the diet of the Romans was mainly cereals, and suggest that there were a variety of wild and cultivated plants available, particularly for rural populations.

Consumption of fish and marine resources in the Roman world is not widely discussed by modern scholars. During the Roman period, fish were considered expensive food items, suggesting that the regular consumption of fish may have been restricted to elite members of society [22]. Two main sources for the types of fish and seafood that were available for consumption in the Graeco-Roman world are Athenaeus' *Deipnosophistae* and the cookbook of Apicius.[1] Fish were also consumed salted (*salsamenta*) and in the form of fish sauces (e.g., *liquamen, garum*), which were not only used for cooking, but also figured extensively in medicinal recipes [14].[2] *Garum* was considered to be the best quality fish sauce and was the one most widely mentioned in ancient recipes [14]. It is not clear how widely *garum* was consumed in the Mediterranean region, and how much was used by those who did consume it regularly. Fish consumption may have been higher along the coastal regions, so that the proportion of fish and seafood in the diets of the people of *Portus Romae* was likely higher than the typical consumption levels for rural farming populations.

Fish and fishing were important economic resources at the ports of Rome, indicated by the existence of corporations of fishermen and fishmongers. It was not only the fish, but also their byproducts, like *garum*, that were the focus of major economic activity along the coastal regions of Italy and the Mediterranean (see Ref. [14]). The dietary regime of the middle-class population from *Portus Romae* would be expected to fall somewhere between that of the urban elite of Imperial Rome and of the rural peasantry in the surrounding countryside.

We know very little about the daily lives (including diet) of the people from *Portus Romae*. Archaeological investigation of the site in the 19th and 20th centuries focused on the harbor and its associated structures, but the site is currently privately owned and excavation of the habitation structures is not possible. Relevant archaeological evidence is available from the nearby city of Ostia.

Table 1
Age and sex distribution of the Isola Sacra skeletal series

Age (yrs)	Sex			
	Unknown	Male	Female	Total
5–15	14	–	–	14
15–30	–	18	11	29
30–45	–	13	8	21
>45	–	17	13	30
Adult	11	–	–	11
Total	25	48	32	105

4. Materials and methods

A sample of 105 femora, consisting of both sexes and all ages (>5 years) was selected for analysis of bone collagen (Table 1).

The age of each subadult skeleton was estimated using the following criteria; development and eruption

[1] Some of the species listed include; squid, octopus, lobster, prawns, sea urchin, scallops, mussels, crayfish, cuttlefish, sturgeon, mackerel, tuna, perch, sardine, tunny, and eel.

[2] *Liquamen* is the general term used for any kind of fish sauce, whereas *garum* is the filtered liquid derived from the original mixture [14]. *Garum* was used to flavor recipes in the place of salt, although it was believed that it also helped the appetite and digestion (ibid.). Mackerel was reportedly the most popular fish used in the preparation of *garum*, but tunny (a type of tuna), sprats, smelts, oysters, sea urchins, sea anemones, and shellfish were also used (ibid.).

of the deciduous and permanent dentition, development of the temporal and occipital bones, development and fusion of the epiphyses, and maximum long bone diaphyseal length ([55], cited in [48]). The adults were aged using the following methods; cranial suture closure, degree and pattern of tooth wear, morphological changes at the sternal rib ends, morphological changes of the pubic symphysis, changes on the auricular surface of the ilium, and trabecular structure of the proximal femur. Long bone measurements and morphological characteristics of the cranium and innominates were used to evaluate the sex of adult skeletons [55]. A detailed analysis of sex and age-related variability in diet of the Isola Sacra sample is forthcoming [42]. Isotopic analysis of weaning in this skeletal sample revealed that infants are fully weaned by 2.5–3 years of age, and by 4–5 years of age $\delta^{13}C$ and $\delta^{15}N$ values are similar to adult values, thus any isotopic traces of breastfeeding in the bone are no longer present in individuals over the age of 5 years [41].

In order to test for dietary differences between coastal and inland populations, we studied a population of 14 adults from a cemetery uncovered during road building by the Azienda Nazionale Autonoma delle Strada (ANAS; Fig. 1), associated with a small rural center of farmers; the age and sex distribution of this sample is not known, but all are adults. We shall refer to this site as the ANAS cemetery, since its exact location is unknown.

In order to estimate the isotopic composition of animal based foods available to residents of *Portus*, we analyzed bone samples from the Isola Sacra necropolis, representing seven different animal species (N=14). Four herbivore species (cow, horse, donkey, and goat/sheep) and three omnivorous species (dog, fox, and pig) are represented. The same procedures for extraction of bone collagen and apatite were used on these samples as for the human samples.

B. Wilkens (University of Sassari, Sardinia) kindly provided ten garum samples for isotopic analysis; 1: from an African amphora (2nd century AD) from the site of Olbia, Sardinia; 2 & 3: also from Olbia, from locally produced amphorae (end of 4th/beginning of 3rd century BC); 4–10: from various amphorae recovered from a 2nd century AD shipwreck near Grado, Italy (N. Adriatic Sea). The samples consisted of small fish bones, brown flakes (probably dried fish), and shells. Some samples included sandy detritus.

5. Extraction procedures

5.1. Collagen

Collagen was extracted from bone following the modified Longin [35] procedure of Chisholm et al. [12]. Cleaned bone samples were treated with repeated washes of 0.25 M HCl until the bone mineral was dissolved. The samples were then treated with 0.1 M NaOH to remove any remaining humic and fulvic acids, gelatinized in warm dilute HCl, filtered, and dried. Approximately 2–3 mg (for $\delta^{13}C$) and 9–13 mg (for $\delta^{15}N$) of the dried collagen was reacted with CuO at 550 °C for 2.5 h, oxidizing the collagen to CO_2, N_2, and H_2O. The samples were then analyzed using a VG SIRA 10 Series II mass spectrometer.

The data are presented in the δ-notation:

$$\delta^{15}N = \{(R_x/R_s) - 1\} \times 1000 \text{ in ‰ (per mille)}$$

where $R = {}^{15}N/{}^{14}N$; x=sample; s=standard (and similarly for $\delta^{13}C$). The standards are PDB (Peedee Belemnite) for $\delta^{13}C$ and atmospheric N_2 (AIR) for $\delta^{15}N$. The precision of analysis is ± 0.1‰ for $\delta^{13}C$ and ± 0.2‰ for $\delta^{15}N$.

5.2. Apatite

The preparation of bone apatite for isotopic analysis followed procedures developed by Sullivan and Krueger [57] and Lee Thorp and van der Merwe [34]. Approximately 0.1 g of bone powder from each sample was treated with 1 M acetic acid, followed by a wash of NaOCl to remove organic material. The treated bone powder (~20–40 mg) was reacted in a sealed tube with 100% H_3PO_4 to produce CO_2. The CO_2 gas was then analyzed on an Optima mass spectrometer. Data are presented as $\delta^{13}C_{ap}$ values with respect to PDB and a precision of ± 0.1‰. Oxygen isotopic data for these samples will be discussed in a later paper.

5.3. Garum

Approximately 0.5–1 g of each garum sample was treated with chloroform+methanol to dissolve any lipids present. The liquid was subsequently poured off and the samples were left to dry for 48 h. Next, they were soaked in 0.25 M HCl to remove surface carbonates, rinsed twice with distilled water, soaked in 0.1 M NaOH, and rinsed another 4 times with distilled water. The samples were dried overnight (at 60 °C), loaded into 9 mm quartz tubes, and placed on a vacuum line for 12 h. After the tubes were sealed, they were placed in an oven at 450 °C for 4 h, cooled, shaken to redistribute the CuO, and then heated for an additional 4 h. Cormie [13] showed that this procedure yields accurate data for $\delta^{13}C$ and $\delta^{15}N$ of collagen but does not liberate CO_2 from bone apatite. The CO_2 and N_2 gases were transferred into separate 6 mm pyrex tubes for analysis on the mass spectrometer. The C:N ratios for the garum samples ranged from 5.01 to 5.97, which is higher than the normal bone ratios, but low enough to indicate that there was still sufficient nitrogen in the samples for analysis.

6. Assessment of bone preservation

The preservation of the isotopic signal in the bone samples was evaluated through a comparison of collagen yield, bone histology, C:N ratios, and crystallinity determined by Fourier transform infra-red (FTIR) analysis. Collagen yields greater than 5% of the original bone weights are expected to provide reliable carbon and nitrogen values [2,53]. Four categories of bone preservation were identified in histological thin sections of the Isola Sacra femora that are used here [49]:

'*Normal*': no evidence of diagenetic alteration of the bone structure, and osteons are clearly visible;
'*Focalized*': foci of structural degradation occur;
'*Amorphous*': more widespread evidence of degradation, lack of visible osteons (much of the destruction observed in both categories is attributed to microbial activity);
'*Mineralized*': evidence of recrystallization of bone tissue.

Bone samples identified as histologically 'normal' tend to have higher collagen yields (average 9.1%) than those categorized as 'focalized' (5.2%), 'amorphous' (3.9%), or 'mineralized' (2.3%). The C:N ratio measures the ratio of carbon to nitrogen in the extracted collagen sample. Well-preserved collagen should yield ratios between 2.9 and 3.6 [16]. We determined C:N ratios for all the samples studied. There are two samples with C:N ratios slightly higher (SCR73, SCR200) than the accepted normal range (2.9–3.6), but their isotopic values are within the normal range of variation for the entire sample. We tested for correlations between C:N ratios, collagen yield, and burial type. There is no significant (P=0.05) relationship between type of burial structure and C:N ratio (Table 2).

A significant positive correlation exists between yield (wt.%) and δ^{13}C for yields lower than 5% (r=0.347, P=0.009), but there is no correlation with yields above 5%, as found by Ambrose [2] and White and Schwarcz [59]. There is no significant correlation found between δ^{15}N values and yield. The correlation between δ^{13}C values and collagen yield were controlled for in subsequent statistical analyses using an analysis of covariance (ANCOVA).

A subset (n=27) of the Isola Sacra femur series was analyzed for diagenetic alteration of bone apatite using Fourier Transform Infrared Spectrometry (FTIR). This technique measures the absorbance spectra of powdered bone samples and is used to evaluate alterations in the crystalline structure of bone. The crystallinity index (CI) of each sample was calculated, using the following formula:

$$CI=(A_{[565]}+A_{[605]})/A_{[595]}$$

where A[x] represents the absorbance at frequency x (in cm^{-1}). This is a measure of the absorbance at two phosphate (PO_4) peaks with vibrational frequencies of 565 and 605 cm^{-1}, divided by the absorbance of the valley between them (595 cm^{-1}). High crystallinity indices in archaeological bone are considered to be indicative of post-mortem crystal growth or dissolution of more soluble crystals [62]. Modern human bone has a CI of 3.1 (ibid.). CI values of the samples range from 2.85 to 4.12, with an average of $3.31 \pm .28$. When these data were compared to Savorè's [49] bone histology evaluations, mineralized samples had the highest CI (4.12), focalized and amorphous samples had intermediate CI (3.57–3.96), and normal samples had the lowest CI values (<3.28).

7. Results

7.1. Collagen

The δ^{13}C data range from −17.8‰ to −19.7‰, ($\bar{x}=-18.8 \pm 0.3$‰). Nitrogen values range from 7.5‰ to 14.4‰ ($\bar{x}=10.8 \pm 1.2$‰). Fig. 2 shows the distribution of data for humans as well as for animal bones recovered at the site. The overall pattern of the Isola Sacra isotopic data indicates the consumption of a terrestrial C_3 diet, combined with a significant contribution of one or more foods with high values of δ^{15}N. This can be seen if we transform the isotopic data for collagen to the presumed average isotopic composition of the diet of each individual, assuming that collagen is enriched with respect to diet by 5‰ in δ^{13}C and by 3‰ in δ^{15}N (trophic level shift) [53].

Fig. 3 shows the resulting diets inferred from the data for Isola Sacra and ANAS. The figure also shows possible marine foods which might have contributed to the diet, as given in Table 3. Some are from the Mediterranean Sea, including species that may have been consumed by the people at *Portus Romae*. There are a wide variety of fish species known to be popular in the ancient Mediterranean diet that are medium to high trophic level feeders, including (among others); turbot, bass, cod/hake, moray eel, sole, torpedo fish, grouper, and dolphin fish [17,43]. Also shown is the estimated composition of the flesh of herbivores as inferred from the δ^{13}C and δ^{15}N analyses of collagen from faunal bones (dashed line); these data were calculated assuming that the δ^{13}C value of flesh is 4‰ lower than that of collagen, whereas the δ^{15}N values are identical.

The inferred diets from the Isola Sacra data form an elongate cluster on Fig. 3. The δ^{13}C values are on average greater than values inferred for the flesh of terrestrial herbivores. Likewise, their δ^{15}N values extend to larger values than would be expected from any terrestrial protein source, but match the range of the expected marine carnivore diet.

Table 2
Isola Sacra and ANAS cemetery data (collagen, carbonate, C:N ratio and yield)

	Sample ID	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C_{ap}$ (‰)	C:N ratio	% Yield
1	SCR 002	−19.2	11.0	−10.1	3.4	3.10
2	SCR 005	–	9.2	−11.1	3.3	3.10
3	SCR 015	−18.6	13.1	−13.9	3.4	8.50
4	SCR 018	−18.9	10.9	−11.3	3.6	4.26
5	SCR 020	−18.9	9.8	–	3.3	11.41
6	SCR 028	−18.6	11.5	–	3.5	2.96
7	SCR 029	−18.8	–	−15.6	3.4	3.83
8	SCR 034	−18.7	10.4	–	3.5	8.54
9	SCR 035	−18.8	8.6	−10.8	3.6	4.11
10	SCR 038	−18.6	12.8	−14.1	3.4	18.08
11	SCR 057	−18.7	11.0	−11.2	3.4	1.68
12	SCR 073	−18.9	11.4	−12.8	3.7	7.39
13	SCR 079	−18.5	11.4	–	3.5	4.17
14	SCR 089	−18.5	11.7	−12.3	3.5	3.34
15	SCR 090	−18.8	11.1	–	3.5	2.45
16	SCR 097	−18.7	11.1	−10.3	3.4	5.28
17	SCR 098	−18.9	11.5	–	3.5	3.35
18	SCR 099	−18.5	9.2	−10.6	3.5	2.75
19	SCR 124	−18.9	9.7	–	3.4	1.24
20	SCR 127	−18.0	11.5	−12.0	3.4	3.03
21	SCR 142	−18.7	11.1	−10.1	3.1	2.79
22	SCR 145	−18.5	8.9	−13.4	3.2	12.23
23	SCR 149	−19.0	10.5	−10.2	3.6	5.33
24	SCR 155	−18.9	11.0	−11.6	3.3	5.16
25	SCR 158	−19.0	10.5	−10.7	3.1	3.72
26	SCR 160	−18.9	11.1	–	3.4	5.93
27	SCR 172	−18.7	11.1	−13.6	3.4	9.66
28	SCR 174	−19.2	9.1	−11.1	3.3	10.16
29	SCR 177	−18.4	12.3		3.2	9.04
30	SCR 178	−18.7	11.1	−10.5	3.4	6.34
31	SCR 179	−18.2	11.2	–	3.3	4.99
32	SCR 180	−18.5	9.8	−11.3	3.4	6.12
33	SCR 188	−18.6	11.1	−9.9	3.3	5.31
34	SCR 193	−19.1	9.8	−11.6	3.3	2.90
35	SCR 200	−19.2	10.7	−10.5	3.7	2.13
36	SCR 208	−19.0	10.9	–	3.4	6.25
37	SCR 211	−18.6	10.5	−11.5	3.3	9.35
38	SCR 213	−18.5	10.8	−10.8	3.4	4.83
39	SCR 220	−18.6	12.0	−11.5	3.4	2.67
40	SCR 237	−19.1	9.6	–	3.3	2.95
41	SCR 239	–	11.9	−10.3	3.3	4.09
42	SCR 258	−18.8	10.4	−11.3	3.4	3.28
43	SCR 265	−18.8	13.0	–	3.3	12.26
44	SCR 280	−18.6	14.4	−12.3	3.2	5.86
45	SCR 295	−18.6	–	−10.7	3.2	2.70
46	SCR 299	−18.6	11.5	–	3.4	3.88
47	SCR 309	−19.2	9.2	−10.4	3.6	2.97
48	SCR 310	−18.6	9.9	−10.1	3.4	1.17
49	SCR 315	−19.3	10.9	−10.8	3.3	2.09
50	SCR 321	−19.5	10.1	−10.6	3.4	3.37
51	SCR 322	−18.7	11.7	−13.6	3.4	9.53
52	SCR 324	−18.7	10.9	–	3.4	2.57
53	SCR 325	−19.0	9.5	−9.8	3.4	4.48
54	SCR 326	−19.0	10.5	–	3.4	4.46
55	SCR 329	−19.1	10.9	–	3.4	2.10
56	SCR 330	−18.8	8.7	–	3.4	5.11
57	SCR 331	−18.6	9.4	−10.9	3.3	3.80
58	SCR 334	−18.9	–	–	3.4	3.27
59	SCR 338	−19.3	8.8	−11.2	3.3	6.81
60	SCR 341	−18.6	10.7	−11.0	3.4	5.51
61	SCR 344	−18.7	11.5	–	3.4	1.99
62	SCR 402	−18.8	11.3	–	3.2	2.42

Table 2 (*continued*)

	Sample ID	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C_{ap}$ (‰)	C:N ratio	% Yield
63	SCR 502	−18.0	12.1	−12.3	3.2	4.21
64	SCR 519	−19.7	10.0	–	3.4	1.64
65	SCR 524	–	11.3	−12.6	3.4	1.46
66	SCR 530	−19.1	10.7	−10.4	3.3	5.86
67	SCR 538	−19.2	10.9	−10.3	3.2	2.52
68	SCR 557	−19.2	10.4	−10.6	3.6	5.16
69	SCR A139	−19.0	10.9	–	3.3	13.19
70	SCR A144	−18.9	10.5	–	3.2	7.62
71	SCR A166	−19.0	11.2	−12.1	3.4	3.05
72	SCR A190	−18.8	9.9	–	3.4	3.87
73	SCR A202	−18.6	11.5	−10.6	3.2	10.79
74	SCR A211	−18.6	9.5	–	3.5	5.73
75	SCR A222	−19.1	10.1	–	3.3	0.86
76	SCR A236	−19.2	13.4	–	3.2	19.22
77	SCR A239	−18.8	11.1	–	3.6	10.22
78	SCR A247	−18.8	10.6	–	3.4	7.23
79	SCR A306	−18.5	10.0	–	3.2	14.11
80	SCR A326	−18.4	12.4	−12.7	3.2	3.62
81	SCR A329a	−19.3	9.9	–	3.4	7.06
82	SCR A357	−19.2	10.8	–	3.2	2.90
83	SCR A360	−18.7	10.9	–	3.4	14.22
84	SCR A362	−19.1	7.5	–	3.5	12.24
85	SCR A364/1	−18.6	11.8	–	3.4	9.51
86	SCR A364/2	−18.5	12.1	–	3.4	12.01
87	SCR A380	−18.8	11.0	–	3.3	1.63
88	SCR A412	−19.0	11.6	–	3.1	3.56
89	SCR A418	−19.0	11.3	−12.5	3.2	5.09
90	SCR A422	−18.5	9.6	–	3.2	4.22
91	SCR A463	−18.6	11.2	–	3.4	5.02
92	SCR 1005/3	−18.7	9.4	−11.9	3.3	4.20
93	SCR 1016/1	−18.6	10.9	–	3.2	2.64
94	SCR 1028/3	−18.5	13.1	–	3.2	12.85
95	SCR 1045	−18.9	–	−12.6	3.2	10.15
96	SCR 1091	−18.7	13.0	–	3.2	4.15
97	SCR 1112	−18.6	10.6	–	3.3	3.67
98	SCR 1133/3	−18.9	11.1	–	3.3	1.32
99	SCR 1139/1	−19.0	10.3	−12.6	3.3	15.74
100	SCR 1139/3	−18.4	11.4	–	3.2	17.14
101	SCR 1147/1	−18.7	10.1	−13.8	3.2	13.30
102	SCR 1175/4	−18.2	11.9	−10.4	3.4	5.33
103	SCR 1210	−17.8	7.8	−11.4	3.3	5.42
104	SCR 1214/2	−19.2	10.6	−11.7	3.2	6.46
105	SCR 1230	−18.8	10.7	–	3.2	9.27
1	ANAS 4	−19.9	6.9	–	3.2	10.18
2	ANAS 5	−19.9	7.9	–	3.2	5.24
3	ANAS 15	−19.5	8.6	–	3.2	3.79
4	ANAS 25	−19.3	10.8	–	3.2	3.62
5	ANAS 31	−19.0	11.0	–	3.2	7.39
6	ANAS 34	−19.6	6.9	–	3.2	11.21
7	ANAS 54	−19.0	11.3	–	3.2	4.70
8	ANAS 77	−19.2	11.1	–	3.2	6.15
9	ANAS 79	−19.2	11.2	–	3.2	5.05
10	ANAS 83	−19.0	11.1	–	3.2	5.31
11	ANAS 84	−19.6	9.0	–	3.2	5.03
12	ANAS 85	−19.6	8.7	–	3.2	7.41
13	ANAS 87	−20.0	7.2	–	3.2	7.12
14	ANAS 93	−18.9	11.3	–	3.2	7.39

The ANAS isotopic data appear to fall in two clusters. One lies in the midst of the data from Isola Sacra sample; the other has $\delta^{15}N$ values lower the mean Isola Sacra data by up to 3‰. This latter cluster falls within the range expected for a terrestrial diet, possibly including the flesh of some terrestrial herbivorous animals as

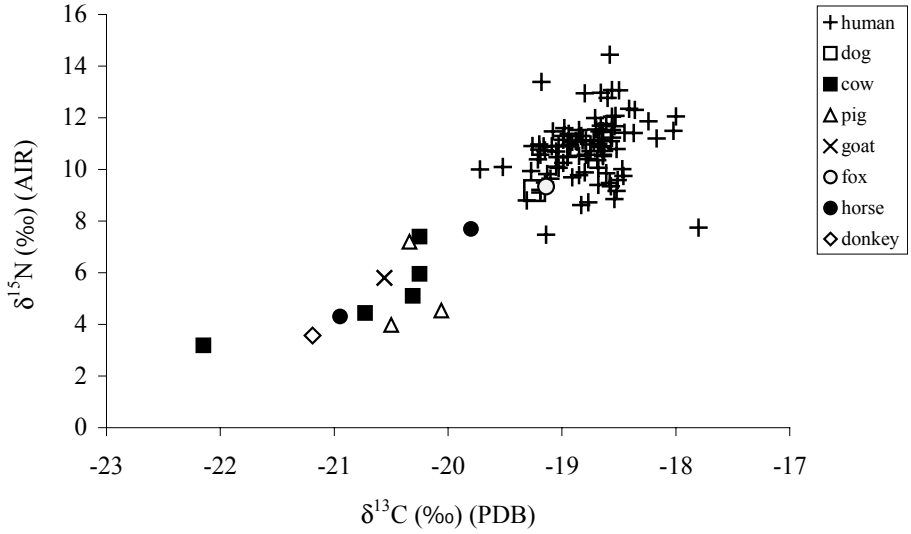

Fig. 2. Graph of $\delta^{13}C$ vs. $\delta^{15}N$ for collagen from humans buried at Isola Sacra, and faunal bones recovered in situ from stratified sites in the cemetery.

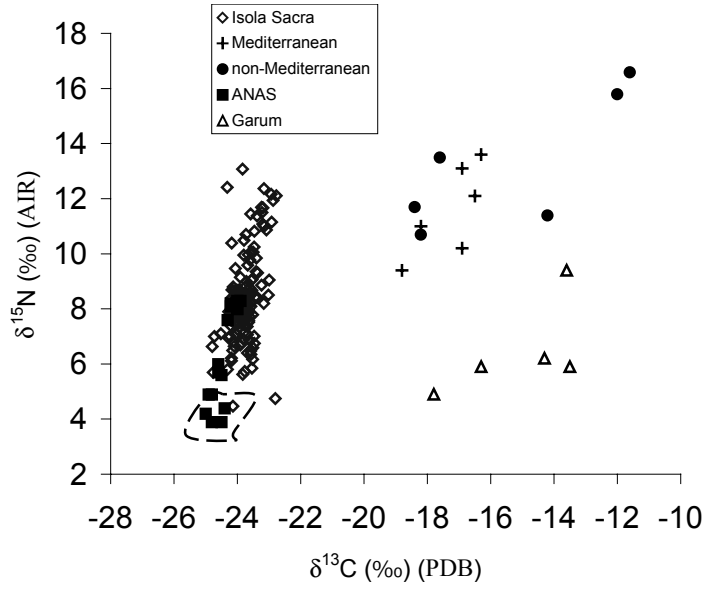

Fig. 3. Inferred isotopic compositions of diets of humans from Isola Sacra (♦) and possible protein sources: +=Mediterranean fish and seafood; ●=non-Mediterranean fish, seafood; Δ=garum; ovoid region=flesh of herbivores assuming $\Delta^{15}N_{flesh\text{-}collagen}$=0‰, $\Delta^{13}C_{flesh\text{-}collagen}$=−4‰.

shown on Fig. 3 (dashed line). The higher $\delta^{15}N$ of the people from the Isola Sacra sample indicates that they were consuming higher trophic foods than some of the people buried at the ANAS cemetery.

7.2. Bone apatite

A subset of the total skeletal population was also analyzed for $\delta^{13}C$ of bone apatite. The carbonate component of apatite may be subject to isotopic exchange with dissolved inorganic carbon of soil water during diagenesis of the bone. Our FTIR study of selected bone samples showed no correlation between $\delta^{13}C$ and crystallinity index. In spite of the apparent risks in using such data, they are valuable because $\delta^{13}C_{ap}$ is determined by the $\delta^{13}C$ of the total diet, whereas the $\delta^{13}C$ of bone collagen is biased towards the protein component of the diet. While tooth enamel is less likely to be diagenetically altered than bone, teeth do not allow us to track life-long changes in diet since they are completely mineralized before adulthood.

Table 3
$\delta^{15}N$ and $\delta^{13}C$ values for marine organisms from published reports (with standard deviations, if reported)

Name	Location*	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Shrimp[a]	J	7.7 ± 0.5	–
Mussel[a] (*Septifer virgatus*)	J	9.0 ± 0.6	–
Squid[b]	P	11.7 ± 0.4	−18.4 ± 0.2
King salmon[c]	AK	12.7	−19.3
Tuna[c]	SC	11.4	−14.2
Walleye surfperch[c]	SC	14.7	−12.4
Common dolphin[c]	SC	15.8	−12.0
Harbor porpoise[c]	SC	16.6	−11.6
Common seabream[d]	M	13.6 ± 0.8	−16.3 ± 0.4
Mediterranean wrasse[d]	M	12.1 ± 0.3	−16.5 ± 0.4
Sea bass (*Serranus scriba*)[d]	M	13.1 ± 0.2	−16.9 ± 0.1
Pacific herring (medium)[e]	BS	13.5 ± 0.1	−17.6 ± 0.1
Atka mackerel (large)[e]	BS	10.7 ± 0.3	−18.2 ± 0.4
Anglerfish[f]	M	11.0 ± 0.0	−18.2 ± 0.0
Mediterranean hake[f]	M	9.4 ± 0.1	−18.8 ± 0.0
Red mullet[f]	M	10.2 ± 0.0	−16.9 ± 0.1

*AK—Alaska; BS—Bering Sea; J—Japan; M—Mediterranean Sea; P—Pacific Ocean; SC—Southern California
[a]Minigawa and Wada [39]
[b]Gould et al. [26]
[c]Schoeninger and DeNiro [50]
[d]Jennings et al. [33]
[e]Kurle and Worthy [32]
[f]Badalamenti et al. [6]

The range of $\delta^{13}C_{ap}$ is −9.5‰ to −15.6‰ ($\bar{x}$ = −11.4 ± 1.2‰). The $\delta^{13}C$ of carbonate of bone apatite is believed to represent the isotopic composition of total diet, but apatite is enriched in ^{13}C by a fractionation Δ_{a-d}, the value of which is not precisely known. Ambrose and Norr [3] used data for laboratory rats fed on precisely known diets to infer that Δ_{a-d}=9.5‰. However, other researchers have proposed values ranging up to 14‰ [58], although higher values observed in ruminants have been attributed to enrichment of metabolic bicarbonate due to loss of ^{12}C-enriched methane from the mid-gut [28]. If we use a value of 13‰ for Δ_{a-d} then we see that the lowest $\delta^{13}C_{ap}$ values (−12‰) are comparable to values expected for a C_3-based diet ($\delta^{13}C$ = −25‰); the heaviest individuals have dietary $\delta^{13}C$ values of −23‰, which would represent a diet including a small amount (c. 10%) of marine foods. There is no correlation between $\delta^{13}C_{ap}$ and $\delta^{13}C_{col}$ (Fig. 4), but this is not surprising given the very small range of both variables.

7.3. Faunal remains

Fig. 2 shows a clear distinction in bone collagen values between the herbivorous animals and the Isola Sacra humans. In addition, the pig values are not in the range expected for omnivores (as they were originally classified), but cluster with other herbivore values, like cow and goat/sheep. This suggests that pigs were being fed, or were eating, a diet similar to other grazing animals. The dog and fox cluster at the lower range of the human data, although the small sample size makes any generalization difficult. While dogs typically display $\delta^{13}C$ and $\delta^{15}N$ values similar to those of humans with whom they are associated (e.g. Refs [9,10]), foxes would not be expected to show such high isotopic values unless they were regularly scavenging on human refuse, although some modern species are opportunistic omnivores.

The $\delta^{13}C$ values of the herbivores are comparable to values obtained from other organisms consuming diets consisting primarily of terrestrial C_3 plants [51]. The average $\delta^{15}N$ value of the humans is ~5.5‰ higher than that of the herbivores, corresponding to a difference of almost two trophic levels [53] (see Table 4). Fig. 2 shows a positive correlation between $\delta^{13}C$ and $\delta^{15}N$ for the faunal collagen samples, even excluding the dog and fox (for which higher values of both parameters would be expected). We do not know the reason for this trend, which is unexpected in herbivores. Only pigs would have been likely to be consuming any of the same higher trophic-level foods as humans.

The $\delta^{13}C_{ap}$ values for the some of the herbivorous fauna (e.g., sheep/goat, horse) are surprisingly high (Table 4); this is unlikely to be due to diagenesis, since this tends to produce lower $\delta^{13}C_{ap}$ values. This corresponds to a diet with a $\delta^{13}C$ of −24 ± 0.8‰, enriched in ^{13}C with respect to C_3 plants. The $\delta^{13}C_{col}$ data for these animals did not show such enrichment. There was no significant difference in $\delta^{13}C_{ap}$ between cow and goat (ruminants with possible methanogenic enrichment in $\delta^{13}C$) as compared to pig and horse, although the cow value is slightly lower.

7.4. Garum

The mean isotopic value for the *garum* samples is also shown on Fig. 3 (mean values are presented in Table 4). The *garum* samples have lower $\delta^{15}N$ values than expected for marine herbivores, which suggests that these samples represent *garum* prepared from low trophic level organisms, presumably extremely small fish like sprat (small herring) or smelt and perhaps some shellfish. This is consistent with the appearance of the samples as well as the reported composition of some *garum* [14]. These data indicate that, despite the reportedly widespread use of *garum* by the ancient Romans, material of this composition could not have contributed significantly to the higher $\delta^{15}N$ values observed in the Isola Sacra samples. The people from Isola Sacra were rather consuming marine organisms at a much higher trophic level, with isotopic values similar to those observed for tuna and salmon. We suspect, however, that these samples of *garum* may not be wholly

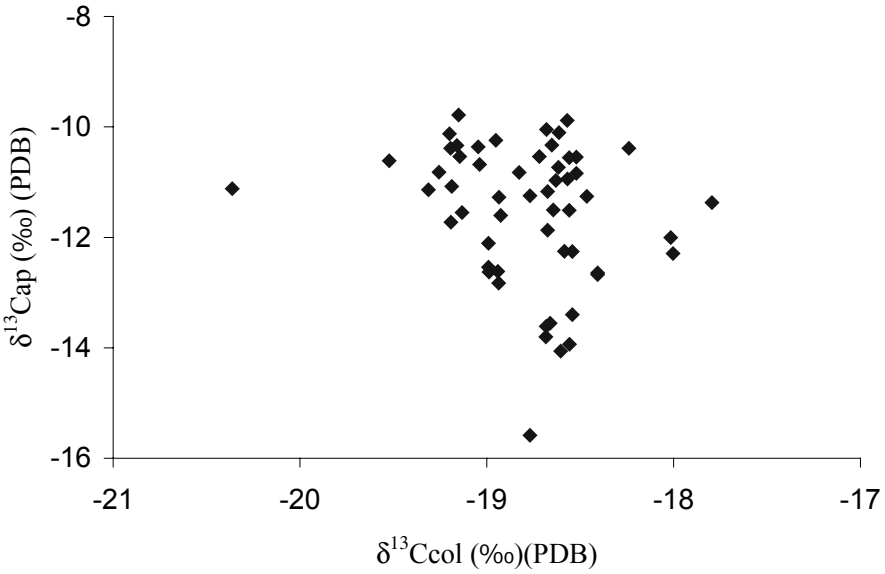

Fig. 4. Plot of $\delta^{13}C_{apatite}$ vs $\delta^{13}C_{collagen}$

Table 4
Comparison of mean isotopic values for human, faunal, and garum samples from Isola Sacra

Sample	N	$\delta^{13}C_{col}$ (‰) PDB	S.D.	N	$\delta^{13}C_{ap}$ (‰) PDB	S.D.	N	$\delta^{15}N$ (‰) AIR	S.D.
Isola Sacra	105	−18.8	0.3	65	−11.4	1.2	103	10.8	1.2
Garum	5	−14.7	0.6	5	–	–	5	6.5	1.7
Cow	4	−20.7	0.8	1	−9.8	–	4	5.2	1.6
Horse	2	−20.4	0.8	1	−10.3	–	2	6.0	2.4
Pig	4	−21.0	1.4	1	−11.4	–	3	5.2	1.7
Sheep/Goat	2	−20.9	0.4	1	−11.4	–	1	5.8	–
Donkey	1	−21.2	–	0	–	–	1	3.6	–
Fox	1	−19.1	–	0	–	–	1	9.3	–
Dog	2	−19.3	0.1	1	−10.4	–	1	9.2	–

representative of the range of isotopic composition of this commodity.

8. Discussion

8.1. Collagen

If a significant proportion of the daily protein intake of the Isola Sacra population had been obtained from the consumption of, for example, flesh of cows and goats (or dairy products), one would expect to see a trophic level enrichment of 3‰ in $\delta^{15}N$ and about 1‰ in $\delta^{13}C$ when comparing collagen of humans and consumed fauna [15]. The mean $\delta^{15}N$ value for the human data is enriched by about 6‰ over the herbivores and approximately 1.5‰ enriched over the dog and fox. The human $\delta^{13}C_{col}$ values are also about 2‰ higher than values for herbivores, showing that consumption of the flesh of terrestrial animals and dairy products alone cannot account for the isotopic enrichment of human collagen at Isola Sacra. Further, if bread or grain products were dominant components of the diet, we would expect to see slightly lower $\delta^{15}N$ values, since the protein content of bread is around 15–18% by weight [1]. We propose that the observed enrichment in $\delta^{15}N$ values was due to the marine component of the human diet.

Marine foods are dominantly protein-rich; it is widely believed that carbon atoms of collagen and other proteins in the human body are preferentially derived from carbon atoms in dietary protein (the so-called "routing" hypothesis, Refs [3,31,54]. On the other hand, terrestrial plant-derived foods would be predominantly rich in carbohydrate and lipid (bread, wine, oil), which should contribute little carbon to the collagen molecules when adequate protein is present in the diet. Based on the routing model, we would expect that a plot of $\delta^{13}C$ vs. $\delta^{15}N$ for human collagen from such a population would show a linear trend between the compositions of terrestrial and marine protein sources. We can see such a trend in the data reported by Richards and Hedges [44], showing a correlation between $\delta^{13}C$ and $\delta^{15}N$ for a British population. Lubell and coworkers [36], studying

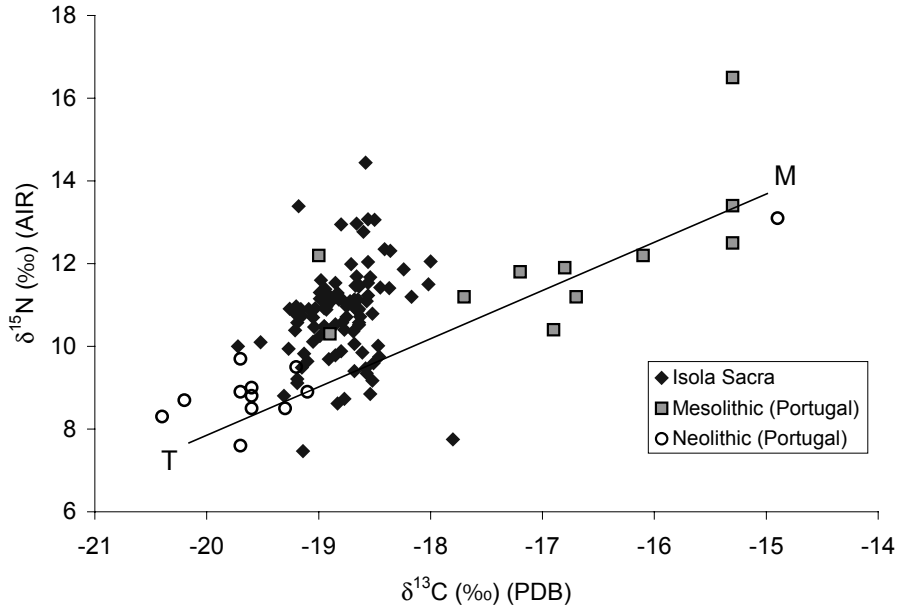

Fig. 5. Plot of $\delta^{13}C_{collagen}$ vs $\delta^{15}N$ for data from Mesolithic and Neolithic human skeletal samples from coastal Portugal [33] compared with data from Isola Sacra. Note steeper slope and generally lower $\delta^{13}C$ values for Isola Sacra at corresponding $\delta^{15}N$ values.

the dietary transition between the Mesolithic and Neolithic periods in Portugal (c. 7000 y BP), also found a linear trend between marine and terrestrial foods; their Mesolithic sample showed a wide range of $\delta^{15}N$ values, interpreted by the authors to indicate a wide range in the proportion of a single type of marine food of intermediate trophic level. Fig. 5 shows a trend line with the points M and T, representing the collagen values of individuals consuming a pure marine (M) or terrestrial (T) diet (after Lubell et al. [36]).

The Mesolithic data of Lubell et al. [36] are consistent with the routing model, showing that both $\delta^{13}C$ and $\delta^{15}N$ of collagen lie on a trajectory between marine and terrestrial sources of protein. In contrast, whereas the data shown in Fig. 3 suggest that marine foods contributed to the diet of the Isola Sacra people, the $\delta^{13}C$ values do not appear to be enriched to the extent expected by the "routing" hypothesis. Typical $\delta^{13}C$ values observed in collagen of people reported by Lubell et al. [36] or Richards and Hedges [44] range between -18 and -13 ‰, compared to the average value for Isola Sacra of -19‰. More strikingly, however, the trend of the Isola Sacra population on Fig. 3 is one of increasing $\delta^{15}N$ without a comparable increase in $\delta^{13}C$. The typical gradient of $\delta^{15}N$ vs. $\delta^{13}C$ in previous studies [36,44] is about 2:1 whereas the gradient for the data from Isola Sacra is about 4:1 (Fig. 5). It seems unlikely therefore that carbon atoms from fish-derived proteins were being used preferentially to construct the collagen molecules. We shall discuss this issue in more detail below.

It is also possible that the moderately higher $\delta^{13}C$ values observed at Isola Sacra were caused by the consumption of C_4 plants. Ancient literary sources refer to the use of millet, a C_4 plant, as animal fodder, but it was considered less desirable for human consumption under normal conditions of food availability [56,60]. This C_4 plant signal would be passed on to humans through the consumption of animals or their byproducts. However, $\delta^{13}C$ values of the faunal remains from Isola Sacra, with the exception of the dog and fox samples, are more negative than those of the humans and fall within the range expected for C_3-consuming terrestrial herbivores.

Stable isotope analysis has also been performed on eleven individuals from a Neolithic ossuary at Alepotrypa Cave, Diros, Greece [40]. This site on the southwestern coast of Greece is close geographically to Isola Sacra, but is much earlier (5000–3200 BC). The authors expected to find a large marine component in the diet, but average $\delta^{13}C$ (-19.9‰) and $\delta^{15}N$ (7.2‰) values instead reflected a predominantly terrestrial C_3 diet, even though the remains of fish and shellfish were recovered from the site (ibid.). The Isola Sacra data, with more positive $\delta^{13}C$ and $\delta^{15}N$ values, show a much stronger influence of marine resources on the isotopic values. In fact, the mean isotopic values from Alepotrypa Cave more closely resemble the 'terrestrial plant consumer' subgroup from the ANAS cemetery.

Comparative isotopic studies of historic samples from Italy, and Europe in general, are rare. At the Roman period site of Poundbury, England, samples from individuals interred in mausolea from the late Roman phase had an average $\delta^{13}C$ and $\delta^{15}N$ values of -18.2 ± 0.3‰ and 10.1 ± 1.0‰, respectively (N=41) [46]. The authors proposed that this signified a diet composed primarily of terrestrial foods, but with a marine contribution to the

overall protein in the diet, and the authors concluded that individuals in the mausolea were higher status members of the population, consuming a more 'Roman' diet composed of marine fish, oysters, or *garum*. In comparing Isola Sacra and Poundbury, we must also recognize the possible effect of $\delta^{13}C$ values of plant food sources being lower by 1–2‰ in northern Europe compared to Italy [45]. In spite of this, the isotopic values between the two Roman sites are remarkably similar and suggest similar diets. The site of Poundbury is located near the SW coast of England and near the modern town of Dorchester, so marine resources were likely easily accessible and plentiful.

Richards and van Klinken [45] examined average human $\delta^{13}C$ values from a variety of European Holocene sites, including sites from Italy (ca. 12,000 BP and later). The $\delta^{13}C$ average obtained for human samples from Italy was -21.3‰ (standard deviation not reported), based on data derived from sites "in the Alps as well as the south" (ibid.:364). Climatic variation in Italy between the two time periods would not have significantly affected isotopic values, so we would have expected similar $\delta^{13}C$ values for collagen of consumers of a diet based on purely terrestrial food sources. This further supports our contention that the higher $\delta^{13}C$ values observed at Isola Sacra are due to the presence of a marine component.

8.2. Apatite

If we assume that Δ_{a-d}=13‰, then the $\delta^{13}C_{ap}$ values (ca. -12 to -10‰) correspond to diets with total $\delta^{13}C$ values ranging from=-25 to -23‰, which would represent a herbivore diet with a small, variable marine component. This is consistent with the discussion of the $\delta^{13}C$–$\delta^{15}N$ covariation in collagen isotopic analyses, and confirms that the main source of carbon atoms (but not nitrogen) was from terrestrial foods, presumably bread plus small amounts of other vegetables, fruits, oil and wine. The average $\delta^{13}C$ of modern olive oils from Italy is reported to be -29.5 ± 0.3‰ [5]. In pre-industrial times this value would have been about 1.5‰ heavier, that is, c. -28.0‰ [38]. Wine is estimated to be about 1‰ lighter than the grapes from which it is formed (C. Hendy, pers. comm., 2002). Both of these dietary components could have contributed to the lower observed $\delta^{13}C_{ap}$ values.

9. Conclusions

The isotopic data indicate that the people at *Portus Romae* ate a mixture of marine and terrestrial resources. This is not surprising considering its location on the coast of Italy and the role that its inhabitants played in maritime trade for the Imperial city of Rome. It is also clear that the marine signal is not due to the reportedly ubiquitous consumption of *garum* in the Roman diet, but rather reflects the consumption of higher trophic level marine organisms. However, it is possible that Isola Sacra represents a biased sample of Romans, that is, people with better than average diets (i.e., a diet consisting of more costly food items like fish); but the data indicate that terrestrial resources (C_3 plants and animals) still formed the bulk of the diet. The comparative data from the ANAS cemetery provides evidence of the dietary diversity within a relatively small geographic area.

We have shown that isotopic data for collagen from Isola Sacra (and for a subset of the ANAS data) follow a $\delta^{15}N$ vs. $\delta^{13}C$ trend quite different from that seen in other studies of marine consumers [36,44], and yet it seems likely that the enrichments in ^{15}N and ^{13}C seen in this population are a result of consumption of marine resources. Why is the $\delta^{13}C$ value of the marine source of protein not equally reflected in the isotopic composition of the collagen, as predicted by the routing hypothesis? Similar trends are seen in other data sets for European sites (see e.g. Ref. [52]). We would propose that the distribution of $\delta^{15}N$ values for collagen that we see in Fig. 3 mainly reflects the source of nitrogen essential for endogenous synthesis of amino acids, whereas carbon atoms used to construct non-essential proteins were derived principally from the plant-derived foods, which had a more limited range of $\delta^{13}C$ values. We note that bread can also provide a significant amount of protein, and some varieties of bread would have been an adequate source of protein. Therefore the addition of small amounts of a protein-rich food such as fish would have had a proportionally small effect on $\delta^{13}C$ values, while having a relatively large effect on $\delta^{15}N$ values. The magnitude of this effect is increased when, in the presence of an inadequate protein supply, a significant fraction of non-essential amino acids (which constitute c. 80% of collagen) would be synthesized from other carbon sources, especially carbohydrates (see Ref. [54]).

Another possible causal factor lies in the apparent range of $\delta^{13}C$ and $\delta^{15}N$ of possible fish sources (Fig. 3). Individuals with the highest $\delta^{15}N$ and $\delta^{13}C$ values may have been preferentially consuming fish with a high trophic level, and vice versa. Thus, the marine component of their diet affected the $\delta^{13}C$, $\delta^{15}N$ values of their collagen, but did not control it.

Analyses of collagen from the ANAS sample could be segregated into two distinct clusters; the individuals with isotopic values similar to the Isola Sacra sample may have been migrant workers who worked on the coast and consumed a diet composed of a greater proportion of marine resources. Curtis [14] described the work of Roman fishermen who would be hired to work for traders and merchants, but who lived in nearby towns. These individuals would have worked for long periods of time on the coast or on ships during peak seasons,

and would have consumed a diet that distinguished them from groups living and eating further inland who were probably involved in agricultural activities and whose isotopic values would indicate the consumption of a large proportion of C_3 plants in their diet. It is also possible that the variability observed in the ANAS sample may reflect status differences within the population.

This study has quantified the contribution of terrestrial and marine resources to the diet of this Roman period population and has demonstrated that marine resources were an important component of their diet.

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