1st International Conference on
Metrology for Archaeology
Benevento, Italy, October 22-23, 2015

Stable isotope analysis reveals the dietary habits of *Paestum* during the Imperial period (2nd to 4th centuries AD)

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***Abstract* – In historical contexts, analyses of carbon and nitrogen stable isotopes can be useful to answer questions on dietary behavior and to crosscheck information, drawn from texts and classical archaeological investigations. In this study, we present an example of this application to the Roman Imperial time at the coastal site of Paestum (southern Italy), one of the most important settlements of Magna Graecia. Stable isotope analyses of carbon and nitrogen were applied to bone collagen extracted from human remains from the necropolis of "Porta Sirena" in Paestum. The necropolis dates back to the 2nd - 4th centuries AD. The hypothesis of fish consumption in the diet of this costal population has been tested. This study aims also to expand our knowledge on diet in Roman Paestum, with particular reference to consumers from the sub-elite.**

I. INTRODUCTION

Stable isotopes analysis of human collagen has proven to be a reliable method for reconstructing the diet of ancient populations (paleodiet), since its early appearance in the late seventy's studies [1, 2]. Isotope composition, i.e. stable isotope ratios of carbon (C) and nitrogen (N), measured as relative deviation from a known standard, is expressed in term of delta notation, from now on $\delta^{13}C$ for C, in the Vienna Pee DEE belemnite (VPDB) scale, and $\delta^{15}N$ for N, in the Air scale. The $\delta^{13}C$ and $\delta^{15}N$ in the body tissues reflect that of the consumed food, as first shown in studies about animals fed on controlled diet [3, 4]. Bone collagen, with its turnover rate of around 3% per year (for a typical male adult), expresses a representative record of an individual's diet [5], albeit between diet and bone collagen there is an offset of 5 ‰ in $\delta^{13}C$ and an offset of 3‰ in $\delta^{15}N$ [6, 7]. Isotopic signatures at the origin of a trophic chain characterize the chain itself (for example, marine or terrestrial). As these signature vary among different trophic chains and it propagates along a chain, it offers reliable clues to distinguish whether or not an individual belongs to a certain food chain or to a certain level of it [8-11]. The increase of $\delta^{13}C$ in human diet arises mainly as a result of consumption of two types of 13C-enriched food. Firstly, marine foods are characterized by $\delta^{13}C$ values ~7‰ higher than terrestrial foods [6]. Secondly, different photosynthetic pathways fractionate carbon isotopes differentially, therefore C4 plants have $\delta^{13}C$ values of their tissues about 17‰ higher than the corresponding components of C3 plants [12, 13]. The most important C4 foods are millet and sorghum in Europe, Africa and Asia. Many of the most important cultigens such as wheat, barley, and rice are C3 plants. Using C isotopes alone these two ^{13}C-enriched sources (C4 plants and marine foods) cannot be distinguished from one another, nor can be revealed the consumption of animal derived protein. Therefore, $\delta^{15}N$ on collagen is necessary ancillary information. Much of the variation in $\delta^{15}N$ largely arises as a result of the trophic level effect: carnivores have higher $\delta^{15}N$values than herbivores, whom they feed on, and the higher is the number of trophic levels between a prey and its predator, the larger is the difference in their $\delta^{15}N$ [11]. The number of trophic levels in the marine and aquatic realms is higher than on terrestrial (since lower trophic-level carnivorous organisms are eaten by higher-level organisms). Therefore, the highest $\delta^{15}N$ values in humans are observed where marine or aquatic foods are important in the diet [7]. Humans that consume only terrestrial protein sources may have collagen $\delta^{13}C$ values of approximately –20‰, and $\delta^{15}N$ values ranging from +5 to +12‰, while humans that consume marine food have collagen $\delta^{13}C$ values close to –12‰ and $\delta^{15}N$ values between +12 and +22‰ [14, 15]. Moreover, humans who consume a mixture of terrestrial animal and marine protein would have isotopic values somewhere between the above end points.

Generally, in a defined historical context, information about foods commonly used in human nutrition is known from archaeological investigations and different models have been implemented in order to untangle the diet of an individual into its different food constituent [16]. But, it results more difficult to obtain precise information about food consumption as between different social, age and gender groups, within a community or region as a whole. In this case, stable isotopes analysis can be useful to confirm the actual adoption of a certain kind of diet, known to be common at a time.

In particular, historical records or textual references about food and diet during the Roman Imperial period explain that fish or terrestrial animal meat was reserved just to an elite of people [17]. During the last decade, a number of publications have addressed this issue, testing historical accounts by means of stable isotope analysis, in different context in the central and southern Italy. They introduced the hypothesis that together with cereals, wine, olive and dry legumes also animal proteins could have been part of the lower classes diet: pork, sheep and goat meat primarily, but also fish when easy accessible for the population, i.e at costal sites. For an introduction to the food availability and diet during the Roman imperial time see Prowse et al., 2004 [17], Killgrove and Tykot, 2013 [18], and the references therein. Killgrove and Tykot, 2013 [18] and Rutgers et al., 2009 [19] studied the city of Rome itself. The first ones investigated two cemeteries just outside the urban walls, Rome-Casal Bertone (2nd-3rd centuries AD) and Rome-Castellaccio Europarco (1st-3rd centuries AD). Rutgers et al., 2009 [19] focused their research on Early Christians buried at the cemetery of St. Callixtus (3rd to 5th centuries AD). However, pioneers in this topic have been Prowse and co-authors [17, 20-22], as they extensively investigated the behaviours of the population from Portus Romae (Trajan's port, 23 km southwest the city of Rome), who was used to bury the corpses in the necropolis of Isola Sacra (1st-3rd centuries). Stable isotope analysis was especially used to look into the diet of the population and how it varied as function of sex age and status [17, 20] and it revealed that the population generally mixed terrestrial resources with marine food, consuming marine organisms of higher trophic level more than the famous *garum*, a fish sauce typical of the elite Roman diet. The nearby inland cemetery, named ANAS, was originally intended as reference site for terrestrial based diet. However, final evidences showed that it had been occupied by two different clusters of individuals, identified for their similarity to Isola Sacra individuals, as one group belonging to the inland site, and another one with individuals that possibly migrated from a coastal zone (ANAS 1 and ANAS 2, respectively for our reference) [17]. Craig et al. [23] moved their research southern and they explored the dietary habits at the coastal sites of Velia. By means of cluster analysis, they revealed that the majority of the population from the Velia necropolis had a diet high in cereals and relatively lower in meat, but they found a group which had consumed more meat and also fish, especially high trophic level fish (Velia 1 and Velia 2, respectively for our reference). About 30 km to the north of Velia, during the excavations conducted in the area of "Porta Sirena" at Paestum (Salerno, Italy), graves belonging to the Roman Imperial Period came to light. The human remains were studied with an anthropological approach, according to the standards of the funerary archaeology [24] and they have been processed for isotope analysis.

In this study, carbon and nitrogen isotope analyses applied to the human remains of the necropolis of "Porta Sirena" in Paestum are presented. Primarily, this investigation has aimed to verify the hypothesis of presence of fish in the diet at this shore site during the Roman Imperial time, especially by comparing coeval sites. Furthermore, since more studies on this topic have been invoked as desirable (see for example Killgrove et al. 2013 [18]), this work is expected to generally contribute to the knowledge on average lower class diet from this time, which is complex and variable in its distribution among different kind of people and different territories.

Fig.1 ***a.*** *Map of south of Italy with the location of the necropolis of "Porta Sirena" at Paestum and the coeval sites considered in the text. The necropolis of Velia is located to the south of Paestum, while to the north, near Rome, on the coast, the cemetery of Isola Sacra. Casa Bertone, Castellaccio Europarco and St Callisto, just outside the city walls of Rome, are too close to Rome be distinguished at this scale.* ***b.*** *Location of the necropolis of "Porta Sirena", outside the ancient walls of Paestum.*

II. EXPERIMENTAL, MATERIALS AND METHODS

A. Site description

The area of the excavation is located along North-South direction of "Porta Sirena" (figure 1), while the eastern and western limits of the zone of operation are respectively represented by the modern road and the walls. The area has been divided into different sectors. The excavation of the necropolis, whose tombs are located at about 10-15 cm depth, have so far returned 73 burials, numbered from 21 to 95 (t21 to t95) (figure 1b).

According to their stratigraphy and the archaeological analysis of the grave goods, the interments have been attributed to a period spanning from the 2th to the 4th centuries AD.

B. Paleodiet analysis

For the scope of this study, 23 out of 73 discovered graves have been sampled for paleodiet analysis. The choice fell on those graves where the human remains were better preserved, preferring those among them who by inhumation and grave goods were more heterogeneous, in order to be as more representative as possible of the whole interred population of the cemetery. The graves in *amphorae*, were excluded from this study, as they were usually used for very young corpses. Long bones, when available, have been preferred to the others, in order to obtain information about lifelong habits of the sampled individuals, and, when possible, the same skeletal element has been chosen for all the individuals, in order to minimize the influence arising from sampling different kind of bones. The list of the sampled bones is reported in table 1. Faunal remains were rare and not properly preserved for classification, for reference purposes two teeth of two different herbivores (possibly a cow and a goat) have been collected at the site, being coeval with the human individuals under investigation.

The samples for the analysis were processed to isolate the organic phase of the sample (collagen) adopting a modified procedure from Longin method (1971) [25]. A fragment of the sample was selected from each specimen. The bone surface was abraded to remove contaminants and it was pulverized. Each sample was then placed in polypropylene test tubes and demineralised in a sequence of acid attacks with hydrochloric acid (HCl) 0.6 M at ambient temperature (20-25°C), interrupted by one alkali attack (NaOH 0.1M) 30 minutes long. Several rinses with de-ionized water were done after each step, before finally oven-drying the samples. For collagen quality test, C and N fractions of collagen dry mass (C% and N%) were measured by an elemental analyser (CN Flash EA 1112, Thermo Scientific, Bremen). Samples were retained for isotope analyses when extracted collagen achieved a yield higher than 1% and an atomic C:N ratio between 2.9 and 3.6 [26-29]. The δ^{15}N and δ^{13}C values were measured concurrently in continuous flow mode by an isotopic ratio mass spectrometer (IRMS), a Delta-Plus connected via CONFLOW II interface to another Flash EA 1112 (Thermo Scientific). Samples were analysed according to the method first described by Preston and Ovens 1985 [30] that is: burned in a single EA red-ox reactor held at 1020°C. The isotopic measurements were calibrated based on the measurement of standards, aiming to set their values on internationally referenced scales (VPDB for C and Air for N) [31]. The analyses were conducted in blocks of 12 samples, maximum. Between one block and the next one, three different reference materials were measured: two, used to calibrate the measurement and the last one used to evaluate the proper conduct of the analysis (target) and the repeatability of the measurement itself. The reference materials used for δ^{15}N analysis calibration were IAEA-N-2 (ammonium sulphate, $\delta^{15}N_{Air}$ = 20.3 ± 0.2‰) and IAEA-N-1 (ammonium sulphate, $\delta^{15}N_{Air}$ = 0.4 ± 0.2‰) [32]. The reference materials used for δ^{13}C analysis were IAEA-CH6 (sucrose, $\delta^{13}C_{VPDB}$ = -10.45 ± 0.03 ‰) and IAEA-CH3 (cellulose, $\delta^{13}C_{VPDB}$ = -24.72 ± 0.04‰) [33]. Typical analytical precision evaluated from repeated measurements of the same sample is 0.1‰ for δ^{13}C and 0.2‰ for δ^{15}N.

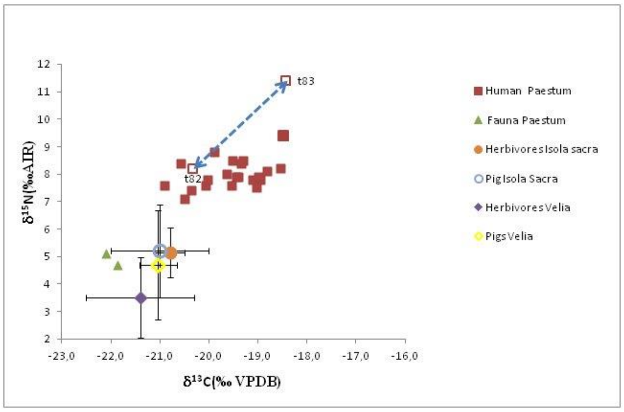

Fig.2 δ^{13}C and δ^{15}N values for humans (squares) and herbivorous fauna (triangles) from Paestum compared with fauna from coeval costal sites of Isola Sacra (circles) and Velia (diamonds), average values with standard deviation. The individuals t82 and t83 are possibly mother and her breast feeding baby.

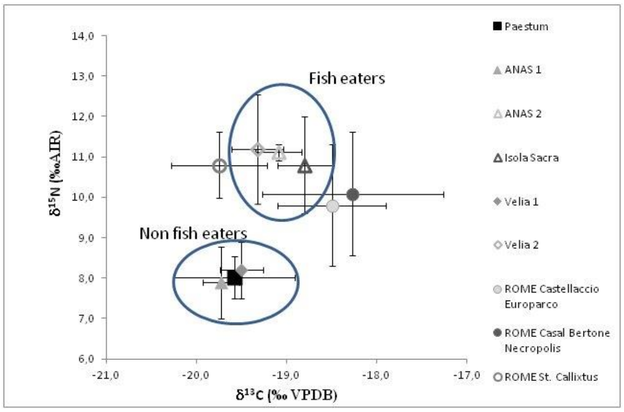

Fig.3 δ^{13}C and δ^{15}N bi-variate plot with the comparison between Paestum and other coeval sites (average values with standard deviation). Isola Sacra (empty dark triangle), ANAS2 (empty light gray triangle) and Velia 2 (empty diamond) have been grouped to identify them as "fish eaters". Velia1 (full diamond) and ANAS1 (full *triangle) are indicated as "non fish eaters". Circles represent the mean values from the cemeteries belonging to the city of Rome. Error bars are standard deviations.*

Sample	C/N	$\delta^{13}C$ (‰VPDB)	$\delta^{15}N$ (‰AIR)	Collagen yield
Herbivorous d1	3.3	-21.9	4.7	3.1%
Herbivorous d2	3.3	-22.1	5.1	1.9%
t24	3.1	-19.0	7.8	2.4%
t26	3.0	-19.5	7.6	3.6%
t34	3.0	-19.3	8.5	2.7%
t38	3.1	-20.9	7.6	2.9%
t46	3.3	-20.5	7.1	1.6%
t47	3.0	-19.4	7.9	1.5%
t53	3.1	-19.1	7.8	2.6%
t58	3.0	-19.0	7.9	2.5%
t64	3.0	-18.5	8.2	4.7%
t65	3.1	-20.4	7.4	3.9%
t68	3.0	-18.5	9.4	3.9%
t69	3.1	-19.5	8.5	1.6%
t70	3.0	-18.8	8.1	3.1%
t72	3.0	-19.4	7.9	1.2%
t76	3.0	-20.1	7.6	2.4%
t77	3.0	-20.0	7.8	3.1%
t80	3.0	-19.0	7.5	1.0%
t81	3.1	-19.6	8.0	1.8%
t82	3.1	-20.3	8.2	1.0%
t83	3.1	-18.4	11.4	2.6%
t84	3.0	-19.9	8.8	3.2%
t85	3.0	-20.6	8.4	2.7%
t86	3.0	-19.3	8.4	3.2%

Tab.1 Isotopic values and collagen quality indicators of human bone and fauna's teeth samples from the necropolis "Porto Sirena" of Paestum.

III. RESULTS AND DISCUSSION

The results of the isotopic analysis are shown in Table 1 as $\delta^{13}C$ and $\delta^{15}N$, together with collagen quality indicators (C/N, collagen yield). The general state of preservation of the sampled bones has been acceptable. Testing the quality of collagen extracted assures the absence of potential contamination effects. From the results obtained, all the samples were retained for analysis, presenting yields above 1% and C/N values spanning from 3.0 to 3.3 [26-29]. Anthropological assessments are not covered by this study; but it is important to consider that the grave goods found in the site were in no case valuable or interesting, confirming the absence of any elite member of the community interred in this necropolis.

Figure 2 represents the $\delta^{13}C$ and $\delta^{15}N$ bi-variate plot which finally allows considerations on the dietary habits. All the results from the necropolis of "Porta Sirena" in Paestum are plotted as compared to the faunal remains, the ones found at the same site (triangles) and the fauna collected at the coeval sites of Velia (diamonds) [23] and Isola Sacra (circles) [17], in particular pigs and herbivores. The isotopic composition of the two fauna samples from Paestum falls well within the variability shown by the coeval fauna from the other sites, both Isola Sacra and Velia.

Coming to humans, the collagen extracted from the bone sampled in the tomb t83 has given delta values clearly very different from those found for other samples. The bone fragments extracted from tomb t83 have been attributed to an individual younger than 1 year, an infant, i.e. a breast-feeding baby. Breastfeeding individuals can be considered "consumers" of their mother's tissues, therefore occupying a higher trophic level than the adults and the weaned children. A ^{15}N enrichment of about +2 and +3‰ and a ^{13}C enrichment of about 1‰ are typical for this trophic shift [34, 35]. This $\delta^{13}C$ and $\delta\ ^{15}N$ offset pattern is consistent with what we find for this individual t83. Being t.83 a child early in life, it is very likely that the individual laying in the same grave (t82) might have been his/her mother. They differ from each other of 1.9 ‰ on the $\delta^{13}C$ scale and of 3.2 ‰ on the $\delta^{15}N$ scale, which is a result that could indicate the trophic shift mother-baby. But this can only be confirmed in response to anthropological analysis (i.e. individuals sex).

If we exclude the sample belonging to the tomb t83, all the $\delta^{13}C$ and $\delta^{15}N$ values are included respectively in the ranges [-20.9‰, -18.5‰] and [+7.1‰, +9.4‰]. According to these result, the diet of the Paestum population buried at the necropolis of "Porta Sirena" can be defined as mainly agricultural-pastoral (although one can not rule out a small (<20%) fish consumption), confirming the indications known from independent archaeological studies [24]. The values obtained, in fact, are consistent with a diet based on the consumption of C3 plants, characteristics of temperate climates, with addition of meat as confirmed by the fact that the average of human population (excluding t83) is about 2.4 ‰ and of 3.1 ‰ higher than herbivores samples on the $\delta^{13}C$ and the $\delta^{15}N$ scale, respectively.

Figure 3 compares the results from Paestum with those from the coeval sites from the central and south of Italy shown for their location on the map of figure 1. Velia is the closest site and it has been the largest investigated site together with Isola Sacra, in term of analysed individuals. In their extensive investigations Craig et al. (2009) [23] for Velia (diamonds) and Prowse et al. (2004) [17] for Isola Sacra (triangles) have been able to distinguish among groups of the population, which had different consume of meat and, especially, of fish in their diet. Here we refer to these categories to further interpret the results of the smaller population sampled at Paestum. The groups which very likely introduced fish in their diets have been here defined for simplicity "*fish eaters*" (Isola Sacra, ANAS 2 and Velia 2), the others have been named "*non fish eaters*" (Velia1, ANAS1). *Fish eaters* at Velia belongs to a small group of 17 individuals (Velia 2, empty symbols), while all individuals from Isola Sacra are thought to introduce to some extent marine food in their diets. In the original study, Isola Sacra samples were compared with individuals buried in a nearby inland place named ANAS (triangle). In the ANAS cemetery just a subset of inhumed individuals was identified with a terrestrial diet (ANAS 1, full symbols), the rest was formed by costal immigrant or seafarers (ANAS 2, full symbols). The δ ^{13}C and δ^{15}N of human individuals found at the necropolis of "Porta Sirena" in Paestum averaged (excluding t83) -19.6‰ (s = 0.7‰, n=22) and +8.0 ‰ (s = 0.5‰, n=22), respectively. On the one hand, the mean δ^{13}C of the *fish eaters* group is -18.9‰ (s=0.4‰, n=126) and the mean δ^{15}N is +10.9 ‰ (s=1.2‰, n=125). These values are significantly higher than the values found at Paestum (two-sample t-test, $p < 0.001$; t=4.7 for the difference in δ^{13}C, t=18.9 for the difference in δ ^{15}N). On the other hand, the human individuals found at the necropolis of "Porta Sirena" in Paestum show unequivocal analogy with the group identified as *non fish eaters*, mainly coming from Velia, whose mean δ^{13}C is -19.5 ‰ (s=0.2‰, n=106) and δ ^{15}N is +8.2‰ (s=0.7‰, n=106), suggesting that these individuals had a negligible or no use of sea food in their diets (two-sample t-test, $p < 0.001$; t=0.6 for the difference in δ^{13}C, t=1.3 for the difference in δ^{15}N). However, it remains the hypothesis that "Porto Sirena" population could eat fish in a minor amount respect to other food, because marine protein could represent up to 20% of total dietary protein without any appreciable change in ^{13}C values and consuming low trophic level marine resources (e.g. garum) could not change significantly ^{15}N signal [17].

A third subset of three sites belongs to the city of Rome: two cemeteries just outside the urban walls, Rome-Casal Bertone (2nd-3rd centuries AD) and Rome-Castellaccio Europarco (1st-3rd centuries AD) and the Early Christian cemetery of St. Callixtus (3rd to 5th centuries AD). Results of the dietary analysis from periurban Casal Bertone and suburban Castellaccio Europarco showed evidences that individuals living closer to the city of Rome were consuming some aquatic resources, those in the suburbium made greater use of millet [18], as could possibly have done some of the individuals buried at Paestum showing higher δ^{13}C values. The access to aquatic resources of Tiber, instead, were used by Rutgers and colleagues (2009) to interpret the comparatively low δ^{13}C values as related to freshwater fish consumption. Beyond this, there is to be noted that the intra-site and inter-site variability existed in the diet of the common people during the Roman Imperial period, Paestum population, however, does not show signs of this complexity, appearing as a simple rural community, in accordance with the classical paradigm that fish or terrestrial animal meat was reserved to elite people, even when sea resources were so close in space. However, we could not bring any evidences of variability of the dietary behavior according to any hierarchical or social status in Paestum. In addition, no particular status distinction among the inhumed individuals was found at the site, as testified, for example, by the kind of tombs and the simple grave goods, found in that.

IV. CONCLUSIONS

This work has confirmed that isotopic techniques can integrate classical archaeological investigations to improve the knowledge of diet in Imperial Roman Paestum. As matter of fact, archaeological study allows to place the site chronologically, identifying analogies with the contemporary and vicinal necropolis of Velia, especially with respect to homogeneity in the poverty of the materials and rituals of burial. The isotopic analysis outlines uniformity even in the diet, identifying a typical agricultural-pastoral diet, although one can not rule out a small (<20%) fish consumption. We can therefore assume a homogeneous social structure, based on a predominantly rural economy, where, despite its proximity, the sea seems not be regarded as a source of food, maybe because it was still not easily accessible. While showing analogies with the coeval and close by site of Velia, isotope analysis revealed also many differences with the city of Rome and its nearby coast.

AKNOWLEDGEMENTS

The original European-funded project, sponsored by the Archaeological Superintendence of Salerno, was done in collaboration with the University of Salerno, (laboratory Mario Napoli, Department of Cultural Heritage).

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