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Using Oxygen and Carbon Isotopic Signatures in order to Infer Dietary Information in Bones from Kythnos Island, Greece

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Abstract. Isotopic studies have been conducted on skeletal remains of ancient populations from Kythnos, an island of the Cyclades in Greece, for the purpose of dietary reconstruction; mostly through carbon and nitrogen isotopic signals of bone collagen, and apatite signatures (oxygen and carbon) as dietary and palaeoenvironmental tools. The basic aims of the present study are to reconstruct the diet of this population and

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to detect possible differentiations between the adult and the childhood/juvenile diet. The results of this study revealed that residents of Kythnos probably consumed C3 and C4 products as their primary protein source. In addition, the collagen results along with the collagen-apatite spacing possibly indicated a detectable consumption of freshwater sources. The isotopic analyses have been conducted at the Stable Isotope and Radiocarbon Unit of INN, NCSR "Demokritos".

Keywords: Skeletal remains, Bone collagen, Dietary, Reconstruction, Isotopic signals

Introduction

Kythnos belongs to the complex of northwestern Cyclades, located between Kea and Serifos. It is an island with a long history and special archaeological interest. The most important site of historical era was Vryokastro, the ancient capital of Kythnos. The fortified polis is situated on the northwest coast of the island and has been continuously inhabited from the beginning of the first millennium B.C. until the 6^{th} – 7^{th} century A.D (Figure 1). The remains of the ancient city occupy an area of approximately 28,5 hectares, including the small islet of Vryokastraki, which was connected to the shore in antiquity by a narrow isthmus. During the systematic investigations which have been in progress since 1990 (survey and subsequent excavations) numerous finds and several ancient structures, such as temples, altars, public buildings, underground cisterns, houses, port facilities, burial monuments, etc., have been brought to light. These discoveries have provided valuable insights into the urban planning of the city and the sociopolitical and economic development of the ancient community.

Important information about the city can be obtained also from the study of the deceased. The ancient city had two cemeteries (Mazarakis Ainian 1980,1994, 2019). The main necropolis occupies a large area outside the southern part of the fortification wall, where several tombs from Classical and Hellenistic periods have been excavated by the Archaeological Service (Samartzidou Orkopoulou 1997; Samartzidou, 2004). From the rescue excavations, sixty-eight graves were uncovered in an area of about 400 square meters. The graves were cut into the bedrock and arranged densely. Among them, only one incrustation in an amphora and a jar burial in a beehive have been found. Unfortunately, the majority of the graves are looted. Out of the total number of burial 41 have been certainly attributed to adults, only 8 to children, and an additional 2 to infants. Due to the various disturbances, only a limited number of skeletons has been well preserved, rendering the identification of the gender and age of the deceased a challenging task. The aim of the ongoing study is to investigate the diet using stable isotopes from bone samples of ancient skeletal material. Bone consists of two parts, the organic phase (collagen) and the inorganic phase (hydroxyapatite carbonate). Both phases are directly intertwined with nutrition. Specifically, the food consumed by the man during his lifetime contains various elements such as carbon and nitrogen which are assimilated in the various tissues of the body such as bones and teeth. These particular tissues are characterized by a very low rate of reorganization and therefore reflect the diet consumed by the individual, on average, during the last ten years of his life. The stable isotope method is used to study the diet of the skeletal finds that have been found in the archaeological site of Kythnos.

Reconstructing the diet of ancient populations using stable carbon and nitrogen isotopes is a well-established method in bioarchaeology. δ^{13}Ccoll values can indicate the type of consumed plants. According to the photosynthetic pathway that they follow, plants are distinguished into three categories: C3 plants, C4 plants, and CAM. C3 plants display carbon values ranging from −24‰ to −36‰ (closed canopy) (Smith & Epstein, 1971; O'Leary, 1981; Heaton 1999). In contrast, C4 plants have $\delta^{13}C_{coll}$ values ranging from −9‰ to −14‰ (Smith & Epstein, 1971), (global mean −12.5‰ (Lee-Thorp, 2008).

CAM plants (cactus) display intermediate values between C3 and C4 plants. Nitrogen-stable isotopes can be used in order to determine the trophic level of the foods consumed and distinguish between marine vs. terrestrial intake. Higher trophic level marine carnivores tend to display elevated nitrogen values (above 10–12‰) (Schoeninger et al., 1983a; Schoeninger et al., 1983b). In addition, δ^{15}N values of marine plants are approximately 4‰ higher than the ones of terrestrial plants (Yoder, 2012). Feeding experiments and anthropological studies have demonstrated that the δ^{15}N offset between dietary protein and human bone collagen is approximately 5.5‰ (Minagawa et al., 1986; Schoeller et al., 1986; Minagawa, 1992; Yoshinaga et al., 1996; Hedges et al., 2009; Huelsemann et al., 2009; O'Connell & Hedges, 1999; O'Connell et al., 2001; Richards, 2001). Hence, the objectives of the present study are to elucidate the dietary habits of Kythnos population.

Figure 1. Aerial photograph of Vryokastro.

Materials and Methods

With just a 5-gram bone sample, a comprehensive range of isotope analyses can be performed, including $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$, ^{14}C, and Sr, providing detailed information about an individual's origin, immigration history, and nutritional profile. Van der Merwe et al. (1982) developed a methodology that distinguishes between different photosynthetic pathways used by plants. These pathways result in different isotopic signatures. There are three main photosynthetic cycles: C3, C4, and CAM. C3 plants, which include trees, rice, cereals, citrus fruits, and legumes, typically have $\delta^{13}C$ isotopic values of around -27 ± 2 ‰. C4 plants, which include tropical grasses, corn, sugarcane, alder, and millet, have an average $\delta^{13}C$ isotopic value of -13 ± 1.2 ‰. The third photosynthetic cycle is used by plants such as cacti and prickly pears that alternate between the two types of photosynthesis, resulting in intermediate values.

Animals that move up the food chain consume plants, which affects the isotopic concentration of the plant that gets incorporated into their bones and teeth. This is also true for humans. If an animal feeds exclusively on C3 plants, it will have a lower $\delta^{13}C$ value compared to one that feeds only on C4. In the former case, the value is around -21 ‰, while in the latter, it is about -7 ‰. Animals that have a mixed diet of C3 and C4 plants should have values that are intermediate. Greece, like most of Europe and North America, has predominantly C3 plants, except for corn and cane sugar, which are crops or imports from the last century. Millet is the only C4 plant that seems to have been cultivated in ancient Greece since the Bronze Age, according to excavations. In order to approach the problem of the geographical origin of the individuals who have been found in Kythnos, as well as the specific diet of the population of ancient Kythnos, the isotopic analysis of a series of archaeological and environmental samples is required, both from Kythnos itself and from neighboring areas. The samples, human remains and animal tissues, have been analyzed for stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$).

Analysis

The isotopic analyses were conducted at the Stable Isotope and Radiocarbon Unit of the Institute of Nanoscience and Nanotechnology, of N.C.S.R Demokritos, in Athens, Greece. Collagen was extracted from the bone samples following Longin (1971) and Bocherens et al. (1991). Approximately 250 mg of each bone sample, after being ultrasonically cleaned, was crushed into small pieces using a mortar and a pestle. The small chunks of bone were soaked in 0.5 M hydrochloric acid (HCI) until all the minerals were dissolved. The remaining samples were then soaked in 0.5 M sodium hydroxide (NaOH) for 20 h, in order to remove any humic contaminants. The samples were afterwards soaked in aqueous solution reaching a temperature of 100 °C, until the collagen could be isolated. Finally, the collagen was subjected to the lyophilization process (freeze-dried). The atomic C:N ratio along with the collagen yields were used in order to determine the quality of collagen preservation. Collagen yields over 1wt% are considered acceptable for carbon and nitrogen values, while the C:N ratio should range between 2.9 and 3.6 (DeNiro, 1985).

Bone collagen samples were analyzed using an Elemental Analyzer (EA1112). The separated CO_2 and N_2 are carried in a helium stream to the mass spectrometer (ThermoScientific Delta V Plus Isotope Ratio Mass Spectrometer) via a Conflo III coupling. The isotopic ratios expressed for carbon as $\delta^{13}C$ versus PDB (a marine carbonate), and for nitrogen as $\delta^{15}N$ versus atmospheric N_2 (AIR):

$$X = [(R_{sample} - R_{standard})/R_{standard}] \cdot 1000 \quad (1)$$

where X is the $\delta^{13}C$ or $\delta^{15}N$ value and R = $^{13}C/^{12}C$ and $^{15}N/^{14}N$ respectively. Analytical precision was 0.1‰ for both $\delta^{13}C$ and $\delta^{15}N$ values.

Results

The results of the isotopic values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ collagen of animal bone, 15 samples, from building 3 located in the archaeological site of Kythnos (7th-5th century BC) and 14 samples of human bone from the classical period and 22 animal specimens and 3 (bones found in a deposit)] found mainly in the deposit are given in Figs. 2 and 3. Bone sampling consists of taking a small piece of bone of the order of a few centimeters. This is a great advantage of the technique because it allows the analysis of a large number of bones without destroying the find, especially in cases where the findings are important and of small quantity. The determination of the relative ratios between the stable isotopes of carbon and nitrogen ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$) is done in collagen extracted from ancient bones.

Regarding the stable isotopes, the values from the analyzes were placed on a $\delta^{13}C$ - $\delta^{15}N$ diagram together with other isotopic values of the Greek area (Panagiotopoulou & Papathanasiou, 2015; Lagia, 2015; Borstad McConnan et al., 2018). The results of the samples, animal tissues, from the same area are placed in the same diagram (Fig.2). If one considers (Richards et al., 1999) that:

1) the value of $\delta^{13}C$ in human bone collagen, when its diet is based exclusively on a terrestrial C3 food chain, is about -20 ‰,
2) the value of $\delta^{13}C$ in human bone collagen, when fed an exclusively marine diet, is approximately -12 ± 1‰, and
3) values for ^{15}N in human bone collagen range from 4 to 10‰ and 10 to 22‰ when fed exclusively terrestrial and marine diets respectively;

One sees that the prices for the samples fall between these two nutritional extremes. Compared to the literature data, the values of Kythnos are more positive in both nitrogen and carbon, showing that their diet, while based mainly on terrestrial plants and animals, also contained a small percentage of marine protein. Furthermore, the enriched isotopic values suggest a greater exploitation of the marine ecosystem for nutrition during the Classic period.

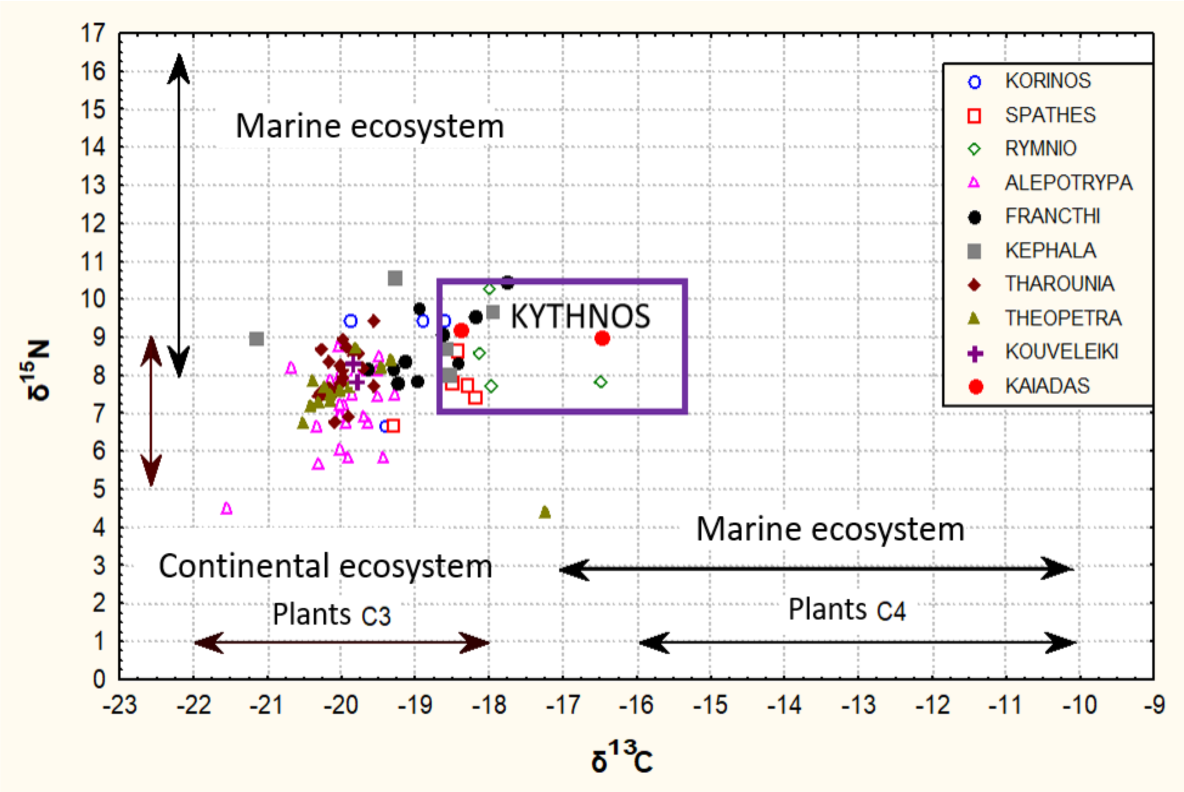

Figure 2. Diagram of the results of isotopic analysis of the Kythnos samples in comparison with isotopic values of human bones from various archaeological sites in Greece. Arrows indicate ranges of values occurring in flora and fauna samples worldwide.

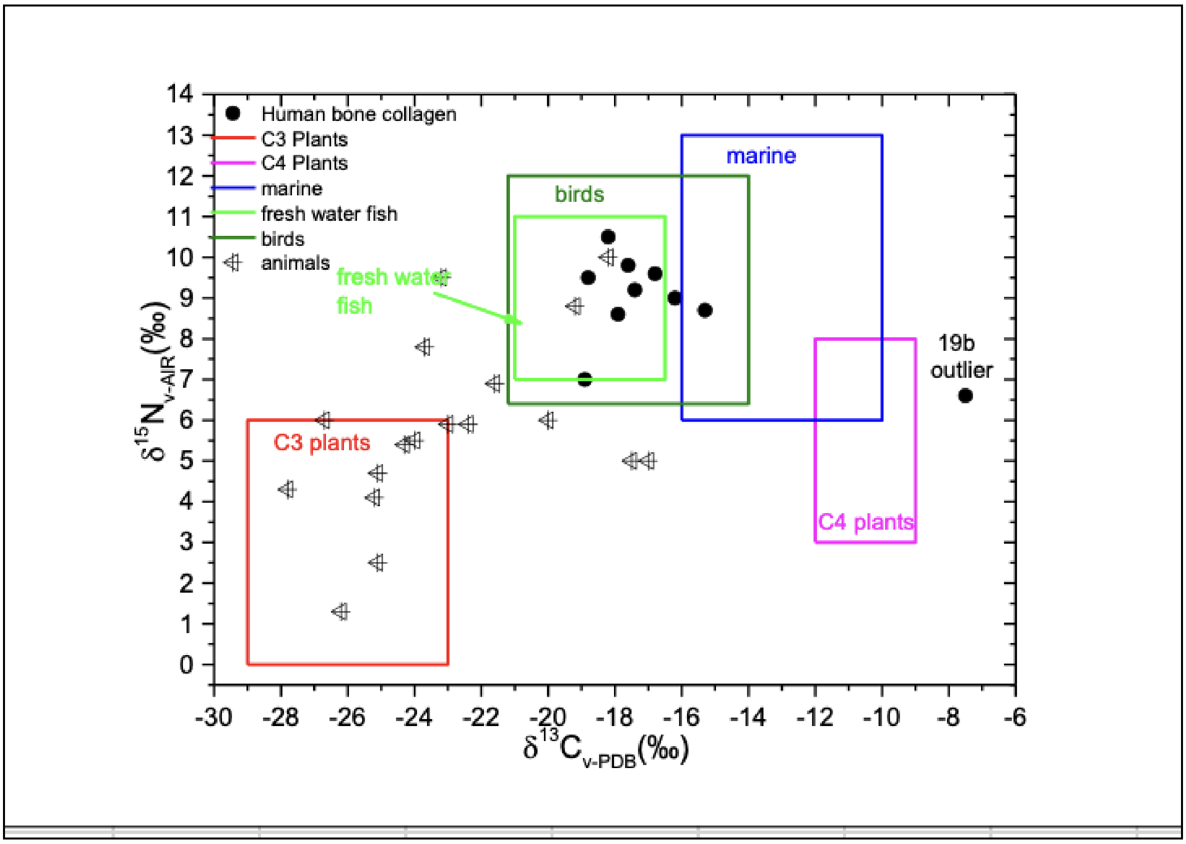

Figure 3. Mean values and error bars ($\delta^{13}C_{CO}$ vs. $\delta^{15}N$) of Kythnos in relation to reconstructed values for floral and faunal items of Greek territory. The isotopic ranges of C3 and C4 plants; C3 animals; Large fish and small fish derive from (Dotsika et al., 2018).

Compared to the literature data, the values of Kythnos are more positive in both nitrogen and carbon, showing that their diet, while based mainly on terrestrial plants and animals, also contained a small percentage of marine protein. Furthermore, the enriched isotopic values suggest a greater exploitation of the marine ecosystem for nutrition during the Classic period.

The isotopic values for all samples range between −15.3 to -18.9‰ and 7 to 10.5 ‰, for $\delta^{13}C_{coll}$ and $\delta^{15}N$ respectively. The mean carbon collagen value is at −17.5 ± 3.3‰ and the $\delta^{15}N$ at 9.1 ± 1.2‰. According to Tykot (2004) a diet completely reliant on C3 terrestrial sources would lead to a collagen carbon value at −21.5‰, thus pointing out to significant inclusion of more ^{13}C enriched food sources.

As presented in Fig. 3., there is a significant diversity among the inhabitants of Kythnos. In general, the individuals are placed above/within the C3 animal range, the animals are placed in the range of small fish and birds, and one sample is placed near the C4 plant range. It seems possible that these individuals consumed small fish, such as sardine or anchovy, which display rather low nitrogen values; 7.1 ± 0.8‰ and 6.7 ± 0.8‰ respectively. Another possibility could be that C4 sources, i.e. millet/corn constituted an important source for the individuals along with C3 items, leading them to elevated carbon values. In addition, unexpectedly elevated nitrogen values could also be attributed to the high consumption of birds, of garum, or to the extensive use of manure (Dotsika & Michael, 2018). It has been observed that a single individual is consuming C4 sources that constitute up to 70% of their total diet. Possibly is an immigrant.

Conclusions

Therefore, these isotopic patterns, $\delta^{13}C$ and $\delta^{15}N$, probably indicate a general diet in which most dietary protein came from resident fauna and flora and partly from marine resources. In a C3 diet type, $\delta^{13}C$ values would mainly reflect a terrestrial diet, while consumption of marine resources would contribute to elevated levels of C and N isotopes. The isotopic reconstruction has provided important information regarding the diet of the Kythnos population. As revealed by the isotopic values, low trophic position freshwater input has possibly been detected in the human diet. However, another possibility could be that C4 sources, i.e. millet/corn constituted an important source for the individuals along with C3 items. According to the data, the island's inhabitants had a varied diet including both C3 and C4 terrestrial food sources, and possibly freshwater items.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgments

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