Chapter 6

Interpreting Stable Isotopic Analyses: Case Studies on Sardinian Prehistory

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Most archaeological applications of isotopic research aimed at tracing diet in the western Mediterranean focus on human bone collagen and overlook environmental factors, which already have been demonstrated to be relevant. In this biocultural context, marine resources and C_4 crops do not appear to have been as important as the proportion of plant versus animal foods. The corresponding smaller range of recorded isotopic variation makes it necessary to devise specific interpretive tools to distinguish environmental effects from dietary information. In this paper, we explore the option of using bone apatite $\delta^{18}O$.

Stable Isotopes: Principles and Interpretation

In many areas of the world, stable carbon and nitrogen isotopic analyses are only recently becoming part of the standard toolkit of bioarchaeologists interested in investigating diet in prehistoric and historic times. Its use in assessing the proportion of marine food and of C_4 plants is well established, and many successful studies have looked at important nutritional transformations around the world involving radical changes in food procurement. Examples are the transitions from Mesolithic fishing to Neolithic farming in Atlantic Europe *(1)*; from foraging to maize farming in the Americas; and the beginning of millet, sorghum, and rice agriculture in Asia and Africa *(2–4)*.

Since the beginning of dietary research through stable isotopes, the main research focus has changed. In the 1960s and 1970s, following the observation of consistent offsets between radiocarbon dates obtained on C_3 plants versus those obtained on maize, estimating maize contribution to diet was the first important anthropological problem to be addressed *(5–7)*. In the 1980s, a new direction of research was concerned with understanding how environmental effects, primarily humidity and temperature, affected isotopic signatures, and with the implications in assessing diet *(8)*, and the complexity of considering whole ecosystems *(9)*. Studies involving transitions from aquatic to terrestrial diets also became important *(10, 11)*. Between the 1980s and the early 1990s, it seems that understanding the different contributions of macronutrients to the synthesis of different tissues has become a critical issue. The first model (scrambling model) suggested that all macronutrients contributed to the synthesis of collagen, which therefore represented the whole diet, while apatite reflected the energy portion *(12)*. Through experimental efforts it became apparent that in reality collagen tended to preferentially be built from protein intake, whereas apatite included, and therefore reflected, all components *(13, 14)*.

Recently we have witnessed an expansion of the field, both in the number of researchers involved and in the degree of specialization. Investigating both principles and particular archaeological contexts is becoming increasingly difficult due to the complexity of the interactions between dietary signature on one hand, and environmental, physiological, and cultural factors contributing to the existence of diverse combinations at different points in time and space. Therefore, the principles are best investigated through the controlled study of living human and animal populations. On the other hand, the complexity of interpreting isotopic values integrating biologically and culturally specific contexts makes a detailed knowledge of the historically specific conditions a crucial element for a faithful reconstruction. Increased specialization needs to be matched by increased integration of scientific, social and historical perspectives.

Oxygen stable isotopic ratios in animal bone and several materials other than human tissues are used to assess environmental conditions, and are

considered a fundamental proxy for paleoclimatic reconstructions. Oxygen isotopes are measured on both phosphate and carbonate *(15)*; for animals that are obligate drinkers, it mainly reflects the isotopic ratios of drinking water, which ultimately derives from rainwater. Although there are several factors contributing to specific $\delta^{18}O$ values, including temperature and the position of air masses, evaporation, and moisture from different sources *(16)*, an overall correlation has been confirmed by studies on the global, continental and local scale *(17, 18)*. Specific archaeological applications using human bone and tooth enamel include the assessment of mobility, residence patterns and the age of weaning. In this paper, we focus on carbon and nitrogen, while oxygen is only used as an interpretive complement.

The principle on which bone chemistry studies are grounded is that, generally speaking, our body composition is made up by the food we consume. The macronutrients we take in are used by the body to build tissues, and in the process they carry along the isotopic signature of their origin. Carbon and nitrogen isotope ratios in tissues (including hair, nails, bone, tooth enamel, flesh and virtually every part of our bodies) change predictably because of differential fractionation. This term refers to the process whereby chemical reactions involving these elements determine the selective uptake of heavier and lighter isotopes in specific ratios. This is due to different atomic mass, which causes the reactions to occur at different rates. Bone tissue is constantly replaced, so that its isotopic composition reflects dietary averages over several years before an individual's death. This means that rather than mirroring the last meals or the last seasons (as do gut contents or isotopes from hair), stable isotopic analysis of bone allows us to assess quantitatively the components of a standard diet for a long period of time.

What is actually measured by mass spectrometry is the ratio between isotopes of the same element ($^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$), where the lighter isotopes are the most common, and the heavier isotopes occur in minimal proportions. Therefore, isotope measurements are expressed as differences (δ) per mil (‰) relative to standards shared by the scientific community, which are PDB for $\delta^{13}C$, AIR for $\delta^{15}N$, and SMOW for $\delta^{18}O$.

Worldwide, a large isotopic difference in $\delta^{13}C$ that is maintained up the food chain is determined by different plant groups: C_3 and C_4. These groups differ in their photosynthetic pathways, resulting in a very distinct signature, averaging about -26‰ in the former and -12‰ in the latter. From an evolutionary standpoint, C_3 plants are the most common and are dominant in temperate latitudes. C_4 plants are mostly grasses that developed traits adaptive to arid, tropical environments. Utilization by humans caused their spread when they held nutritional significance, as in the case of maize, millet, and sorghum.

It has been shown by controlled-feeding experiments on mammals that the organic and mineral portion of bone (collagen and hydroxyapatite, or apatite) do not reflect the same macronutrients in the same proportion *(13, 19)*. Bone

collagen reflects the protein portion of the diet best, because it is mainly synthesized from ingested protein. Bone apatite is a more comprehensive indicator of diet since it is produced from proteins, carbohydrates and lipids. This means that if there is no nutritional imbalance *(20)*, collagen in humans will mostly reflect foods of animal origin, which are much richer in protein, and will reflect plants only if meat, dairy and fish were scarce, so that vegetal proteins were used to synthesize tissues. Apatite will reflect all three macronutrients *(13, 21)*, depending on their relative proportion to each other. However, the details of the mechanisms regulating the isotopic fractionation among diet on one hand, and collagen and apatite on the other, are complex and not fully understood *(22)*. It appears that the lower the amount of protein in the diet, the more carbohydrates and lipids will contribute to collagen composition, so that its isotopic composition *(20, 23)* may in some cases be a combination of both 'scrambling' and selective routing.

Due to physiology, the selective isotopic uptake in consumers varies by tissue type. In bone, the tissue archaeologically most important, $\delta^{13}C$ values shift about +5‰ from the vegetal food source in collagen, and about +12‰ in apatite. Therefore, values for herbivores in C_3 ecosystems should be around -21‰ for collagen and around -14‰ in apatite. Pure C_4-feeders would show $\delta^{13}C$ values around -7‰ in collagen and around 0‰ in apatite. Going up the food chain, $\delta^{13}C$ values show a much smaller difference, not more than 2‰, so that this element is not the best tracer to quantify the importance of nutritional resources from different trophic levels for human diet. Human values for fully terrestrial C_3 ecosystems would typically be around -20‰ (collagen) and -12‰ (apatite), with differences related to the extent of their carnivory. Nitrogen represents the best choice for this purpose, since average differences between consumed and consumer are usually around 3–5‰. Nitrogen is fixed or absorbed by plants, and its values are also passed on up the food chain. Values for plants are therefore around $\delta^{15}N$ 0–4‰, and consequently herbivores' about 4–8‰ and carnivores' about 8–12‰.

Marine ecosystems have much longer food chains, so that the range of variation in $\delta^{15}N$ is much wider. Marine predators show values up to 20‰ in collagen, while fish signatures are generally higher than terrestrial animals *(10, 11)*. Marine $\delta^{13}C$ values also are often enriched, resulting in overlap with the C_4 plants range, so that the best way of setting them apart, and the standard way of presenting the values, is by means of a plot where $\delta^{13}C$ and $\delta^{15}N$ are the x and y coordinates. High $\delta^{13}C$ and low $\delta^{15}N$ indicates C_4 plant-based diets, while high $\delta^{13}C$ and high $\delta^{15}N$ indicates marine protein-based diets.

Lacustrine ecosystems, due to reservoir effects that are particular to individual water bodies, can show remarkable variation, potentially overlapping with both marine and terrestrial values. The reservoir effect applies to some extent also to smaller circumscribed seas such as the Mediterranean.

Stable Isotopes and Diet in Western Mediterranean Prehistory

If we are to correctly interpret the isotopic values in dietary terms in the context of western Mediterranean prehistory, we need to consider the specific resources available and especially those that archaeology has documented as being used as food items. Our focus is on later prehistory.

Food production and consumption between the 6th and the 2nd millennium B.C. is characterized by the spread of the Neolithic suite of domesticated animals and crops, which apparently were adopted in different tempos and combinations in different regional contexts *(24, 25)*. While some areas apparently relied on foraging until relatively late, others seem to have adopted quickly and thoroughly the Neolithic economic patterns. It is believed that in the Mediterranean reliance on fishing did not reach the same importance it did in the Atlantic and Baltic areas *(1)*, although the quality of the evidence is not homogeneous.

Domesticated animals and crops spread from the Near East, mostly through coastal routes along Greece, the Italian Peninsula on both the Tyrrhenian and Adriatic sides, and then down the coast of the Iberian Peninsula and the Corsican-Sardinian complex. Due to environmental factors, on the African side, Neolithicization took the form of a shift to pastoralism rather than agriculture. It seems that at several locations domesticated animals, namely sheep, goat, pig and cattle, were adopted before farming. The importance of agriculture was highly variable in the 6th and 5th millennia B.C., and became more generalized only later, while in some areas pastoralism possibly became more important starting in the 4th millennium. This trend may have been favored by climate change, and/or by the exploitation of secondary animal products such as milk and labor in the form of plow traction *(26, 27)*. The effects of these technological innovations, though, must have varied according to both environmental and cultural contexts.

According to the typical way of reading the values, it seems to be confirmed by isotopic analyses (Figure 1) that in the Late Neolithic through Bronze Age exploitation of seafood was not important. This is not surprising in inland sites in Neolithic Anatolia, such as Çatalhöyük *(28)*, but appears to be the case also in insular settings such as Bronze Age Crete. At Mycenae, only the high status individuals show values that indicate some consumption of marine food, and in Greece as a whole, an explicit comparison of inland and coastal sites spanning the entire Neolithic (late 7th to early 4th millennium B.C.) revealed that the protein component of the diet was relatively homogeneous and based on plants and animals, with no enriched values providing clear evidence for substantial fish intake *(29, 30)*. In the central-western Mediterranean, similar $\delta^{13}C$ and $\delta^{15}N$ values compatible with terrestrial C_3 protein have been documented in coastal environments such as the Maltese archipelago from around 4000 B.C. to the 3rd millennium B.C. *(31)*. This pattern does not change if we look at data from the

Balearic islands between the 4th millennium B.C. to the 1st century A.D. *(32, 33)*, and from several locations on the Italian peninsula dating to Neolithic through Copper Ages *(34)*. While this could be disguised by seafood values specific to the Mediterranean that are much more terrestrial-like *(35–37)*, the general conclusion is that the consumption of marine resources was negligible, and that, given the little range of isotopic values, it is impossible to assess the contribution of animal versus plant foods to the diet analyzing collagen alone.

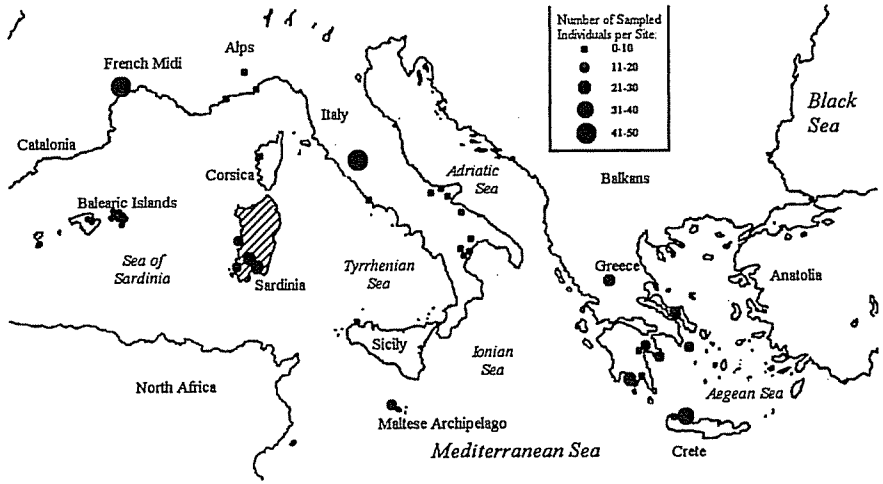

Figure 1. Map of the central-western Mediterranean showing the location of Sardinia and the prehistoric sites that have been analyzed for $\delta^{15}N$ and $\delta^{13}C$ in the whole area

Unlike East Asia, Africa and the Americas, where the presence of C_4 crops makes it easier to detect the beginnings and importance of agriculture, the cereals domesticated in the Near East and spread in the Mediterranean yield $\delta^{13}C$ and $\delta^{15}N$ values similar to wild plants. Since marine resources do not seem to represent a large component of the diet, the majority of western Mediterranean collagen values lie within $\delta^{15}N$ 9.0 ± 1.0‰ and $\delta^{13}C$ 19.3 ± 1.0‰.

Apatite values and the spacing between collagen and apatite are therefore very useful in addressing what becomes the main nutritional question in prehistoric Mediterranean diet and economy: the relative proportion of animal and vegetal foods. This corresponds to the importance of agriculture versus animal husbandry, with all the implications this holds in terms of ethnographically documented correlations involving society and culture. The

spacing between collagen and apatite $\delta^{13}C$ can be used, in fact, to 'triangulate' a dietary reconstruction with separate interpretations of collagen and apatite. Larger spacings have been found to correlate with herbivores, smaller spacings with carnivores *(21, 38)*.

Another way of improving the data is by considering environmental factors affecting the isotopic signature. This is particularly useful when a faunal and botanical isotopic baseline is not available. This is the best way of detecting ecosystem-wide shifts, which when comparing human groups can be mistaken for dietary change or variation. It is critical, therefore, to establish whenever possible a baseline that is site-specific, or at least represents the region during a given period. Analyses of faunal remains provide a good estimate both of the animals themselves and the plants they consume. Unfortunately, faunal remains are not always directly associated with human burials, and often there are no faunal remains from sites close enough in space and time, or for various reasons they are not accessible.

Case Studies on Sardinia

Sampling Criteria and Selected Populations

Skeletal collections were selected for sampling to address specific research questions as part of a larger project aimed at understanding variation between coastal and inland environments and change of economic practices over time. In this chapter we use the first results to make some interpretive considerations.

Samples were collected in summer 2003 and fall 2004 from the Ex GIL, a storage facility of the Soprintendenza in Sardara (province Medio Campidano, then Cagliari); in the Old Museum in Cagliari; and at the storage unit of the Dipartimento di Biologia Sperimentale, Sezione Antropologia, in Monserrato (province Cagliari), using a portable drill. Attention was paid to preserving as much as possible the integrity of the remains.

The large majority of skeletal remains from prehistoric Sardinia come from collective burials where skeletal elements were not articulated nor associated in any way deemed significant by the excavators. It was therefore necessary to select one skeletal element in order to insure that the same individuals were not being sampled more than once. Crania were found to be the best choice, since they hold the highest potential to identify the individual's sex, age and pathologies. While we know of different turnover rates in different skeletal elements and within the same element, the specific times have not been documented. Growth speed is believed to affect intra-bone variability mostly in

subadults, while by adult age isotopic values should be fairly similar across the skeleton. The possibility of substantial variation has been investigated in humans for $\delta^{15}N$ *(39)*, and no substantial variation has been found, so confirming its value for reconstructing diet change and weaning practices, which is being done routinely by comparing bone and tooth enamel, or through tooth enamel microsampling, less often with bone alone *(40)*. For this study, samples were removed from the cranial vault whenever possible, and in a few cases, from the mandible, maxilla or long bone, to ensure the homogeneity and overall comparability of averages between groups. Authorizations for removal and transport to the United States of America for preparation and analyses were granted according to the regulations, and prepared for analyses by mass spectrometry according to the procedures explained below.

The four sites span roughly 2500 years, between the early 4th and the mid-2nd millennium B.C. Geography and environment show some diversity and similarity (reference for chronology and geographic location are in Figure 2). San Benedetto *(41–43)* is a necropolis of tombs carved into the rock dating to the early part of the Late Neolithic; it is within one day's walk from the coast, but the very steep and rugged terrain in between makes it unlikely that a human group living nearby could have relied on marine food. The group shows very homogeneous morphology, so that the possibility of an endogamic community has been suggested.

Padru Jossu *(44–46)* is a rock-carved tomb, located inland on the outskirts of a wide alluvial plain where a lake was present until the 1800s. Two phases were sampled, dating to the Bell Beaker phase (2500–2200 B.C.) and to the Early Bronze Age (2200–1900 B.C.). Animal remains, interpreted as funerary offerings, were recovered; the relative species frequency in the two represented phases changes from a prevalence of sheep/goat to a higher quantity of cattle and pig specimens, which has been interpreted as an increase in the importance of agriculture as opposed to sheep tending.

Is Calitas *(47–49)* is a pit grave dating to the Early Bronze Age (2200–1900 B.C.) in an inland, hilly area near the coastal plain surrounding the Gulf of Cagliari. It yielded a few articulated skeletons and a large number of scattered bones, with associated grave goods including beads, pottery, stone and metal tools.

Is Aruttas is the only coastal site tested. Skeletal materials were recovered in a cave a few hundred meters from the shore, located in a marshy and sandy area on the central-west coast of Sardinia. The stratigraphic context was disrupted by looters, and pottery sherds found associated were attributed to the final phase of the Late Neolithic. One radiocarbon date, though, shifted the chronology to the Middle Bronze Age (1600–1300 B.C.). From the physical fitness and low caries of the recovered human remains, a well balanced diet has been inferred *(43, 50, 51)*.

NEOLITHIC	Middle	Bonu Ighinu	ca. 4700-4000 BC
	Late	Ozieri	ca. 4000-3200 BC
COPPER AGE	Early	Sub-Ozieri, Filigosa, Abealzu	ca. 3200-2700 BC
	Middle	Monte Claro	ca. 2700-2200 BC
	Late	Bell Beaker A	
BRONZE AGE	Early	Bell Beaker B/ Bonnannaro A	ca. 2200-1900 BC
	Middle	Bonnannaro B	ca. 1900-1600 BC
		Nuragic I	ca. 1600-1300 BC

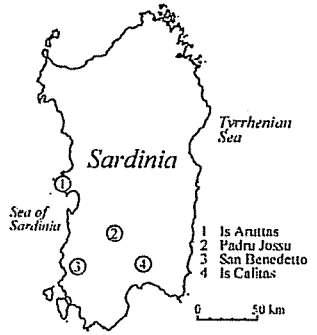

Figure 2. Left: Chronological table of Sardinian prehistory from the Middle Neolithic to the Middle Bronze Age, based on calibrated radiocarbon dates. Right: Map of Sardinia with the location of the four sites where the skeletal remains tested were recovered.

Sample Preparation

Approximately 1 gram of whole bone was selected per each individual, physically cleaned when needed, ultrasonically cleaned and dried. The preparation is a variation of well established procedures *(14, 52)*. After removal of 10 milligrams of bone powder by drilling and milling, collagen was isolated by demineralization with low-concentration HCl in two or more ~24-hour baths, depending on the necessity for more reaction. These baths were preceded and followed by soaking for ~24 hours in NaOH to remove humic acid contaminants. Finally, samples were treated with a 2:1:0.8 mixture of methanol, chloroform, and water to remove lipids, again for ~24 hours. The use of weak HCl (2%) allows the recovery of collagen even from bone that is physically degraded. Sample preservation and reliability can be visually assessed throughout the process, unlike during procedures that involve turning bone into powder *(6, 53)*. Collagen pseudomorphs are the end result; they are oven-dried and weighed to obtain yields. The reliability of collagen samples is commonly assessed using parameters, including the ratio of C:N in the collagen and the quantity of N and C in collagen *(52)*. C:N ratios are not available for this paper so we rely on visual assessment, collagen yields, and consistency of peak area ratios during stable isotopic analysis. Yields under 1% usually indicate poor preservation, and the likelihood that the remaining material may have become fractionated. On the other hand consistency of yields and no relationship between yields and isotopic values suggests that the signal is preserved. Atypical peak area ratios (relative to

the majority of other samples in a run) measured during isotopic analysis are also a sign of poor preservation. Measured collagen yields showed more variation between than within sites; there was no substantial stable isotopic difference within each site between lower- and higher-collagen yielding samples, which indicates that diagenesis did not affect isotopic values significantly.

To isolate the apatite, 10 mg of bone powder are treated with a ~72-hour bath in sodium hypochlorite which dissolves the organic portion; non-biogenic carbonate is removed by soaking the sample for ~24 hours in a 1M buffered acetic acid/sodium acetate solution, and attention is paid to be as consistent as possible with sample to solution quantity ratio and soaking times *(54)*. Bone apatite remains less accurate and reliable if compared to collagen and particularly to tooth enamel, because removal of carbonate derived from recrystallization of exogenous carbonates leaked from the soil matrix may not be complete *(55, 56)*. Nevertheless, the procedures used, and the assessment of the integrity of samples through the yields *(57)* measured after each preparation treatment can be used as an indication of reliability. It has been shown that bone mineral has the potential to retain original isotopic signatures, and it has been suggested that in certain conditions recrystallization can even favor this preservation, rather than contaminating the sample *(58)*.

As explained elsewhere *(6, 59)*, one milligram per sample of the end product of collagen preparation is placed in tin capsules and analyzed in continuous flow mode, using a Carlo-Erba 2500 Series II CHN analyzer coupled with a ThermoFinnigan Delta Plus XL stable isotope ratio mass spectrometer. As for the apatite samples, one milligram of purified powder is analyzed on another ThermoFinnigan Delta Plus XL mass spectrometer, in dual-inlet configuration, equipped with a Kiel III individual acid bath carbonate system. Both mass spectrometers are located at the University of South Florida, St. Petersburg campus. Samples of an isotopically known working lab standard (urea for collagen and Carrera marble for apatite samples) are included at the beginning, middle and end of each run of samples. Continuous Flow-IRMS precision (2σ) is typically better than ±0.3‰ for $\delta^{15}N$ and ±0.2‰ for $\delta^{13}C$. Dual-Inlet-IRMS precision (2σ) is typically better than ±0.04‰ for $\delta^{13}C$ and ±0.06‰ for $\delta^{18}O$.

Results

Average collagen values by site range between $\delta^{13}C‰ = -19.5$ at San Benedetto to −18.6 at Is Aruttas, and between $\delta^{15}N‰ = 9.4$ at San Benedetto and 10.8 at Padru Jossu phase A. Apatite $\delta^{13}C‰$ values are between −13.8 at San Benedetto and −10.4 at Is Calitas and Is Aruttas, while the $\delta^{18}O‰$ values are between −4.6 at Is Calitas and −1.2 at Is Aruttas (Figure 3).

Table I. Average Stable Isotopic Values for the Five Analyzed Groups

	Collagen			*Apatite*		
Sites/phases	*n*	$\delta^{13}C‰$	$\delta^{15}N‰$	*n*	$\delta^{13}C‰$	$\delta^{18}O‰$
San Benedetto	16	-19.5±0.2	9.4±0.5	16	-13.8±1.1	-4.3±0.3
Padru Jossu A	9	-18.8±0.2	10.8±0.8	9	-12.0±1.2	-3.2±0.7
Padru Jossu B	13	-18.9±0.4	10.1±0.9	13	-11.3±0.8	-2.6±0.6
Is Calitas	26	-19.2±0.4	10.4±0.9	29	-10.4±0.5	-4.6±0.4
Is Aruttas	11	-18.6±0.3	10.4±0.9	10	-10.4±2.6	-1.2±0.8

By plotting the collagen values with relative standard deviation, we can observe a large overlap. The most distinct group is San Benedetto, which for its more negative values of both $\delta^{13}C‰$ and $\delta^{15}N‰$ should be interpreted as the site with the lowest consumption of animal protein. Is Aruttas shows the most positive $\delta^{13}C‰$ values and fairly high $\delta^{15}N‰$. Since the site is on the coast, we would interpret this difference as due to a limited intake of marine foods, while at Padru Jossu A and Is Calitas this would be more compatible with higher consumption of food of terrestrial animal origin. We must emphasize, in addition to the overlap between the groups, the closeness of their signatures if considered within the larger picture. Figure 4 shows the reference values for the main categories of food for western Europe, and the range covered by Figure 3. Clearly, most protein from all groups came from terrestrial animals, and the seafood contribution may have been limited to none.

Apatite $\delta^{13}C‰$ values, as shown in Figure 5, have more distinct patterns. San Benedetto, the Late Neolithic group, stands out for its more negative values. The chart is again showing a parameter indicating the trophic level of the protein component (collagen $\delta^{15}N‰$), but this time plotted with the value resulting from the whole diet (apatite $\delta^{13}C‰$). The interpretation of apatite values and the resulting $\delta^{13}C‰$ spacing between collagen and apatite is complex and not fully understood *(22)*. One reason for enriched apatite $\delta^{13}C‰$ in ecosystems where C_4 crops are available and important is likely to be their substantial consumption. However, as we have discussed, this seems unlikely in the context of prehistoric Sardinia where all domesticated plants were C_3 until the Iron Age. Large differences cannot be attributed to heavy reliance on marine food, since this would be apparent from collagen as well. Values are indeed consistent with data from several locations in Italy *(34)*. Among possible alternative factors is a substantial difference in lipid content in the diet, and/or a difference in lipid origin, and/or a difference in carbohydrate relative quantities. However, discussing this aspect would require a separate paper and will not be dealt with here. The point we want to highlight is instead the importance of environmental variation in the interpretation of tightly clustered isotopic values, which we discuss below.

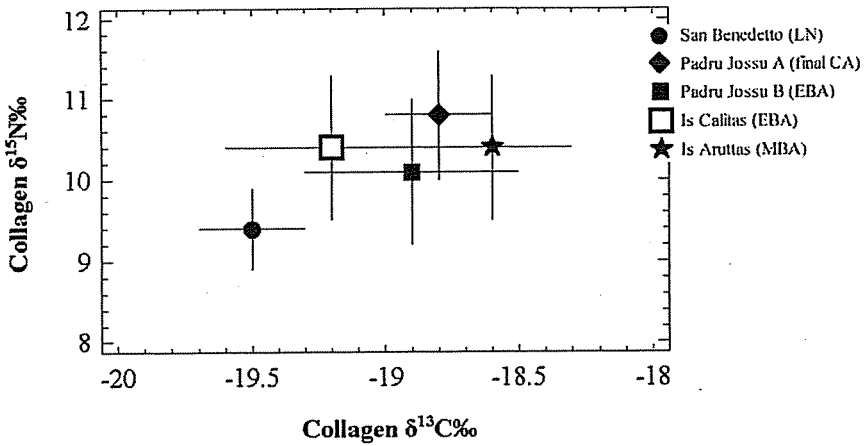

Figure 3.Plot of average collagen $\delta^{15}N$ and $\delta^{13}C$ values for the five human groups analyzed, with standard deviation.

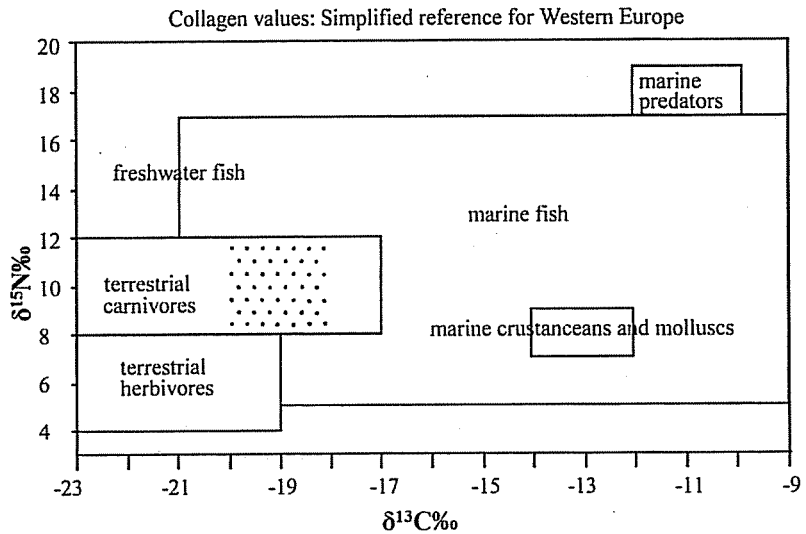

Figure 4 .Plot of reference collagen $\delta^{15}N$ and $\delta^{13}C$ values for western Europe. The range of the plot in Figure 3 is indicated by the dotted texture.

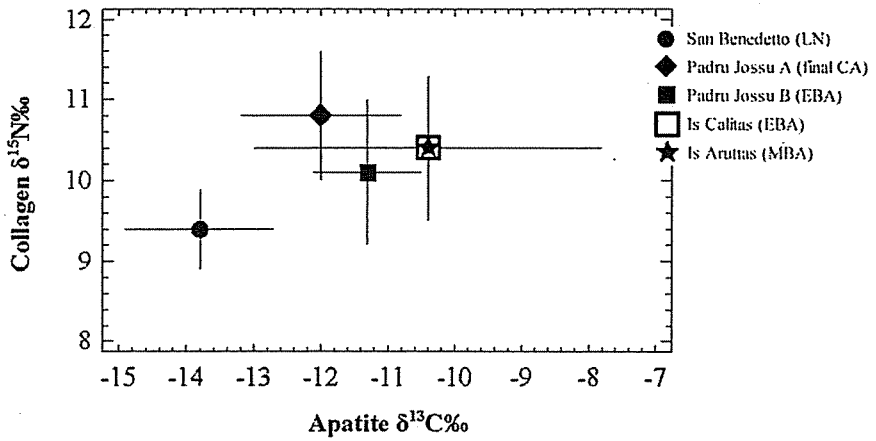

Figure 5. Plot of average collagen $\delta^{15}N$ and apatite $\delta^{13}C$ values for the five human groups analyzed, with standard deviation.

Discussion

It is well established that isotopic values are affected by climate in direct or indirect ways. There are also studies concerning the importance of environmental variation over time and space in interpreting dietary information. Variation in $\delta^{13}C$ in wood charcoal has been found to correlate to elevation within a given region *(14)*. Variation in $\delta^{13}C$ according to climate during the Holocene has been specifically investigated for wide areas from Northwestern Europe to the Near East and North Africa *(60)*. This has demonstrated a clear regional variation, in some cases stronger than temporal variation. When comparing Spain with northern European countries during the entire Holocene, isotopic values were consistently less negative in the former than in the latter, with no overlap for the last 10,000 years. Fluctuations over time corresponded across the studied area, reflecting continent-wide climatic phenomena *(60, 61)*.

Similar variation in $\delta^{13}C$, observed commonly in cave sediments and routinely used along with $\delta^{18}O$ for paleoclimatic reconstruction, has been studied for horse bone collagen and tooth enamel on a larger chronological scale, and found to correlate with climatic trends in the last 40,000 years. It has been argued that this variation may stem from different phenomena: changes in atmospheric CO_2 concentration, and/or secondary effects due to $\delta^{13}C$ depletion in forested environments *(62, 63)*. Regardless of the cause, these isotopic differences would be maintained through the food chain from plants to herbivores (and omnivores), and from herbivores to carnivores (and omnivores).

Studies of isotopic fractionation in several plant species have demonstrated its correlation with water availability *(64–66)*.

Concerning the correlation of $\delta^{15}N$ with climate, there have been observations on specific areas, where an inverse relationship with altitude was documented *(67)*. In the last decade, a substantial body of evidence on $\delta^{15}N$ change over time has being collected, and its relationship with climatic conditions is becoming clear *(62)*. More specifically, the dataset considered by Schwarcz and coworkers is used to explore the correlation with precipitation, which appears to be inverse and statistically strong *(68)*.

What is most important is that all these ecosystem-wide shifts are transferred from the lower trophic levels up to the several groups of consumers. Considering collagen $\delta^{15}N$ and the case of our Sardinian dataset, if we assume an identical diet we should nevertheless expect some degree of variation according to precipitation. This, from a synchronic perspective, involves altitude and geomorphology. Based on present-day rainfall, which is ~750 mm/year at San Benedetto and ~550 mm/year at the remaining sites, if we apply an approximate ratio of ~$\delta^{15}N$ 1‰ per 100 mm/year of rain, dietary interpretation changes substantially. The San Benedetto group, falling above the line describing climatic variation, would show higher consumption of animal protein. On the other hand, $\delta^{15}N$ values for Padru Jossu, Is Calitas and Is Aruttas can be explained by climatic variation alone (Figure 6).

The problem is that we are still dealing with small isotopic differences. Since the groups span over 2,000 years we must also consider climate change. Among the main stable isotopes, $\delta^{18}O$ is the most commonly used for paleoclimatic reconstruction. Its correlation to both temperature and precipitation has been long established. A correlation between biogenic $\delta^{18}O$ in water-dependent mammals and meteoric water $\delta^{18}O$ has been documented *(69, 70)*, so we assume that the isotopic signature should be preserved from the atmosphere to bone carbonate and phosphate, passing through rainwater and drinking water. The relationship itself between $\delta^{18}O$ with $\delta^{13}C$ has been detected in tree leaves *(71, 72)*; in horse teeth through microsampling, which has shown remarkable seasonal covariation *(73)*; and in lacustrine environment on molluscs *(74)*.

Isotopic signatures of groundwater and water reservoirs may alter the $\delta^{18}O$ values of rainwater, and the physiology of different species can be very complex and often not comparable. In addition, their $\delta^{18}O$ signature is derived through different pathways, whereas humans derive the majority of it from drinking water. Despite these specific mechanisms, what is important is the general ecosystem-wide variation. With this premise, and under the condition derived from archaeological evidence that diet at Sardinian prehistoric sites was based on the same resource pool that included C_3 plants and animals but no C_4 plants or marine resources, we can plot collagen $\delta^{13}C$, collagen $\delta^{15}N$ and apatite $\delta^{13}C$ against apatite $\delta^{18}O$ as an environmental proxy, isolating the real dietary signal.

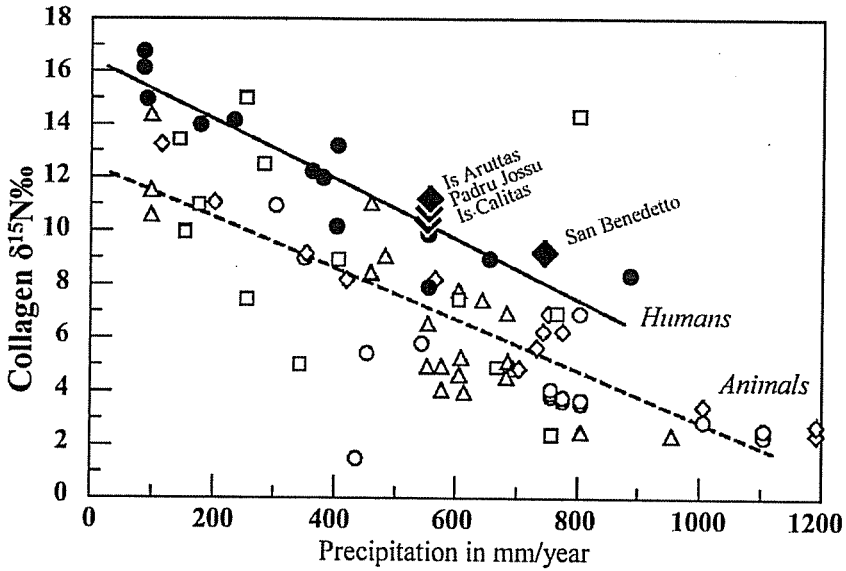

Figure 6. Plot of average human (filled circles) and animal (empty symbols) collagen $\delta^{15}N$ and precipitation values from several sites across the world reprinted after Schwarcz and coworkers (68), Fig. 1, p. 630, with permission from Elsevier. Average values from Sardinia (filled diamonds) have been added to the original plot according to present-day precipitation.

Plotting apatite $\delta^{18}O$ and collagen $\delta^{15}N$ allows us to visually appreciate how much variation is due to climatic factors and by comparison how much is due to diet (Figure 7). The line in the chart is not a regression line, but indicates only the slope of the covariation between $\delta^{15}N$ and $\delta^{18}O$ related to rainfall. Its purpose is to allow a comparison among sites. It is obtained by combining two correlations to precipitation. One is the correlation between collagen $\delta^{15}N$ and precipitation measured by Schwarcz and coworkers *(68)* estimated at ~1‰/100 mm/year. The other one is the correlation between $\delta^{18}O$ and precipitation by Bar-Matthews and coworkers, which is measured on non-human, biogenic carbonates *(75)* estimated by the authors to ~1‰/200 mm/year. This results in a slope of $\delta^{15}N$ ~2‰ = $\delta^{18}O$ ~1‰ which signifies the covariation between $\delta^{15}N$ and $\delta^{18}O$ that is related to climate. The dietary difference between two groups would correspond to the distance from any given slope line. Interpreting the data in this manner, the human group at San Benedetto appears to have roughly as much animal protein in the diet as Padru Jossu A, both having the same distance to the climatic regression line. The values of Is Calitas show enrichment,

reflecting a higher animal protein proportion, while those of Padru Jossu B and particularly Is Aruttas are the lowest amount of all groups.

Plotting collagen $\delta^{13}C$ against apatite $\delta^{18}O$ should again set apart the signatures due to dietary protein and climatic variation (Figure 8). The values appear to be linearly correlated and they indicate a small difference in the intake of animal protein. In this case we can read a trophic level effect from $\delta^{13}C$ values by comparing Is Calitas to San Benedetto and Padru Jossu A to Padru Jossu B. The first of each couple shows less animal protein consumption than the second, which is in agreement with the $\delta^{15}N$ interpretation.

Finally, plotting apatite $\delta^{13}C$ against apatite $\delta^{18}O$ (Figure 9) shows a strong linear correlation between the two coordinates, except for the Is Calitas group, which stands out for its enriched apatite $\delta^{13}C$. Since apatite reflects the whole diet, this comparison would lead to the interpretation that this human group is the only one with a substantial dietary distance from the rest. As discussed above, the meaning of apatite values is complex due to its mirroring the signature of all macronutrients, and it can have multiple causes. In accordance with the previous plots, we could argue that a high consumption of animal protein, which is isotopically heavier than plant carbohydrates (~-21‰ vs. ~-25‰), could explain the less negative $\delta^{13}C$. The common explanation that connects smaller $\delta^{13}C$ collagen-apatite spacing to the amount of consumed lipids would in this case contradict the high quantity of animal protein apparent in the previous plots.

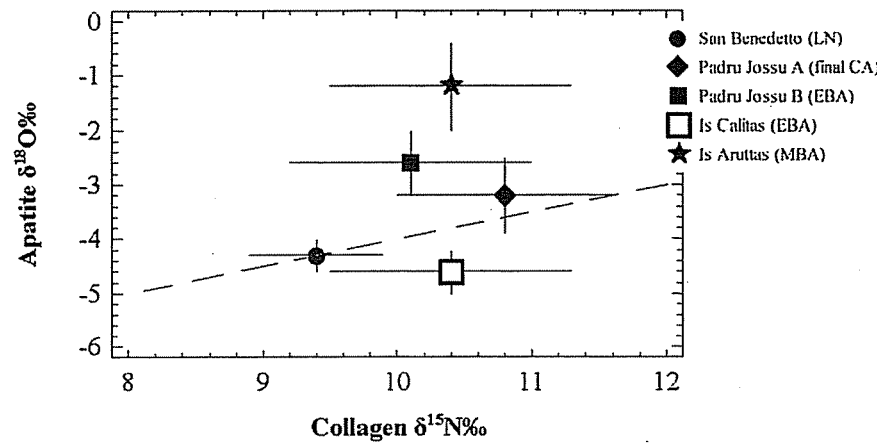

Figure 7. Plot of average collagen $\delta^{15}N$ and apatite $\delta^{18}O$. The regression line is the slope obtained from a rough approximation of the correlation between precipitation and both $\delta^{15}N$ and $\delta^{18}O$. Distance from the line would correspond to dietary variation, with more animal protein to the right and less to the left. As an example, at San Benedetto this quantity would be less than at Is Calitas.

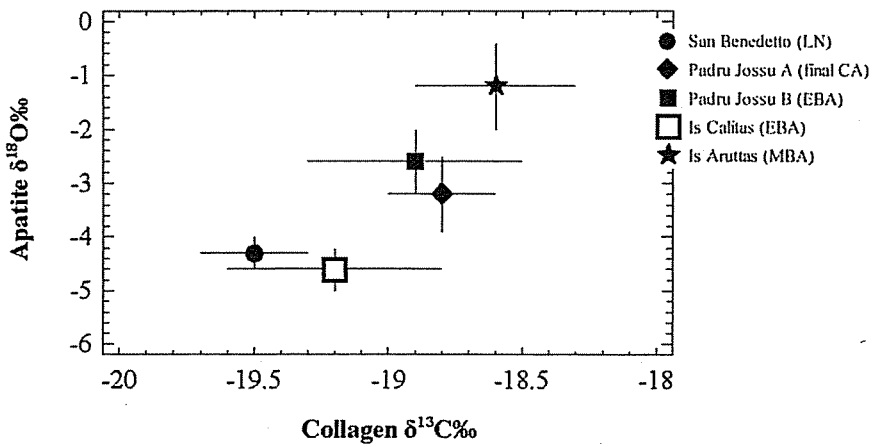

Figure 8. Plot of average collagen $\delta^{13}C$ and apatite $\delta^{18}O$. Assuming the alignment indicates environmentally-related covariation of the two coordinates, $\delta^{13}C$ values that are less negative relative to the alignment would indicate higher consumption of animal protein.

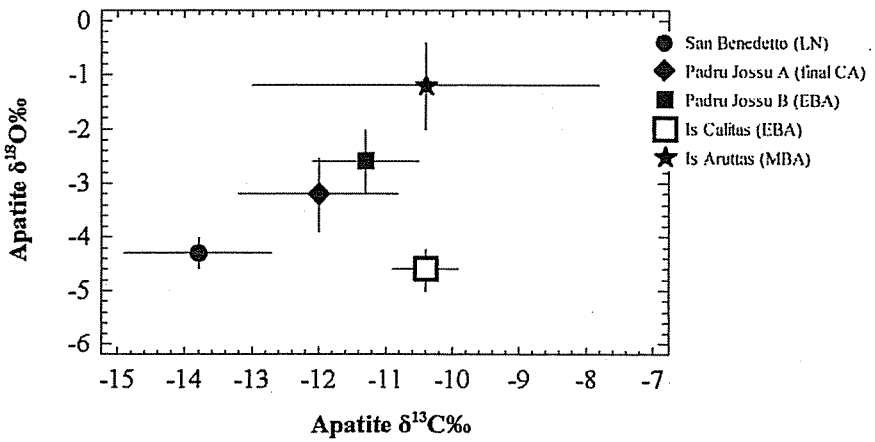

Figure 9. Plot of average apatite $\delta^{13}C$ and $\delta^{18}O$. Assuming that the alignment indicates environmentally-related covariation of the two coordinates as for Figure 8, $\delta^{13}C$ values that are less negative relative to the alignment would indicate dietary difference. In this case, since the plotted dietary indicator is apatite $\delta^{13}C$, the difference involves the whole diet. The group clearly apart would therefore be Is Calitas.

This procedure is to be considered an exploration of alternative directions to detect shifts of climatic origin affecting whole ecosystems and get closer to the actual dietary information, when animal and plant reference values from the same context are not available. We emphasize that the most direct and reliable method remains the isotopic analysis of faunal and botanical remains from the same context, since other factors like the isotopic signature of groundwater and water reservoirs may alter the $\delta^{18}O$ values and therefore the correlation between $\delta^{18}O$, $\delta^{13}C$ and $\delta^{15}N$.

Conclusions

In order to reconstruct human diet from bone tissue, direct isotopic analysis of animal and plant remains from the same archaeological context is the most reliable way to detect isotopic shifts involving the whole ecosystem due to environmental variation. Since this is often impossible for the lack of these control samples, we have explored the use of $\delta^{18}O$ to assess the environmentally induced variation in $\delta^{13}C$ and $\delta^{15}N$ values from collagen and apatite, and assess the dietary information they represent. This can be done assuming a scarce nutritional role of marine resources and the absence of C_4 crops, as seems to be the case in the western Mediterranean and specifically in the Sardinian Neolithic and Bronze Age.

After reconstructing diet by means of plotting collagen $\delta^{13}C$, collagen $\delta^{15}N$ and apatite $\delta^{13}C$ with each other, we plotted each with $\delta^{18}O$. Since all of them partially depend on climatic factors, while $\delta^{18}O$ should reflect climate more faithfully, we would expect a straight line if diet is identical for all groups. Values that are not along this line are interpreted as depending on differences in diet.

From the collagen values we assessed that the protein component of the diet was mainly supplied by terrestrial animal sources among all the populations and periods. Moreover, marine resources were not nutritionally as important, confirming the previous data available for the central and western Mediterranean. Comparing the five groups analyzed so far, San Benedetto appears to reflect the lowest animal protein consumption, Padru Jossu A the highest, and Is Aruttas possible limited consumption of seafood. The apatite $\delta^{13}C$ values showed a more sensible difference between San Benedetto and the remaining sites, to be attributed to more plant carbohydrates and/or lipid consumption.

Interpreting the plots of collagen $\delta^{15}N$, collagen $\delta^{13}C$ and apatite $\delta^{13}C$ with $\delta^{18}O$ leads us to more accurate reconstructions: it seems that the San Benedetto, Padru Jossu B and Is Aruttas groups all had a lower average consumption of animal protein than the Padru Jossu A and Is Calitas groups. Is Aruttas values are not indicative of any animal protein intake (whether terrestrial or marine).

higher than the remaining sites, and may instead have been the group with the highest consumption of plant protein. Is Calitas shows the highest consumption of animal products, which affects visibly even the apatite signature when plotted against $\delta^{18}O$.

Drawing conclusions of paleoeconomy requires the integration of several other lines of evidence, which is not in the scope of this paper. Based on the comprehensive interpretation of the isotopic results presented, the Late Neolithic group (San Benedetto), the Early Bronze Age group at Padru Jossu (B), and the Middle Bronze Age group (Is Aruttas) seem to have relied more on farming, while the Late Copper Age group (Padru Jossu A) and the Early Bronze Age group at Is Calitas emphasized reliance on animal husbandry. More specifically, San Benedetto seems to be the most agricultural, and Is Calitas seems to be the most pastoral group. This interpretation is in line with the expected dietary and economic change over time in Sardinian prehistory.

To test these conclusions from both a paleoeconomic and a methodological perspective, analyses of faunal and human samples are critical. As we have highlighted throughout our discussion, documenting isotopic variation over the different trophic levels of whole ecosystems is and remains the best way to reconstruct dietary variation reliably. We suggest the possibility that, in the specific ecological and cultural context of late western Mediterranean prehistory, using $\delta^{18}O$ helps us to disentangle dietary and environmental signature and therefore detect more faithfully nutritional and economic practices.

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Chapter 7

Bitumen in Neolithic Iran: Biomolecular and Isotopic Evidence

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This paper presents the results of the chemical analysis of materials recovered from two of the earliest agricultural villages in southwestern Iran and a late Neolithic pastoral encampment in nearby Khuzistan. Gas chromatography - mass spectrometry (GC-MS) revealed biomarker compounds characteristic of bitumen in residues from ceramic vessels supporting the excavators' contention that the interior surfaces of some vessels were coated with a thin layer of such material and confirmed that 'fragments' collected during excavation were indeed bitumen. Biomolecular and isotopic (δD and δ^{13}C) analysis of the bitumen indicated that the sources utilized lie in the Susa and Deh Luran regions of southwestern Iran.