Chapter 10

An isotopic study of palaeodiet at the Circle and the Xemxija tombs

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10.1. Introduction

A key aim of the *FRAGSUS Project* was to improve understanding of the subsistence patterns and changes of the ancient residents of the Maltese Islands. As detailed in the introduction of Volume 1, food supply and dietary health were identified as important aspects of the sustainability of the prehistoric community. The goal has been to elucidate the relationship between the remarkable prehistoric cultures of the Maltese Islands and the landscape upon which human life depended, especially over two millennia of unstable environmental conditions. It is the notion of time that is particularly significant in this study, and the *FRAGSUS Project* has focused particular interest on dietary changes associated with the degradation of the island's soils, which was a natural process, but one likely accelerated with the pressures of intensifying agricultural practices (Volume 1, Chapters 2 and 3). In this chapter, we present an analysis of the palaeodietary inferences that can be drawn from stable isotopic analysis of human and animal tissue.

10.2. Background: previous work

Richards *et al.* (2001) measured carbon and nitrogen isotopic values in collagen samples from two individuals from the rock-cut tomb at the Circle, and five from the cave system. They concluded that there was no significant input of marine protein, nor any detectable dietary differences between the two time periods, and that 'Neolithic'-style agriculture was the subsistence base for the population. Lai, O'Connell and Tykot, reporting on their results in Stoddart *et al.* (2009a), expanded upon this initial study with carbon and nitrogen isotopic analyses of 24 individuals made in tandem with AMS radiocarbon dates, and four additional measurements of $\delta^{13}C_{coll}$, $\delta^{15}N_{coll}$, $\delta^{13}C_{apa}$ and $\delta^{18}O$ from bioapatite, of which the latter is a climatic and demographic proxy (discussed in Chapter 9). Their results confirmed the findings of Richards *et al.* (2001), and highlighted the rather substantial dietary differences between individuals from the site. They also detected a trend of decreasing collagen carbon isotopic values over time, although the significance of this trend could not be assessed given the relatively small number of samples. The lack of comparative faunal data to provide a baseline for the human isotope measurements hampered the ability of these earlier studies to quantify the relative importance of meat in the diet, and to exclude fully the possibility of some limited amount of fish.

10.3. Food sources in prehistoric Malta

Recent excavations by the *FRAGSUS Project* at prehistoric sites in Malta have unearthed a wealth of new archaeobotanical and faunal data. These consisted of assemblages constituting the traditional Neolithic 'package': wheat, barley, lentil and pea, and cattle, ovicaprines (sheep and goat, largely indistinguishable from each other) and pigs. Barley and wheat seemed to have been the most important food crops and ovicaprines were by far the most commonplace animal (see Volume 2, Chapter 9) and their slaughter patterns are consistent with a dairy economy.

Fish bones were only encountered sporadically in the faunal remains from prehistoric Malta, although shellfish (particularly limpets) are very commonplace, albeit in low numbers. This suggests that they were a regular part of the diet, but had limited significance in terms of their calorific value. The lack of fish bones on the archaeological sites, which seems surprising for an archipelago such as Malta, does not exclude

Table 10.1. *Comparative terrestrial faunal isotope data from the Maltese Islands.*

Code	$\delta^{13}C_{coll}$	$\delta^{15}N_{coll}$	C:N ratio	Site	Species	Island	Period	Reference
UBA-37689	-20.8	5.8	3.20	Santa Verna	*Bos*	Gozo	Skorba	This study
UBA-37665	-20.6	6.7	3.24	Kordin III	*Ovis*	Malta	Mġarr	This study
UBA-37669	-20.5	6.9	3.23	Kordin III	*Ovis*	Malta	Mġarr	This study
UBA-29833	-20.6	11.1	3.23	Taċ-Ċawla	Ovicaprine	Gozo	Ġgantija	This study
UBA-37681	-20.5	8.5	3.22	Taċ-Ċawla	*Bos*	Gozo	Ġgantija	This study
UBA-29835	-21	6.7	3.22	Taċ-Ċawla	*Bos*	Gozo	Tarxien	This study
UBA-29836	-20.1	7.1	3.22	Taċ-Ċawla	*Sus*	Gozo	Tarxien	This study
UBA-31711	-19.5	6.8	3.23	Taċ-Ċawla	*Sus*	Gozo	Tarxien	This study
UBA-37683	-21.1	6.9	3.23	Taċ-Ċawla	*Ovis*	Gozo	Tarxien	This study
OxA-27687	-20.6	6.7	3.3	The Circle (Context 714)	Ovicaprine	Gozo	Tarxien	Malone *et al.* 2009d
UBA-10385	-21.7	4.23	3.1	The Circle (Context 1206)	*Bos*	Gozo	Tarxien	Malone *et al.* 2009d

Table 10.2. *Comparative stable isotope measurements from ancient fish bone collagen from the Mediterranean region.*

Species	Site	$\delta^{15}N_{coll}$ (‰)	$\delta^{13}C_{coll}$ (‰)	Period	Reference
Sparus sp.	Santa Maira (Spain)	8.6	-15.2	Mesolithic	Salazar-Garcia *et al.* 2014
Mugil sp.	Santa Maira (Spain)	8.5	-15.2	Mesolithic	Salazar-Garcia *et al.* 2014
Sparidae (n=5)	Pompei (Italy)	6.7 ± 1.1	-14.6 ± 0.6	Roman period	Craig *et al.* 2013
Epinephelus marginatus	Cova des Riuets (Formentera)	10.1	-10.5	Bronze Age	Garcia-Guixé *et al.* 2010
Pagellus erythrinus	Cova des Riuets (Formentera)	8.2	-11	Bronze age	Garcia-Guixé *et al.* 2010
Sphyraena sphyraena	Cova des Riuets (Formentera)	9.4	-12.4	Bronze age	Garcia-Guixé *et al.* 2010

the possibility that they were an important part of the diet. Fish could have been consumed differently from terrestrial animals and their bones do have different taphonomic properties; together, these factors could render them less visible in the archaeological record. Therefore, a key objective of the stable isotope study was to clarify the stable isotope results and allow for an independent check on whether marine protein featured prominently in the diets of those buried at the Circle.

Measurements of carbon and nitrogen isotope ratios from animal bones, taken in tandem with the *FRAGSUS Project*'s programme of radiocarbon dating, provide a baseline for interpreting the results from human tissue (Tables 10.1 and 10.2).

10.3.1. Tooth enamel samples

Enamel surfaces were first cleaned by ablation prior to sampling. Approximately 6 to 10 mg of enamel were sampled by drilling enamel powder using a hand-held drill with a diamond-tipped drill bit. Sampling was performed in a vertical line along the crown. Enamel powder was pre-treated following Balasse (2002); samples were treated with 2–3% aqueous sodium hypochlorite (0.1 ml/mg) for 24 hrs at 4°C and then rinsed. They were then treated with 0.1M acetic acid (0.1 ml/mg) for four hours at room temperature, rinsed again and freeze-dried. Four modern horse dental standards were treated and analysed in the same manner to provide a control. The samples were analysed using an automated gas bench interfaced with a Thermo Finnigan MAT253 isotope-ratio mass spectrometer at the University of Cambridge. Carbon and oxygen isotopic ratios were measured on the delta scale against the international standard VPDB scales. Analytical error for this instrument has been recorded as less than 0.10‰ for oxygen and 0.08‰ for carbon.

10.3.2. Bone collagen

The samples of collagen in the current study were extracted from the roots of human molar teeth using the method described by Reimer *et al.* (2015). All samples were also ^{14}C dated using AMS (Chapter 3); the isotope results discussed here were obtained on a separate line using IRMS at the 14CHRONO centre at Queen's University Belfast. Results are reported as delta values relative to the VPDB (carbon) and AIR (nitrogen) scales. IRMS machine uncertainties are sig$\delta^{13}C$=0.22, sig$\delta^{15}N$=0.15.

10.4. Data analysis

The data were first analysed using standard tools in the R environment for statistical computing, which was used to calculate summary statistics for various groupings of the data, and to project the data into their principal components. To study trends in palaeodiet with time, linear regression models were applied using palaeodietary isotope results and their associated radiocarbon dates (Chapter 3). However, because of the radiocarbon history of the atmosphere, radiocarbon measurements have a complex probability structure once they have been 'calibrated' into calendar time and cannot be reduced to a reliable point estimate for inclusion in a linear model. To circumvent this natural limitation of the radiocarbon technique, we incorporated radiocarbon uncertainty by bootstrapping 500 individual linear regression models, each built from repeated 'Monte Carlo' samples drawn from the probability density functions of each radiocarbon date. The IntCal13 (Reimer *et al.* 2013) calibration dataset was used to calculate these using ***rowcal*** (McLaughlin 2019). The resulting set of 500 linear models were used to estimate a plausible range of correlation coefficients and R-squared values, using the mean and standard deviation of these statistics for each model. In this way we could assess the significance of any temporal trend apparent in the data. This is a new method for extracting information from paired radiocarbon / stable isotope samples developed for this project.

We also explored a Bayesian mixing model as a first step towards interpreting these results in the context of what is known about the isotopic content of dietary sources, and how these are routed through the food web. This approach depends on prior information about both the isotopic content of the foods people were eating, and how it was metabolized by their bodies. This exercise can only be considered preliminary, as data for dietary sources are still scant. We now have a reasonably good set of data for prehistoric Maltese terrestrial animals, measured as part of this study, although their results were rather variable. Unfortunately, few direct measurements of terrestrial plants and marine animals have been made (although see below, promising studies have emerged around lipid residue characterization in prehistoric pottery (Debone Spiteri and Craig, 2011)). The lack of suitable material from our excavations undertaken by *FRAGSUS* and at the Circle, namely the small size and sparse quantity of the charred seeds, meant priority was given to securing an AMS date in preference to stable isotope measurements. Without a direct AMS date, any stable isotope measurements of charred seeds would not be a reliable indicator of conditions in prehistoric Malta because we could not be sure about their chronology. This situation is a result of the poor stratigraphic integrity of seeds in the rather loose soil profiles and the large amount of intrusive material that results. Indeed, we have detected significant quantities of this material in the sites excavated by the *FRAGSUS Project* in Malta and Gozo (see Volume 2, Chapter 9).

For the purposes of modelling food sources, we considered a 'concentration-independent' estimate of dietary protein, where the quantity of protein in the bulk of the source food is not considered during the mixture modelling process. This was done using the Bayesian mixing model simmr 0.4.5 (Parnell 2021), which was used to infer the proportions of food sources represented by the stable isotope data. Offsets for the enrichment of source isotopes took the values of 0.8±0.5‰ for ^{13}C and 4±1.0‰ for ^{15}N (cf Knipper *et al.* 2020; Styring *et al.* 2017 Hedges & Reynard 2007). For the central Mediterranean, the relatively small number of published source values for marine foods inevitably limits the scope of this exercise, as the bones of fish and marine mammals tend to be rare and poorly preserved on prehistoric sites. A review of the available literature illustrates how variable the isotope values from fish were, so for the purposes of this preliminary assessment a figure of 8.4±2.1‰ $\delta^{15}N_{coll}$ and -13.5±2.1‰ $\delta^{13}C_{coll}$ was used for fish (based on Table 10.3), 7±1.5 $\delta^{15}N_{coll}$ and -20.6±0.6‰ $\delta^{13}C_{coll}$ for terrestrial herbivores (this study), and 4.5±2.5‰ $\delta^{15}N_{coll}$ and -23.5±2‰ $\delta^{13}C_{coll}$ for terrestrial plants (based on averaging results from Knipper *et al.* 2020 & Vaiglova *et al.* 2014). The potentially complicating factor of C_4 pathway plants does not apply in Malta in the Temple Period (see Volume 2, Chapter 9).

10.5. Results

The average (mean, standard deviation) results for each site are shown in Table 10.3. Full details of each sample are given in Appendix Tables A2.3 & A2.4 and visualized in Figure 10.1.

Table 10.3. *Summary results for each site. A full list detailing the results from each sample appears in Appendix Tables A2.3 & A2.4. *Note that not all isotope measurements were made for each element – see Appendix Table A2.3. Also note that at the Circle the minimum number of individuals in the isotope study is less than this figure.*

Site	N elements*	Mean $\delta^{13}C_{coll}$ (‰)	S.d.	Mean $\delta^{15}N_{coll}$l (‰)	S.d.	Mean $\delta^{13}C_{apa}$ (‰)	S.d.
The Circle rock-cut tomb	18	-19.3	0.29	10.4	1.59	-12.5	0.88
The Circle main cave system	206	-19.3	0.36	10.7	0.98	-13.2	2.29
Xemxija	5	-19.3	0.23	10.3	0.26	-13.7	0.67

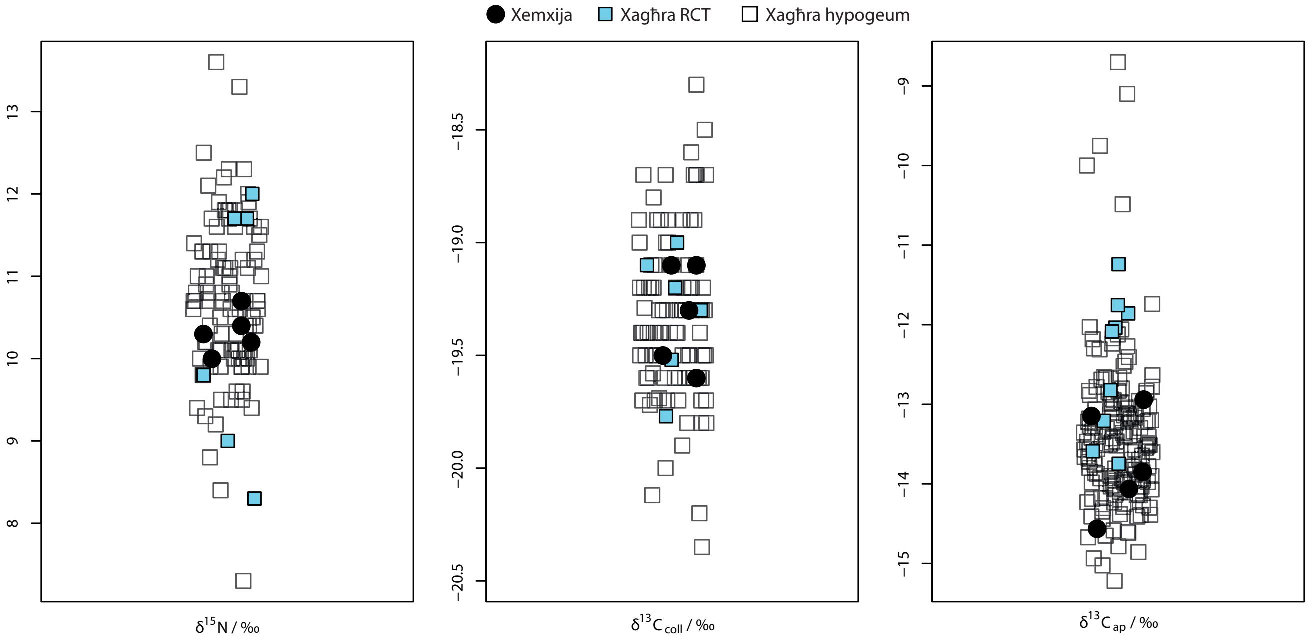


Figure 10.1. *Stripcharts indicating the range and distribution of carbon and nitrogen isotopes in collagen from tooth dentine, and tooth enamel bioapatite carbon isotopic values at the Circle and Xemxija. The x-axis is random jitter allowing the variance of the data to be visualized.*

In Figure 10.2 the results from collagen samples are visualized and compared with faunal samples from the Maltese Islands, and other human populations in the wider central Mediterranean region. The results indicate that both the Circle and the Xemxija individuals are consistently more enriched in nitrogen-15 compared with individuals from peninsular Italy, or indeed from neighbouring Sicily. One individual, identified as UB-10375 from Context (714) could be read as a recent immigrant to Malta as their diet is atypical, or a simply a statistical outlier as their isotopic measurements were made during earlier work with less refined laboratory protocols.

The offsets between the δ^{15}N$_{\text{coll}}$ and δ^{13}C$_{\text{coll}}$ animal mean values and the means of the three human groups show that the diet was consistent between the

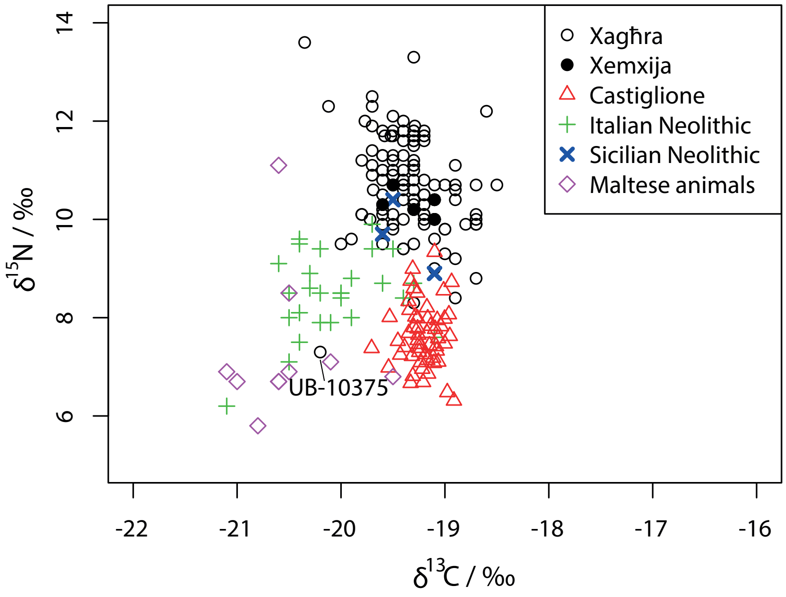


Figure 10.2. *Biplot of bone collagen carbon and nitrogen isotopic values from Circle and Xemxija, plotted with comparative data for the Neolithic of peninsular Italy and prehistoric Sicily, and Castiglione, an early Bronze Age site in southern Sicily (Varalli* et al. *2014).*

Table 10.4 *Offsets between the $\delta^{15}N_{coll}$ and $\delta^{13}C_{coll}$ human and animal values.*

Site	$\Delta^{13}C_{human-animal}$ (‰)	$\Delta^{15}N_{human-animal}$ (‰)
Circle (rock-cut tomb)	1.3	3.4
Circle main cave / hypogeum	1.3	3.7
Xemxija	1.3	3.3

rock-cut tomb and the main Tarxien phase burials, and also between Xagħra and Xemxija. These offsets (Table 10.4) are consistent with a mixed diet based on terrestrial animals and plant protein. The importance of animals in particular is suggested by the degree that the offsets fall with the established range of values that indicate a step to higher trophic levels (Minagawa and Wada 1984; Schoeninger & DeNiro 1984; Bocherens & Drucker 2003).

To investigate this in more detail, the results of our preliminary attempt at Bayesian mixture modelling of source foods are shown in Figure 10.3. The results clearly indicate a small but significant contribution from marine protein, as well as the likelihood that animal foods were the dominant sources of protein in the palaeodiet at Xagħra. The same pattern holds for the individuals from Xemxija and indeed for the earlier rock-cut tomb at Xagħra, although with less certainty because of the smaller sample size.

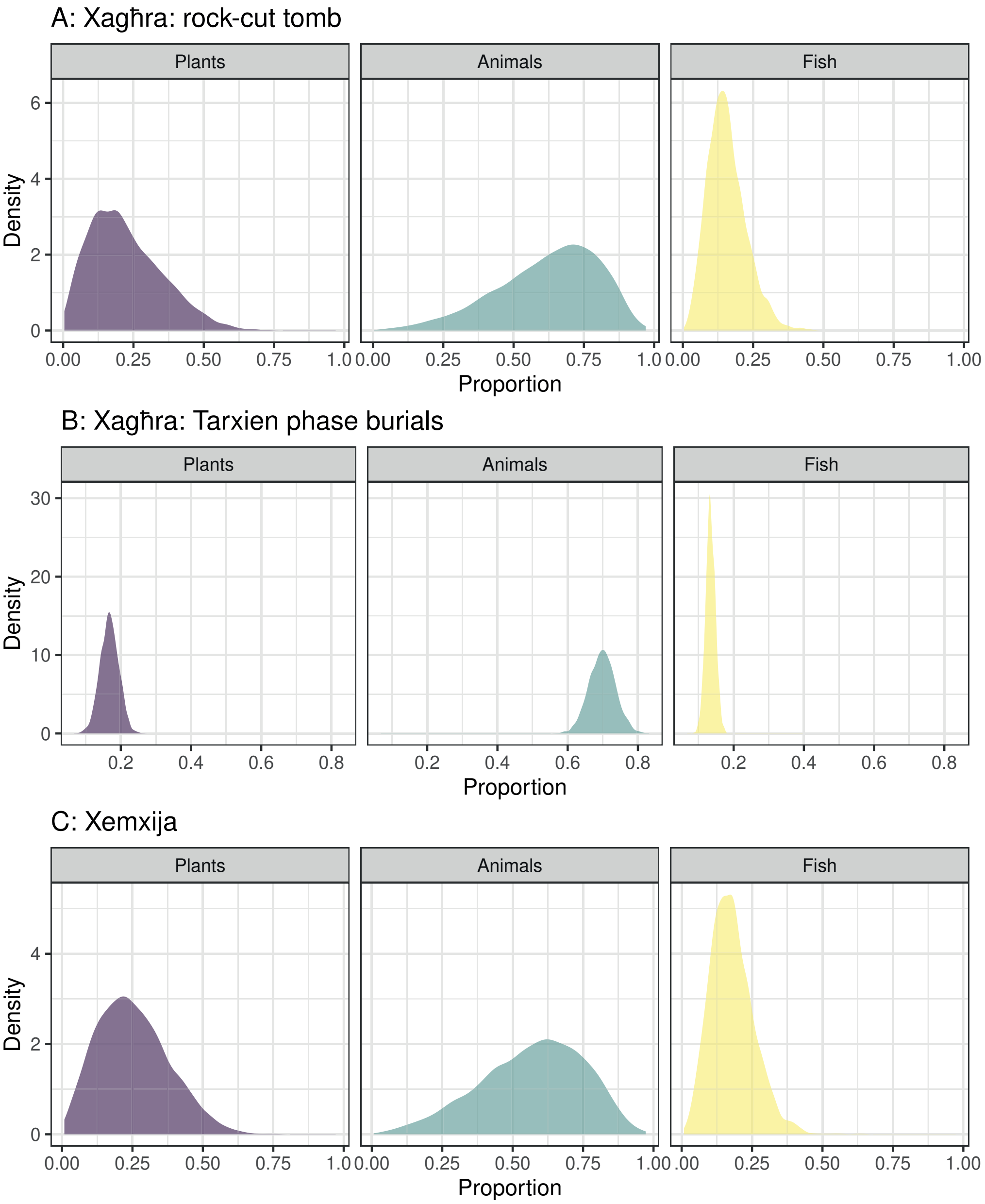


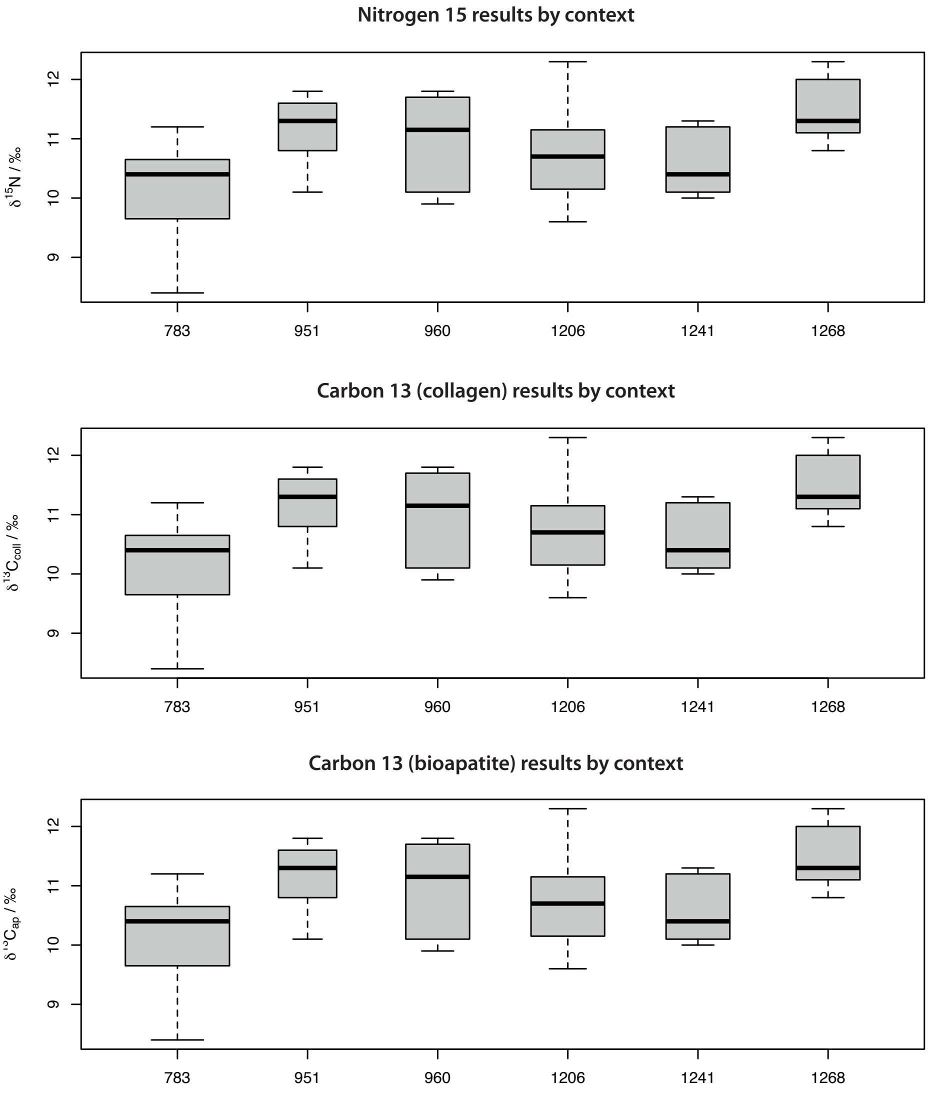
Figure 10.3. *Modelled contribution of terrestrial plants, terrestrial animals and marine fish to the palaeodiet at the Tarxien phase burials at Xagħra calculated using Bayesian mixing model.*

Table 10.5. *Number of samples of human bone from well-sampled contexts subjected to isotopic analysis.*

Context	Number ($\delta^{15}N_{coll}$ and $\delta^{13}C_{coll}$)	Number ($\delta^{13}C_{apa}$)
783	16	36
951	5	41
960	6	9
1206	8	19
1241	6	5
1268	6	9

The large number of samples from certain contexts (Table 10.5) provides an opportunity to test whether some parts of the site contained human remains with palaeodietary signatures that differed significantly from the average results from across the site. In the event, however, the readings are remarkably homogenous, aside from statistical noise caused by the smaller sample size in certain contexts (Fig. 10.4). One possible exception is ^{15}N values from Context (783), which tend to be lower than average, which probably reflects a chronological pattern, as discussed below.

Time-dependent changes in nitrogen isotope values can be caused by palaeoenvironmental changes, especially soil development and aridity, so a detailed look at this variable is called for. Turning first to faunal remains, using the bootstrapping method detailed above (§10.4) to develop the data into a time-series, there is an apparent spike in the ^{15}N enrichment around 3300 cal. BC (Ġgantija period), apparent in samples of both sheep/goat and, to a lesser degree, cattle from Taċ-Ċawla. Although more samples would be required to confirm this pattern, it suggests that there was an episode of aridity that drove ^{15}N enrichment of herbivore remains to very high levels. An alternative explanation


Nitrogen 15 results by context

Carbon 13 (collagen) results by context

Carbon 13 (bioapatite) results by context

Figure 10.4. *Boxplots of the main isotopic results by context (width of the boxes is proportional to the sample size).*

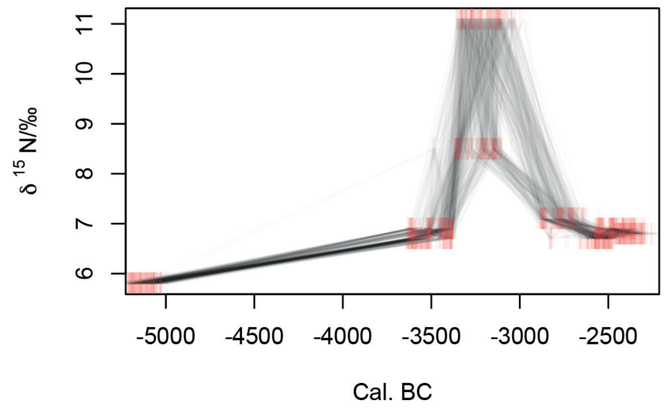


Figure 10.5. *Nitrogen isotopic values of animal bone collagen, plotted as a time series using multiple bootstraps of the radiocarbon dates to represent chronological uncertainty. The spike around 3300* BC *during the Ġgantija period is very noticeable and may imply either heavily manuring or an episode of extreme aridity although further work will be needed to assess the significance of this.*

is manuring (Bogaard *et al.* 2013), although this seems unlikely, as we would then expect to also find the signal in human remains from the period. In the event, however, the results the burials of this period from the rock-cut tomb are not significantly different from the later Tarxien-period individuals from the cave complex.

There is a slight but significant trend towards less enrichment of nitrogen-15 during that intensive phase of Tarxien-period mortuary activity. Using a linear regression model we can estimate that the average $\delta^{15}N_{coll}$ value fell from around 11‰ to 10‰ over between 2900 and 2400 cal. BC. The regression is statistically significant ($p<0.0005$) and explains between 11% and 16% of the variability, with the confidence envelope caused by the inherent uncertainties of the radiocarbon dating process (Figure 10.6).

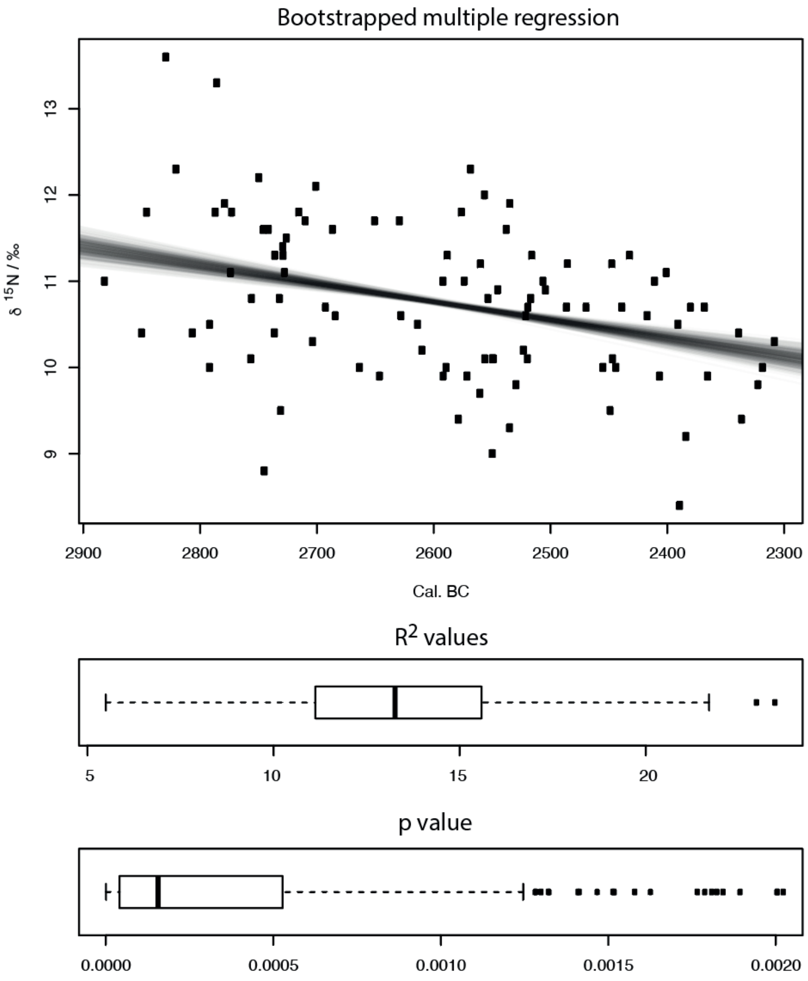


Figure 10.6. *Multiple regression modelling the dependence of nitrogen isotope ratios on time. The results indicate a significant linear trend of decreasing enrichment explaining approximately 13% of the variability in the data (R^2 values).*

10.6. Discussion and conclusion

It is clear that there is a palaeodietary signal that is distinct to the Maltese Islands. Within Malta, our study has found little difference between sites and time periods, aside from the subtle trend in nitrogen isotope ratios discussed above. One important finding is that the results from the Xemxija tombs are very similar to those from the Circle. Until this study, any generalizations about prehistoric human diet in the Maltese Islands have, to date, been made using results from the Circle alone. When we consider the dietary sources and their offsets, the highly elevated δ^{15}N values for the Ġgantija period skew any potential inference. The similarity of the Ġgantija period individuals from the rock-cut tomb to the main cave system would suggest that whatever was causing the elevation of δ^{15}N in animal bones at this time did not affect humans; perhaps because animals did not feature prominently in the human diet and were foddered on separate crops to humans.

The human-animal offsets and the results of the source mixing modelling suggest that animal protein was more important than plant protein during the Temple Period in Malta. We cannot at present make a distinction between meat and dairy in this regard, other than point to zooarchaeological evidence of slaughter patterns consistent with a dairy economy (see Volume 2, Chapter 9). Future modelling work can avail of the additional information provided by our $\delta^{13}C_{apa}$ data, especially if a more extensive database for food sources can be assembled, in attempts to understand other components in the total diet, such as carbohydrate intake, which may present a more balanced picture of the relative importance of plants and animals. However, it remains that meat and/or dairy was of critical importance in Malta during the Temple Period, and this reiterates the fundamental relationship between people and domesticated animals that was key to sustaining island life. Dairy products have been reported from the early levels of Skorba by lipid analysis (Debono Spiteri *et al.* 2011; 2016) and an extension of such research combined with proteomic analysis would be invaluable in providing another line of evidence for dairy intake into the diet of prehistoric Malta. Could this factor have given rise to occasional occurrences of obesity (seen in the prehistoric art)? If some individuals had access to relatively high levels of dairy after impoverishment of diet in their early life course (§8.6.2.8), this could have led to the corpulence for which the artistic record is so famous.

It is also clear that throughout the Temple Period, marine food was much less important to these island residents than the foods grown and raised on the island. However, given the results of the Bayesian isotopic mixing modelling, the sea cannot be completely excluded as a secondary source of nourishment. This finding aligns with the archaeological data discussed in Volume 2, Chapter 9. *FRAGSUS Project* excavations at Taċ-Ċawla and Santa Verna produced a very small number of fish bones, but shellfish remains were ubiquitous in archaeological strata across the island, and the bones of marine birds are also quite numerous, several of which were modified into simple artefacts (see McCormick and Hamilton-Dyer in Volume 2, Chapter 9). The question of whether marine foods were eaten regularly, in small quantities, or whether they provided a major base of subsistence at during times of the year when other resources were scarce, must be left for future research.

Comparing the different strands of our analysis, aside from two outliers, the offset between $\delta^{13}C_{apa}$ and $\delta^{13}C_{col}$ for paired measurements was seemingly random, normally distributed around a mean value of -6‰. Outliers UBA-32038 and UBA-32039, both from Context (951) have significantly lower $\delta^{13}C_{apa}$ relative to their $\delta^{13}C_{col}$ values (-9‰ and -8‰ respectively), although this offset is not as extreme as the -10‰ measured during previous work for the samples BR2 and BR4 by Lai, O'Connell and Tykot (2009). Therefore, it seems the proxies are telling us different things, although no clear chronological or context-dependent pattern is forthcoming from either sets of data, the results are difficult to interpret beyond the exclusion of marine diet.

Compared with other regions, the nitrogen-15 results are most intriguing. These set Malta apart from peninsular Italy, and even neighbouring Sicily, reflecting the more arid climate and poorer soil development. The trend detected at the Circle in nitrogen-15 could reflect changing dietary practices, such as a gradual decline of the importance of meat and milk in the diet, or environmental factors. The latter interpretation is slightly at odds with geoarchaeological work undertaken by the *FRAGSUS Project* on Gozo, which suggests that a gradual aridification trend operated throughout the period considered here (Volume 1, Chapter 5).

More research will be needed to resolve the ultimate cause of this interesting pattern, and this should primarily focus on measurement of animal bone samples. This may prove challenging as, during the current phase of work, we attempted analysis of a further 22 animal bones from the archaeological sites excavated by the project, but all failed because of low collagen preservation (see Volume 2, Chapter 2). Despite these uncertainties and gaps that remain in our knowledge, the results discussed here nonetheless reveal a dynamic relationship between environment, agriculture and diet that was no doubt central to the lived experience of the individuals whose remains we study.