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Evaluating Marine Diets Through Radiocarbon Dating and Stable Isotope Analysis of Victims of the AD79 Eruption of Vesuvius

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ABSTRACT The stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values of bone collagen are frequently used in paleodietary studies to assess the marine contribution to an individual's diet. Surprisingly, the relationship between stable isotope these values characteristics and the percentage of marine foods in diet has never been effectively demonstrated. To clarify this relationship, the stable isotope values and radiocarbon dates of nine humans and one sheep from Herculaneum, all who perished simultaneously during the AD 79 eruption of Vesuvius, were determined. Significant differences were found in the radiocarbon dates which are attributable to the incorporation of "old" carbon from the marine reservoir. The magnitude of the observed differences was linearly correlated with both $\delta^{13}C$ and $\delta^{15}N$ values allowing the response of each isotope to increasing marine carbon in collagen to be independently verified. Regression analyses showed that for every 1‰ enrichment in $\delta^{13}C$ and $\delta^{15}N$, 56 years and 34 years were added to the radiocarbon age, respectively. Predictions of the maximum marine reservoir age differed considerably depending on which stable isotope was considered. This discrepancy is attributed to some degree of macronutrient scrambling whereby nitrogen from marine protein is preferentially incorporated in collagen over marine carbon. It is suggested that the macronutrient scrambling explains the observed relationship between $\delta^{13}C$ and $\delta^{15}N$ from Roman coastal sites and should be considered when interpreting any diet which is not dominated by protein. Nevertheless, without knowing the degree of macronutrient scrambling in different dietary scenarios, the accuracy of dietary reconstructions is severely compromised. Am J Phys Anthropol 152:345–352, 2013.

Charting variability in marine consumption is an important goal of archaeological research (Anderson et al., 2010). Since its first application over 3 decades ago (Schoeninger et al., 1983), the analysis of carbon and nitrogen stable isotopes from the protein fraction (collagen) of human bone has been at the forefront of this endeavor. This approach most commonly uses the ratio of ^{13}C to ^{12}C ($\delta^{13}C$) in collagen to directly gauge the degree of marine versus terrestrial foods that were consumed several years before death, especially when other ^{13}C enriched or depleted dietary sources, such as C_4 plants or freshwater fish, are absent. For example, this assumption has been used to support Upper Palaeolithic evidence for a reliance of marine foods (Richards et al., 2005), widespread decline in marine consumption with the arrival of agriculture in Europe (Richards et al., 2003), resurgence in the early medieval period (e.g., Barrett and Richards, 2004), and also to identify variability within populations (e.g., Müldner et al., 2009). More recently, some potential limitations of this approach have been identified (Hedges, 2004; Milner et al., 2004) and the interpretation of isotope data continues to generate debate (Barberena and Borrero, 2005; Milner et al., 2006; Richards and Schulting, 2006), partly due to the absence of independent verification. Notably, the precise relationship between $\delta^{13}C$ and the degree of marine consumption has never been demonstrated.

It is often assumed that only carbon from dietary protein contributes to collagen, and therefore, that $\delta^{13}C$ is linearly and positively correlated with increasing amounts of marine protein in diet (e.g., Richards and Hedges, 1999). From this assumption, the degree of marine protein consumption in humans has been estimated by linear interpolation from theoretical endpoints corresponding to 100% marine and 100% terrestrial diets. However, the relationship between dietary protein $\delta^{13}C$ and bone collagen $\delta^{13}C$ is likely to be more complex than as described by this simple linear relationship (Hedges, 2004). This is because other dietary macronutrients, i.e., carbohydrate and lipid, may also contribute carbon to collagen (Ambrose and Norr, 1993; Jim et al., 2004; Jim et al., 2006) and because these may be

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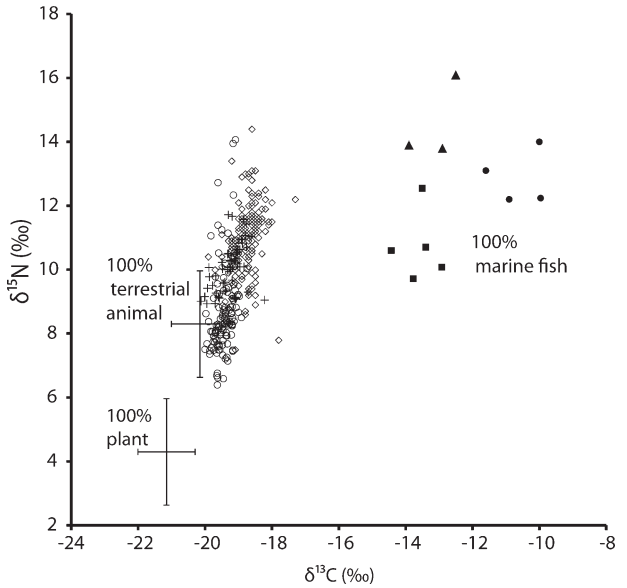

Fig. 1. Stable carbon and nitrogen isotope analysis of humans from three Imperial Roman coastal populations against theoretical dietary endpoints. Human data are from Isola Sacra (open diamonds; $n = 167$, (Prowse et al., 2004) (Crowe et al., 2010)); Velia (open circles, $n = 117$, (Craig et al., 2009), Herculaneum (crosses, $n = 72$, this study). The theoretical dietary end-points for terrestrial plants is equal to the isotopic range of terrestrial herbivores at these sites ($n = 25$), the terrestrial animals range is derived by adjusting the terrestrial herbivore range for isotopic fractionation (+1‰ for $\delta^{13}C$ and +4‰ for $\delta^{15}N$). Measurements of individual fish (see Table 2) are shown also adjusted for isotopic fractionation, these are *Thunnus spp.* (filled triangles); Sparidae (filled squares) or are unknown (filled circles).

variably depleted in ^{13}C compared to dietary protein (Tieszen and Fagre, 1993). This more complex model of macronutrient scrambling has been proposed to interpret human stable isotope data from several hundred individuals buried in Roman necropoleis along the Tyrrhenian coast of Italy (Prowse et al., 2004; Craig et al., 2009; Crowe et al., 2010). These data are characterized by a limited range of $\delta^{13}C$ values, mostly within the terrestrial range, but a much wider range of $\delta^{15}N$ values (Fig. 1), some of which are higher than can be explained from consumption of terrestrial protein alone. Both here and at other Roman coastal sites, it has been postulated that the high $\delta^{15}N$ values are due to consumption of marine protein, as this source is enriched in $\delta^{15}N$ (Fig. 1), whereas the carbon values are heavily influenced by ^{13}C depleted terrestrial macronutrient sources (Prowse et al., 2004; Craig et al., 2009; Keenleyside et al., 2009), particularly grain carbohydrates, which from historical sources are known to have dominated Roman diets (Garnsey, 1999). Consequently, the standard linear interpretation of $\delta^{13}C$ values, without considering these additional carbon sources, underestimates the marine dietary contribution.

If correct, these findings have profound implications for understanding marine consumption in the past and require the size of the effect to be quantified. While applicable to most humans accessing coastal resources, a critical reinterpretation of carbon stable isotope values would seem especially warranted where carbohydrate consumption might also have been high, which extends well beyond Roman Mediterranean contexts. There are also potential implications for radiocarbon dating human remains, as $\delta^{13}C$ is commonly used to predict the amount of "old" carbon derived from the marine reservoir in order to correct dates through mixed calibration (e.g., Barrett and Richards, 2004). Without knowing the precise relationship between $\delta^{13}C$ and the proportion of marine carbon in collagen, which is complicated if there are multiple isotopically enriched or depleted dietary carbon sources, the accuracy of any reservoir correction is compromised.

Currently, the evidence for extensive macronutrient scrambling is tentative and other explanations, such as the consumption of high trophic level freshwater fish, have been put forward to explain ^{15}N enrichment in bone collagen from Italian Roman contexts (Rutgers et al., 2009) and elsewhere (Müldner and Richards, 2005). Also, in reality, dietary protein must contribute the majority of carbon to collagen, as the majority of collagen amino acids cannot be synthesized from other sources. For example, animal feeding experiments have established that at least 50% of carbon in collagen is directly routed from dietary protein (Ambrose and Norr, 1993; Jim et al., 2006; Fernandes et al., 2012), and an even greater percentage is anticipated for higher (>20%) protein diets or, as recently suggested, the degree of routing may be relatively constant (e.g., 74%; Fernandes et al., 2012). There are also physiological reasons why metabolized carbon from dietary lipid, the component likely to be most depleted in $\delta^{13}C$, cannot make a substantial contribution to collagen (Schwarcz, 2002). Nevertheless, as we show below through modeling exercises, even a small degree of scrambling is liable to have a dramatic effect on the interpretation of $\delta^{13}C$ values, yet the potential effects are rarely considered when interpreting human stable isotope values.

To provide a major step forward in the precision of estimating marine consumption from stable isotope values, here we present high precision AMS dates and $\delta^{13}C$ and $\delta^{15}N$ determinations of nine adult humans from the Roman coastal town of Herculaneum, each securely attributable to the AD 79 eruption of Vesuvius (Bisel, 1991; Capasso, 2000, Mastrolorenzo et al., 2001). The presence of fishing boats and other equipment (Steffy, 1985; Bekker-Nielsen, 2003) and the remains of fish in the sewers (Wallace-Hadrill, 2011) demonstrate that marine foods were available to and consumed by the inhabitants of this coastal town. Therefore, it is reasonable to expect that ^{14}C ages should differ between individuals only according to the amount of carbon derived from the marine reservoir during collagen synthesis, which spans a limited period prior to death (Hedges et al., 2007). A bone sample from a domestic sheep (*Ovis aries*) from the same context as the humans and also attributable to the AD79 eruption was also analyzed to provide a secure terrestrial endpoint for comparison. The independent relationship between the difference in the apparent ^{14}C age and the relative enrichment in collagen ^{13}C and ^{15}N provides a rare opportunity to evaluate different models for carbon flow from dietary macronutrients to collagen.

METHODS

Bone samples from nine humans and one sheep were selected from a large assemblage of remains found in each of several vaulted storehouses, or fornici, within the coastal town of Herculaneum (Fig. 2; Bisel, 1991;

Capasso, 2000; Mastrolorenzo et al., 2001). Ribs were selected from adult humans (>20 years old). Because of variation in the rate of collagen turn-over during life, as observed in femoral bone (Hedges et al., 2007), differences in the age of the individual at the time of death may cause a small discrepancy in the observed radiocarbon dates. The rate of collagen turnover specifically in rib bone is unknown, although histomorphological studies have shown that ribs remodel at a much faster rate than femora (Mulhern, 2000; Cho and Stout, 2011). Therefore, by selecting rib samples the collagen analyzed is more likely to have been synthesized closer to the time of death (i.e., AD 79) thus minimizing any age related differences.

Fig. 2. Map showing approximate locations of Imperial Roman sites referred to in the text.

Each sample was cleaned, pulverized and collagen was extracted by acid demineralization and gelatinization using standard protocols (Craig et al., 2009), which included ultrafiltration to prepare a high molecular weight (>30 kDa) collagen fraction (Brown et al., 1988). $\delta^{15}N$ and $\delta^{13}C$ were determined in triplicate by isotope ratio mass spectrometry (EA-IRMS), using a Thermo Finnigan Delta Plus XL continuous flow mass spectrometer coupled to a Flash EA 1112 elemental analyzer, with an instrument precision of <0.2 for each isotope, on at least two separate collagen extracts from each bone. Uncertainty was calculated from the mean $\delta^{13}C$ and $\delta^{15}N$ values obtained from each replicate extraction (Table 1). Human and animal bone samples were AMS dated in duplicate (ORAU, Oxford, UK) to obtain a high precision date. Following standard procedures (Bowman, 1990), the date ranges from each sample were checked for internal consistency, combined and the uncertainties on the measurements were calculated (Table 1). For comparison, collagen was also prepared and analyzed by EA-IRMS from a further 63 adult humans from Herculaneum (all dating to AD79) and a further five samples of sea bream (Sparidae) from the nearby and contemporaneous site of Pompeii. Statistica (v.7) was used to compute all statistics. Uncertainties in regression parameters were used to calculate confidence intervals on predicted measurements by extrapolation.

RESULTS AND DISCUSSION

The remaining stable isotope data comprising 72 adults from Herculaneum and the comparative archaeological fish specimens are reported in Supporting Information Table 2.

Collagen was adequately preserved in each sample (>2% by weight), and the elemental data are within the acceptable range (Van Klinken, 1999). The maximum observed difference in the ^{14}C ages between individuals was 86 years despite the fact that all individuals perished simultaneously. As only rib samples from adults were chosen (Table 1), many of whom were the same age, and the precision on each date was less than 20 radiocarbon years (Table 1), the discrepancy in dates is

TABLE 1. Summary of AMS dates and $\delta^{13}C$ and $\delta^{15}N$ measurements of human and animal bones from Herculaneum

Sample	Bone Element	Age at death (y)	Lab code, ^{14}C BP (±1 SE)	Mean weighted ^{14}C (y BP)	±1 SE. (y)	%C	%N	Atom C:N	$\delta^{13}C$ (‰)	±1SE (‰)	$\delta^{15}N$ (‰)	±1SE (‰)
F12I28	Human rib	35–40	OxA-25360 (2002±22) OxA-25548 (1958±27)	1982	18	42.4	15.2	3.2	−19.89	0.05	8.89	0.04
F10II11	Human rib	30–35	OxA-25365 (2001±14) OxA-25553 (1974±28)	1988	19	41.9	14.7	3.3	−19.7	0.03	9.31	0.49
F10I28	Human rib	35–40	OxA-25363 (2011±20) OxA-25551 (1972±27)	1993	18	41.6	15	3.2	−19.65	0.03	9.16	0.17
F12I3	Human rib	25–30	OxA-25362 (1985±25) OxA-25550 (2034±28)	2007	19	43.8	15.5	3.3	−19.67	0.19	10.09	0.31
F10I16	Human rib	35–40	OxA-25361 (2043±30) OxA-25549 (1984±27)	2016	18	43.7	15.3	3.3	−19.79	0.09	10.09	0.02
F9I13	Human rib	40–50	OxA-25364 (2070±24) OxA-25552 (2022±27)	2048	18	42.6	15.3	3.2	−19.12	0.11	10.76	0.22
F12I23	Human rib	40–45	OxA-25368 (2026±25) OxA-25556 (2075±28)	2049	19	43.7	15.5	3.3	−18.57	0.1	10.93	0.12
F7 10	Human rib	35–40	OxA-25367 (2056±25) OxA-25555 (2061±27)	2058	18	42.7	15.6	3.2	−18.75	0.16	10.63	0.09
F9I9	Human rib	20–25	OxA-25366 (2074±26) OxA-25554 (2045±28)	2061	19	43.4	15.6	3.3	−18.8	0.12	11.45	0.18
EF8	Sheep metatarsal	n/a	OxA-25369 (1975±25) OxA-26144 (1960±26)	1975	25	42.3	15.3	3.2	−21.86	0.18	7.79	0.15

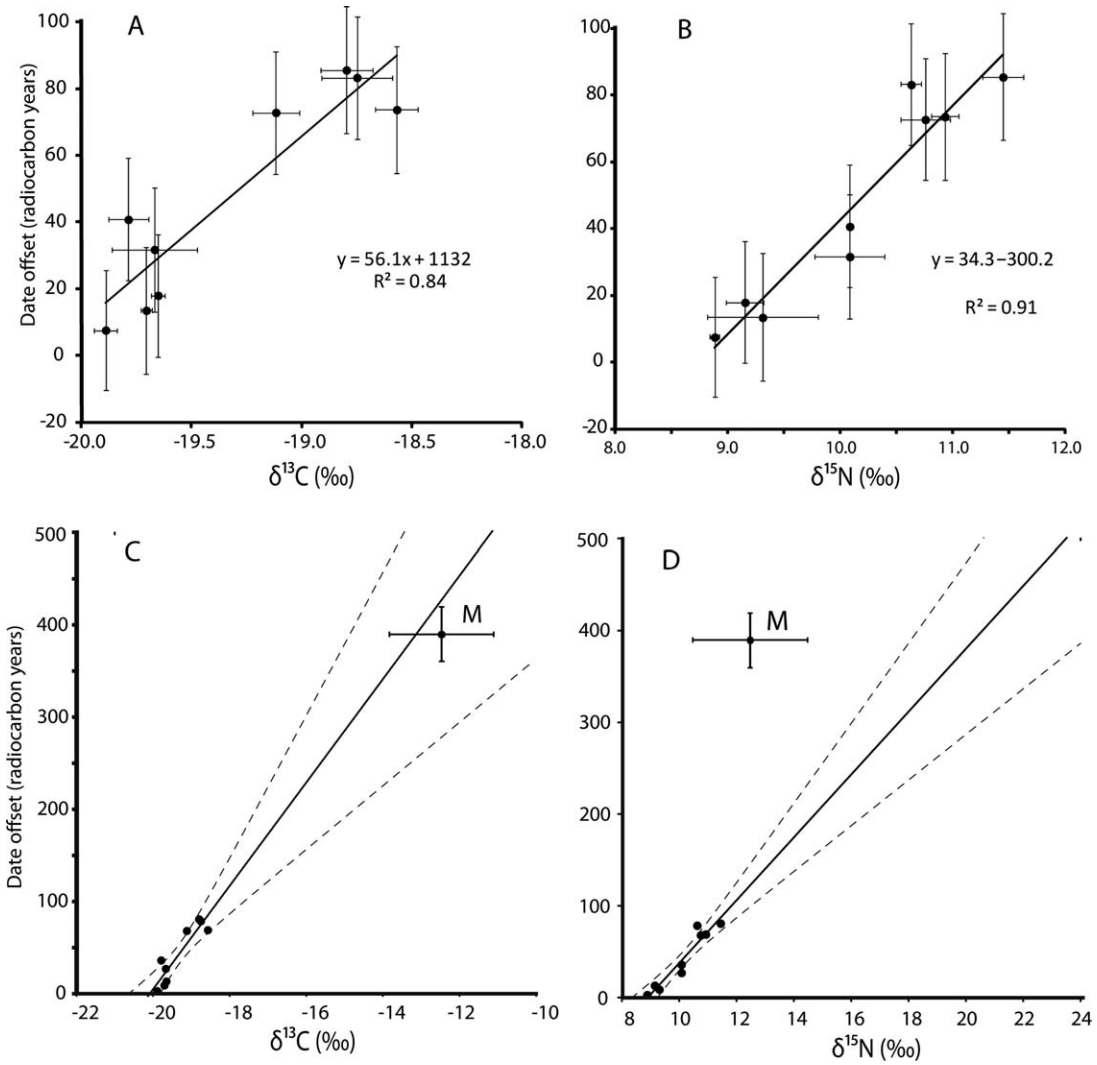

Fig. 3. Plots of $\delta^{13}C$ and $\delta^{15}N$ against ^{14}C offset attributable to the marine reservoir effect. **A** and **B:** These show the regression line, equation and standard errors on each measurement. **C** and **D:** These show the values of $\delta^{13}C$ and $\delta^{15}N$ predicted from the regression equation, including 95% confidence bands, over 500 radiocarbon years to encompass to the maximum marine reservoir correction for 100% marine consumers. *M* is the value expected from a 100% marine consumer after correcting the observed mean value for Roman marine fish ($\pm 1\sigma$) for isotopic fractionation (+1‰ for $\delta^{13}C$ and +4‰ for $\delta^{15}N$) and using the maximum marine reservoir age for the region (i.e., 390 ± 30 y).

best explained by variable consumption of marine foods and the incorporation of "old" carbon from the marine reservoir. AMS dates obtained from the chronologically "youngest" individual (F12I28) are statistically indistinguishable from those from the terrestrial animal (sheep) bone (mean = 1979 ± 14.6 yr; $T = 0.05$; d$f = 1$, $\chi^2 = 3.84$). Differences between the mean date from the sheep bone (i.e., 1975 BP) and the mean dates of each human sample (date offset) were plotted against both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 3A,B) and found to be remarkably well linearly correlated ($R^2 = 0.84$, $P = <0.001$ for $\delta^{13}C$; $R^2 = 0.91$, $P = <0.001$ for $\delta^{15}N$; Fig. 3A,B). With increasing $\delta^{13}C$ and $\delta^{15}N$, the human bones produce increasingly older dates, providing compelling evidence that both isotopes enrich due to marine consumption.

The slope (β) of the regression equations that best describe these relationships, shows that every 1‰ enrichment in $\delta^{13}C$ and $\delta^{15}N$ adds 56 years ($t[7]=6.0$, $P = <0.05$) and 34 years ($t[7] = 8.5$, $P = <0.05$), respectively to the radiocarbon age. By extrapolation to the theoretical isotopic endpoints of a 100% marine consumer, we are able to predict the marine reservoir age (R) from each regression equation. Marine endpoints of −12.5‰ for $\delta^{13}C$ and 12.4‰ for $\delta^{15}N$ can be estimated by correcting the mean value of Mediterranean marine fish from the Roman period (Fig. 1, Table 2) by +1‰ and +4‰ (Bocherens and Drucker, 2003) respectively for diet to collagen fractionation ($\Delta^{15}N_{diet\text{-}collagen}$). From the $\delta^{13}C$ regression equation and $\delta^{13}C$ marine endpoint, the date offset of a 100% marine consumer (R) is predicted to be 430 years, which despite large errors corresponds well with the best estimate of the marine reservoir age for this region of 390 ± 30 years (Fig. 3C). The latter were obtained from dating marine shells from the Tyrrhenian Sea of known calendrical age (Reimer and McCormac, 2006). Therefore, as expected, $\delta^{13}C$ in collagen provides an accurate estimate of the amount of marine carbon in collagen and is a suitable linearly

TABLE 2. Summary of mean and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ measurements of marine fauna from Imperial Roman Sites in the Mediterranean

Site	Classification	*n*	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	Reference
Velia, Italy	*Thunnus spp.*	3	−14.1(±0.7)	10.6(±1.3)	Craig et al. 2009
Velia, Italy	Unknown	1	−12.6	9.1	Craig et al. 2009
Pompeii, Italy	Sparidae	5	−14.6(±0.6)	6.7(±1.1)	This study
Leptiminus, Tunisia	Unknown	2	−11.5(±0.6)	9.1(±1.3)	Keenleyside et al. 2009
Katedrala Sv Stošije	Unknown	1	−11.0	8.2	Lightfoot et al., 2012

proportional proxy for correcting radiocarbon dates for the marine reservoir effect. Other than the observed marine and terrestrial fauna, no other isotopically enriched or depleted dietary sources of carbon (e.g., C_4 plants or freshwater fish) are needed to explain the observed human values. While the finding does not preclude macronutrient scrambling, it also implies that any carbon in collagen derived from terrestrial carbohydrate or lipid is of similar isotopic composition to that from terrestrial protein. As lipids are depleted in ^{13}C compared to protein from the same source (Tieszen and Fagre, 1993), they do not seem to significantly contribute carbon to collagen, at least in this dietary example.

Linear extrapolation of the regression equation to the $\delta^{15}N$ value of a 100% marine consumer produces an *R* value of just 127 ± 24 (2 s.d.) years. This predicted *R* value is significantly lower than the marine reservoir age estimated from dating known age shell (Reimer and McCormac, 2006, Fig. 3D). Conceivably, the marine $\delta^{15}N$ endpoint (12.5‰) could be underestimated. A greater $\Delta^{15}N_{diet\text{-}collagen}$ of 6‰ has been tentatively suggested for humans based on controlled dietary studies (O'Connell et al., 2012), and other dietary sources, such as fermented fish products which are expected to be enriched specifically in ^{15}N through loss of ammonia, could also be considered given their abundance during the Imperial Roman period (Curtis, 1984; Curtis, 1988). Nevertheless, we estimate that the minimum endpoint needed to produce an offset equitable with the observed shell value is 18‰ at 95% confidence, whereas the maximum theoretically obtainable, estimated by correcting the most ^{15}N enriched Mediterranean fish collagen so far observed (a mature tuna from the site of Velia; Fig. 1) for $\Delta^{15}N_{diet\text{-}collagen}$ is only 16‰. We also note that the Mediterranean fish from the Roman period (n = 12, Table 2) are consistent with isotope measurements of Mediterranean fish and marine mammals from all periods (Jennings et al., 1997; Lightfoot et al., 2011; Lelli et al., 2012; Lightfoot et al., 2011). Here, we suggest that a more parsimonious explanation for the discrepancy between the curves of $\delta^{15}N$ and $\delta^{13}C$ versus date offset is due to macronutrient scrambling.

Examining elementary models to explain macronutrient sources of carbon in collagen

We can illustrate the effects of different degrees of scrambling by modeling the response of $\delta^{13}C$ against the percentage of marine protein in total dietary protein in a theoretical dietary scenario (Fig. 4). The scenario and the assumptions used are described in detail in the Supporting Information but essentially consist of three dietary sources; terrestrial plants, terrestrial animals, and marine fish, with the $\delta^{15}N$ and $\delta^{13}C$ values of each source derived from measurements of faunal bone collagen from Italian Roman contexts (Prowse et al., 2004; Craig et al., 2009). As nitrogen in collagen can only be derived from dietary protein, $\delta^{15}N$ must provide a reliable estimation of the proportion of marine protein in total dietary protein and is used to derive the *x* axes values in Figure 4. The actual values are dependent on the $\delta^{15}N$ marine and terrestrial endpoints, and hence, the source of terrestrial protein (i.e., meat vs. plant), so for the purposes of the model, 0% and 20% meat contributions are considered with the maximum achievable $\delta^{15}N$

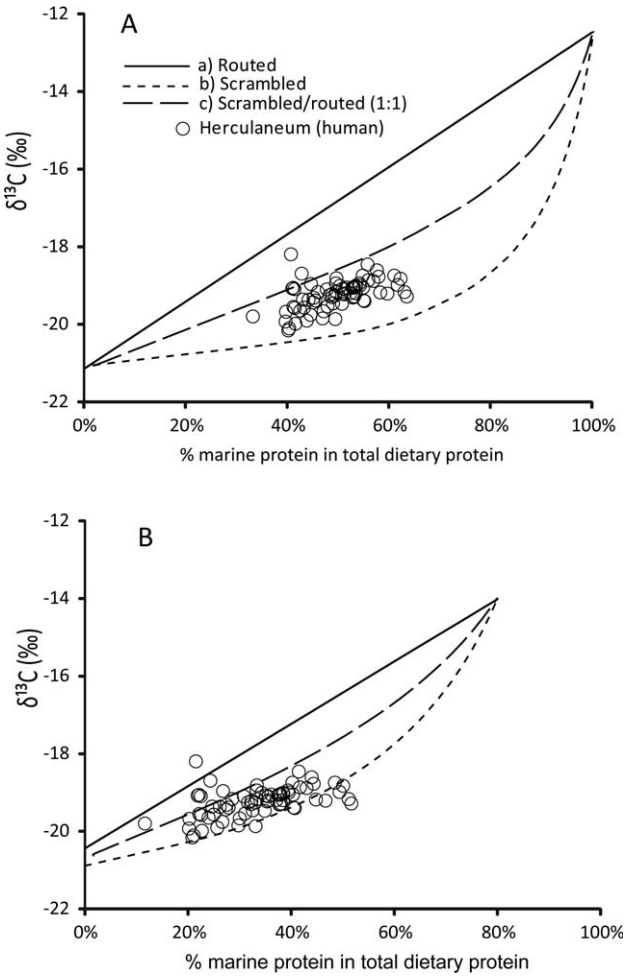

Fig. 4. Predictions of collagen $\delta^{13}C$ with increasing contribution of marine protein to diet for different models of biosynthesis. **A**: assumes no contribution from terrestrial animal protein. **B**: assumes a 20% dietary contribution from terrestrial animal protein. Also shown are the observed $\delta^{13}C$ values and estimates of % marine protein for adult humans from Herculaneum. The latter are estimated from the observed $\delta^{15}N$ values linearly interpolated against the terrestrial and marine endpoints.

endpoint of 16‰ and a $\Delta^{15}N_{diet\text{-}collagen}$ of 4‰. The $\delta^{13}C$ values are determined according to three models (a,b,c) that assume different degrees of scrambling (0%, 100%, and 50%) and therefore different contributions of carbon from dietary protein (plant, terrestrial, and marine) and carbohydrate (plant) to collagen.

For no scrambling, i.e., 100% routing of protein to collagen, carbon and nitrogen are derived from the same source and so both are linearly and positively correlated (Fig. 4, model a). For 100% scrambling (model b), carbon in collagen is derived equally from all dietary macronutrients which in this scenario includes a substantial additional contribution of carbon from plant carbohydrate. It is evident that macronutrient scrambling produces a nonlinear relationship between $\delta^{13}C$ and marine protein (as a fraction of total dietary protein) or $\delta^{15}N$, the slope increasing with increasing marine consumption (Fig. 4). A more realistic combined model of mixed routing and scrambling (model c) also produces a nonlinear relationship (Fig. 4). For example, if 50% of collagen carbon is assumed to be derived from dietary protein (as for essential amino acids), and 50% is from all dietary carbon, then the initial slope of the curve is approximately twice as great as for protein routing alone (model a), using the same dietary end-points.

When all 72 adult humans' values from Herculaneum are plotted, using estimations of marine protein consumption from the observed $\delta^{15}N$ values (*x*-axes, Fig. 4), it is clear that macronutrient scrambling (b) or (c) better describes the slope of observed data while direct routing (a) provides an unsatisfactory explanation. The effect of including terrestrial meat consumption is noticeable but small, and does not alter these qualitative features. Without accounting for a non-protein contribution of carbon to diet, $\delta^{13}C$ values of low to moderate marine consumers will be wrongly interpreted and underestimate the marine protein contribution to dietary protein.

Implications for understanding Roman diets in the Mediterranean and beyond

Assuming that $\delta^{15}N$ and $\delta^{13}C$ are not necessary linearly correlated over the full dietary range, due to macronutrient scrambling, then each isotope must be interpreted separately. For example at Herculaneum, the $\delta^{13}C$ the range for all 72 humans is -20.2‰ to -18.2‰ (Fig. 1). We can interpret this to infer that across the sample up to 30% of carbon in collagen (mean 15%) was derived from marine foods (Fig. 5), using the regression equation and the maximum marine reservoir age (Fig. 3C). Assuming that carbon was derived for all dietary sources equally, this figure approximates to the marine contribution to diet by weight. The terrestrial meat contribution must be <20% by weight, as modeling a more substantial contribution produces values inconsistent with the observed human range for any of the models in the dietary scenario. This is because marine foods would more substantially enrich the consumer's $\delta^{13}C$ if mixed only with other protein rich animal products. Therefore, plant foods must make up the remainder of the diet, estimated to be at least 50% by weight and in the majority of cases greater than 75%. These ranges are at least consistent with estimates from the historical record, which often stress the dominance of cereal carbohydrate to Roman diets (e.g., Foxhall and Forbes, 1982; Garnsey, 1983). In reality, the marine dietary contribution by weight, which as estimated is comparable to some modern small island developing states (FAOSTAT, 2012), may be lower depending on the degree that carbon in collagen was preferably routed from dietary protein.

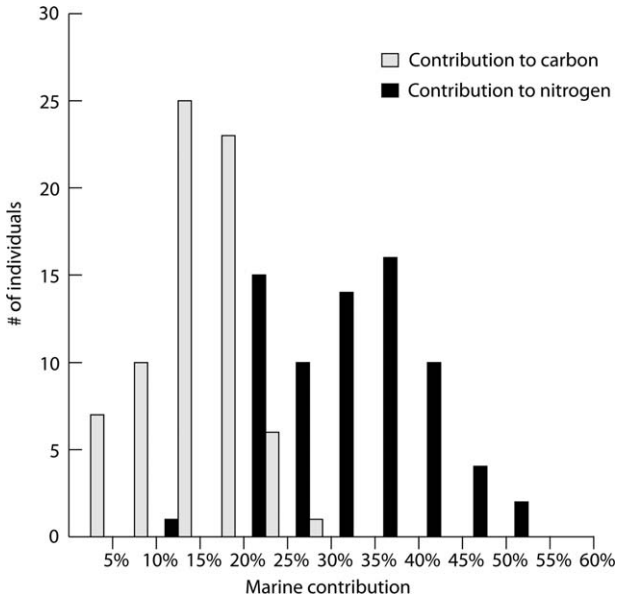

Fig. 5. Predicted % marine contribution to collagen carbon and nitrogen of the AD79 Herculaneum adult population. The % contribution to nitrogen is derived from linear interpolation of the observed $\delta^{15}N$ values of 72 adults against the mean marine and terrestrial endpoints. Estimates of % contribution to carbon are from the regression equation, derived from AMS dating nine individuals and assuming a maximum reservoir age of 390 years use.

In contrast to carbon isotopes, $\delta^{15}N$ provides an independent estimate of the % protein contribution of marine foods to total dietary protein rather than total diet. The range of $\delta^{15}N$ at Herculaneum is $8.2-11.7$‰, from which we can estimate the marine contribution to protein to be 10–50% (median = 34%) with a 20% meat diet or 30–65% without (median = 50%), when calculated from the $\delta^{15}N$ terrestrial and marine endpoints (Fig. 1). Thus, while the amount of marine derived carbon in collagen is relatively small, the amount of marine derived nitrogen is much greater, due to the overall greater protein content of marine foods compared to terrestrial. If a greater $\Delta^{15}N_{diet\text{-}collagen}$ value is assumed (e.g., 6‰; O'Connell et al., 2012) then the estimated marine contribution to total protein is substantially reduced to 0–15% with a 20% meat diet and 20–40% without.

Assuming macronutrient scrambling and that the marine and terrestrial endpoints are consistent proxies, we can interpret the stable isotope values of other Mediterranean coastal populations. For example, at the Roman site of Velia in Italy some individuals have $\delta^{15}N$ values of about 14‰ (Fig. 1), which may be interpreted to imply that a high proportion of their dietary protein (ca. 80%) was derived from a marine source assuming a $\Delta^{15}N_{diet\text{-}collagen}$ of 4‰ or at least 50% with a $\Delta^{15}N_{diet\text{-}collagen}$ of 6‰. While this may seem very high, the marine contribution to total diet could still have been relatively modest (ca. 16% by weight) with carbohydrate making up the shortfall, as shown by a relatively

minor enrichment (ca. 1‰) in ^{13}C. Similarly, stable isotope data from the Gravettian human buried at Arene Candide (Pettitt et al., 2003) on the Italian Ligurian coast also supports a much more substantial marine contribution to dietary protein (ca. 60–70%) compared to whole diet (ca. 40%), again a low protein dietary source would have to provide the remainder. Provided the endpoints are equitable, this may imply a greater plant contribution to Palaeolithic diets than previously thought.

For other populations where isotope analysis has been extensively carried out to investigate the degree of marine consumption, the applicability of macronutrient scrambling needs to be carefully considered. For Mesolithic hunter-fisher-gatherers and early Neolithic "farmers" of the Atlantic and Baltic coasts, the relationship between $\delta^{13}C$ and $\delta^{15}N$, in some cases (e.g. Lubell et al., 1994), shows a much shallower curve than for the coastal Mediterranean Roman populations, suggesting that both isotopes equally reflect the source of dietary protein. Here, the diets of both Neolithic and Mesolithic groups might be dominated by protein. Yet for the majority of cases, the response of $\delta^{13}C$ and $\delta^{15}N$ to marine consumption is difficult to establish due to limited data sets, in which case, changes in carbohydrate consumption need to be considered before temporal trends in marine consumption can be confidently made. If, as the examples above imply, the degree of protein routing varies with the population studied, additional sources of information will be required. Such information could come from other isotope systems (e.g., $\delta\ ^{34}S$), bone fractions such as biogenic apatite given rare cases of adequate preservation (Fernandes et al. 2012) and especially $\delta^{13}C$ and $\delta^{15}N$ of individual amino acids (e.g., Choy et al., 2010), which are traceable to different dietary components.

CONCLUSIONS

Through high precision dating of known age material, for the first time we have been able to show how collagen $\delta^{15}N$ and $\delta^{13}C$ respond to increased marine consumption in a human population. We argue that the nonlinear relationship between $\delta^{13}C$ and marine protein consumption resolves many puzzling interpretational issues of fish consumption, especially in the Roman Mediterranean. Notably, significant amounts of marine protein were consumed by some individuals but these made a much smaller contribution to the carbon in collagen due to macronutrient scrambling and high carbohydrate consumption. This is the most likely explanation for the discrepancy in the curves shown between $\delta^{15}N$ and $\delta^{13}C$ versus AMS dates. More generally, interpretations of stable isotope data must establish whether in estimating fish consumption, one is evaluating consumption in terms of weight of fish as a percentage of the whole diet (taken as dry weight, and so approximately equivalent to percentage carbon in the diet); or whether consumption is taken as weight of fish protein as a percentage of the total dietary protein. While this is simply a convention, it must be used consistently, both for $\delta^{15}N$ and $\delta^{13}C$ data. We suggest that cases where collagen $\delta^{13}C$ reflects dietary protein only are likely to be unusual—typically for high protein diets as for some hunter-gatherers. For most populations, an accurate dietary reconstruction, in terms of contribution of marine foods by weight or calories, is impeded without knowing the degree of carbon routing versus scrambling, which cannot be ascertained from the bulk isotope characteristics of collagen alone.

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