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Muge Mesolithic samples analyzed in Canada, including previously unpublished stable isotope data

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Recently, we reported that the dating of an Arruda individual to 7550 ± 100 BP (Beta-127451) seems to have been erroneous (Jackes et al. 2014). A sample from Arruda 6 (Porto) was redated and the new date was found to have an improved fit with the known range of Muge Mesolithic individuals. We also wanted to understand whether the difference in the $\delta^{13}C$ value was of any significance. Because the question arose as to whether our Muge $\delta^{13}C$ values were from IRMS (isotope-ratio mass spectrometry) or were the ion exchange values associated with the AMS dating process, we asked Beta Analytic: "Would it be true that the $\delta^{13}C$ values we have for the Beta dates of this period are the AMS measurements?" (MJ in litt. 7 December 2014). Chris Patrick, Deputy Director/Technical Manager Beta Analytic, Inc. replied (in litt. 8 December 2014):

> This sample was done as a radiometric sample (using liquid scintillation)* done on bone collagen that has been treated with alkali also. The $\delta^{13}C$ value of -19 was an assumed value for typical bone. It was NOT measured directly on the sample. At this time not all of our samples were having the $\delta^{13}C$ measured directly - the submitter had to opt to pay for it. (*Beta protocol, but it was certainly an AMS date)

The sample had been processed in 1999. Reference to the data (Umbelino 2007: 82, Table 4) suggests that varying $\delta^{13}C$ values were in fact given for three Muge bones, from Amoreira 7, Arruda 6, and Moita 16, which had sequential Beta numbers. We will discuss this further below. However, the response from Beta Analytic pointed to a completely different problem that might arise with $\delta^{13}C$ data that are not fully documented.

There is obviously a need to clarify exactly where and when Muge Mesolithic stable isotope analyses were done: we provide that information and add to it by presenting stable isotope data that have remained unpublished. In view of continuing confusion in publications (e.g. Bicho et al. 2013) regarding some Muge sample analyses, we will amplify information on samples and at the same time demonstrate that none of the stable isotope data are derived from the ion exchange values associated with the AMS dating process. It is likely, however, that the Beta values from 1999 are associated with AMS measurement of $\delta^{13}C$.

The importance of recording this is because $\delta^{13}C$ measurements coming with AMS ^{14}C dates were not necessarily reliable for dietary studies. This is stated clearly by Taylor and Bar Yosef (2014:117) when they note that the variability between IRMS- and AMS-based values is, on average, and "in most cases, less than 2‰, [but] it has been shown that, with some samples, it can be as much as 10‰. Because of this, the use of $\delta^{13}C$ values obtained on AMS instruments cannot be used for the purpose of inferring dietary or other isotopic-based environmental signals." They go on to say "...it might be prudent to check with the laboratory.....to confirm the source of the $\delta^{13}C$ values."

Our initial stable isotope values were published in Lubell et al. (1994, Table 1) where the collagen extraction and stable isotope analysis methods were outlined by Schwarcz. The work was done in the 1980s in Schwarcz's laboratory at McMaster University prior to each extracted collagen sample being sent to the IsoTrace Laboratory at the University of Toronto for AMS dating.

Sample	IsoTrace #	Date (BP)	%C	%N	% collagen	$\delta^{13}C$	$\delta^{15}N$	atomic %C/N
Moita 22	TO-131	7240 ± 70	50.3		3.2	-16.1	12.2	
Moita 24	TO-132	7180 ± 70	52.1		2.5	-16.8	11.9	
Moita 29	TO-133	7200 ± 70	58.0		6.3	-16.9	10.4	
Moita 41	TO-134	7160 ± 80	55.3		5.6	-16.7	11.2	
Moita"CT"	TO-135	6810 ± 70	40.4		3.4	-15.3	13.4	
cervid pelvis with Moita 7					3.3	-20.7	3.9	
Arruda A	TO-354	6970 ± 60	49.2		3.66	-18.98	12.2	
Arruda D	TO-355	6780 ± 80	45.79	16.26	8.51	-18.9	10.3	3.29
Arruda N	TO-356	6360 ± 80	44.77	15.97	7.39	-15.3	12.5	3.27
Arruda 42	TO-359	6960 ± 70	44.15	15.7	5.34	-17.2	11.8	3.28
Arruda III	TO-360	6990 ± 110	41.15	13.83	2.56	-17.7	11.2	3.47

Table 1: Available data for samples from Moita do Sebastião and Cabeço da Arruda from the Museu Geológico, Lisbon including a previously unpublished cervid. All human samples were ribs. Stable isotope data from McMaster University (Moita reported in March 1985 and Arruda in September 1987), and %C values for Arruda samples provided by Martin Knyf (23 March 1992).

Figure 2 in Jackes et al. (2001:418), shows data on the atomic %C/N ratio and the collagen percentage dry weight provided in 1999 by Henry Schwarcz and Martin Knyf for nine limestone cave samples, two rockshelter samples and 16 midden samples. All known information for the Muge samples is given in Table 1 here. The higher atomic %C/N ratio given for Arrruda III leads us to expect the same for the Moita samples that had poor collagen preservation as shown in Table 1. The Moita C/N ratios are no longer available: Schwarcz stated that all Moita C/N ratios fell within the 2.9-3.6 range (Lubell et al. 1994), no doubt meaning the acceptable range for the atomic %CN (i.e., C/N multiplied by 1.1666667).

Martin Knyf (in litt. 2 July 2015) suggests that initially, at the time the Moita analyses were undertaken, the percent carbon was calculated with a vacuum line and a manometer, but nitrogen was not easily measured. Thereafter, collagen samples were analyzed for total carbon and nitrogen content using a Carlo-Erba CHN Elemental Analyzer in the laboratory of Dr. A. Oakes, University of Guelph. Nitrogen data had to be recorded manually, and some records are incomplete.

Discrepant data from different sources has caused confusion, but the percent carbon and nitrogen we report in Table 1 must have come from the University of Guelph. It is clear that the results were not achieved through comparable methods, and the Moita human mean of 51.22%C ($\sigma = 6.0$) based on manometer readings is likely to be too high. There are 16 Portuguese Mesolithic and post-Mesolithic samples for which we have fairly consistent McMaster data, together with Guelph data. Comparison of the data for these 16 most consistent samples gives a mean for Guelph analyses of 41%C ($\sigma = 1.5$): the McMaster mean was 47.7%C ($\sigma = 6.8$).

The next set of Portuguese stable isotope analyses done at McMaster University was for Eugénia Cunha (University of Coimbra) on bone samples from the Museu de Antropologia e Pré-História Mendes Corrêa, Porto. From the $\delta^{13}C$ values and the % collagen dry weight reported to us in early 1999, we can identify the Muge samples as the same as those reported by Umbelino (2007:82, Table 4, see also page 53), but they do not include the samples associated with the three Beta dates referred to above (Umbelino 2007:79, Table 1). The dated samples have varied $\delta^{13}C$ values, consistent with those cited here, although they are no doubt derived from the AMS measurements. In addition, Arruda 6 has been redated, and a new stable isotope analysis gave a new $\delta^{13}C$ value of -16.6, rather than -19.0 (Jackes et al. 2014).

The next set of work followed excavations by Rolão and Roksandic at Arruda and Amoreira, the dates being reported by IsoTrace in June 2002 (Table 2). We will not include TO-10218 in the following table: it has been cited in the literature (e.g. Bicho et al. 2013) but was replaced by a re-analysis as TO-11819-R (Detry 2007:37; Meiklejohn et al. 2009). The original stable isotope analysis was done at McMaster (-17.087 for $\delta^{13}C$, but with no reading for $\delta^{15}N$), and extracted collagen was sent to IsoTrace for dating. The redated sample was analyzed for $\delta^{13}C$ and $\delta^{15}N$ at the University of Waterloo. The "R" identification for the TO date recognizes that the TO-11819 sample was dated again, in early 2005, because of poor collagen preservation (first processing yielded only 0.14% collagen). Reprocessing provided somewhat better quality collagen (% collagen ~1.4), allowing for the more accurate date of TO-11819-R.

Sample	IsoTrace #	Date (BP)	%C	%N	% collagen	$\delta^{13}C$	$\delta^{15}N$	atomic % C/N	comment
Arruda 2000 Sk. 2[a]	TO-10216	7040±60	37.8	13.8		-17.87	10.60	3.2	from lowest level cleaning, undisturbed: tibial midshaft
Arruda 2000 Sk. 1[a]	TO-10217	6620±60	16.2	5.6		-18.10	10.46	3.38	rib fragments from upper level disturbed burial
Amoreira 2000-01	TO-11819-R	7300±80	21.81[b]			-16.38[b]			replaces TO-10218: right ribs of child
Amoreira 2001 bone 46			11.8	3.1	1.44	-21.82	4.88	4.44	weathered fragment of large shaft
Amoreira 2001 bone 139	TO-10225	6550±70	30.1	10.4	5.7	-20.06	8.15	3.38	rib fragment
Amoreira 2001 bone 140			39.7	14.5	3.46	-19.30		3.19	rib fragment (same individual as above)

[a] Data for % collagen not available (Martin Knyf, in litt. 23 June 2015)
[b] Analysis at University of Waterloo, EIL #108981, 2005 average of two runs

Table 2: Samples from the 2000 and 2001 excavations directed by José Rolão at Cabeço da Arruda and Cabeço da Amoreira, including one bone with an unacceptable C/N ratio of 4.44.

The sample that provided the TO-11819-R date is one of two small children from Muge that have been analyzed. The stable isotope value reported in Table 2 is from the second of two attempts by EIL, the first providing fuller information associated with what was a clearly erroneous date. Because of the importance of this burial, and the multiple attempts at collagen extraction, both at McMaster University and at IsoTrace, the sample is at present being reprocessed with methods not available to us a decade and more ago (Jackes et al. n.d.).

Table 3 presents unpublished data on Muge samples for which extraction was done at the University of Alberta. Stable isotopes were analyzed at the University of Waterloo, Environmental Isotope Laboratory in 2004.

Sample	Waterloo EIL #	%C	%N	% collagen	$\delta^{13}C$	$\delta^{15}N$	atomic % C/N
Moita 7 rib	107880	40.34	13.67	5.5	-16.83	10.94	3.44
	107880	40.23	13.7		-16.67	11.02	3.43
	average	**40.29**	**13.67**		**-16.75**	**10.98**	**3.44**
Arruda 40 long bone fragment	84086	42.14	14.72	2.8	-16.78	12.66	3.34
	84086	42.57	14.83		-16.89	12.15	3.35
	average	**42.35**	**14.77**		**-16.84**	**12.41**	**3.35**
Moita 42 rib	84087	42.39	14.95	7.2	-16.15	11.63	3.31
	84087	42.72	15.1		-15.72	11.95	3.30
	average	**42.55**	**15.0**		**-15.94**	**11.79**	**3.31**

Table 3: Previously unpublished stable isotope values on human bones from the Museu Geológico, Lisbon.

As shown in Table 4, further Muge Mesolithic samples were processed at IsoTrace, with stable isotope analyses of mammal bones reported in 2005. All samples were from Amoreira trench CAM-01, an area of mixed human and faunal bone to a depth of 70 cm (Rolão and Roksandic 2007). The atomic %C/N ratios indicate poor preservation, although considerably better than that of Amoreira 2001 bone 46 reported in Table 2 above, a weathered robust long bone fragment, from square A1-2, well separated from the materials firmly identified as human (Rolão and Roksandic 2007, Fig. 15.4). Bone 46 fitted with no other fragment (Roksandic in litt. 15 June 2005), was a small fragment, and very weathered, unlike all other bones in the trench (Roksandic, 2006).

Date (BP)	IsoTrace #	Waterloo #	Square/ Level	%C	%N	$\delta^{13}C$	$\delta^{15}N$	atomic % C/N
5710±170	TO-11860	103258	A1-1/L2	5.9	1.74	-21.48	5.86	3.96
5970±70	TO-11861	103259	A1-3/L3	18.55	5.8	-19.61	8.51	3.73
6990 ±60	TO-11862	103260	A1-3/L4	23.2	7.48	-19.52	7.56	3.62
		103260 repeat	A1-3/L4	24.11	7.89	-19.19	7.74	3.57

Table 4: Mammal bones from Cabeço da Amoreira, Trench CAM-01. Submission by Mirjana Roksandic, cited with permission.

In summary, we have provided all available data on the Muge Mesolithic bone samples analyzed in Canada over many decades, including unpublished information, and announced the re-analysis of a problematic sample of some significance, a small child *in situ* in the basal sands at Cabeço da Amoreira. We have emphasized the importance of providing full information on human bone samples analyzed over a span of many years and confirmed that none of our stable isotope data derive from the AMS dating process.

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