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Mesolithic human occupation and seasonality: sclerochronology, $\delta^{18}\text{O}$ isotope geochemistry, and diagenesis verification by Raman and LA-ICP-MS analysis of *Argyrosomus regius* (meagre) *sagittae* otoliths from layer 1 of Cabeço da Amoreira Mesolithic shell midden (Muge, Portugal)

AQ1

Rita AQ2 Dias, 1 

Email ritadupontdias@gmail.com

Juan Estrella-Martínez, 2

Paul Butler, 2

Alexandra Nederbragt, 3

Ian R. Hall, 3

Pedro Barrulas, 4

Anne France Maurer, 4

Ana Mafalda Cardeira, 4,5

José Mirão, 4

Cleia Detry, 6

Nuno Bicho, 1

¹ Interdisciplinary Center for Archaeology and Evolution of Human Behavior (ICArEHB) AQ3, Universidade do Algarve, Faro, Portugal

² School of Ocean Sciences, Bangor University, Bangor, Wales, UK

³ School of Earth and Ocean Sciences, Cardiff University, Cardiff, Wales, UK

⁴ Hercules Laboratory, Universidade de Évora, Évora, Portugal

⁵ Artistic Studies Research Center (CIEBA/FBAUL), Largo da Academia Nacional de Belas-Artes, 1249-058 Lisbon, Portugal

⁶ Faculdade de Letras da Universidade de Lisboa, Centro de Arqueologia da Universidade de Lisboa (UNIARQ), Lisbon, Portugal

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Abstract

We present preliminary sclerochronological analysis on 15 *Argyrosomus regius* (meagre) otoliths collected from two different human occupation levels from the Cabeço da Amoreira shell midden (Muge valley, Portugal). The otoliths were sectioned and observed under a reflected light stereomicroscope to examine seasonal growth rings. Carbonates collected from individual growth rings subsampled with a micromill sampling device were analyzed for their stable oxygen isotope content with the objective of determining the predominant season of capture and therefore season of site use, environmental conditions, and sites of resource procurement (local versus regional). The otoliths' stable isotope records show clear seasonality and a season of capture estimate that seems consistent with a "good season" (warmer season, i.e., from spring to late summer/beginning of autumn in this area) site use in the last occupation layer (the great majority of otoliths are from layer 1), except for four samples giving heavier oxygen isotope ($\delta^{18}\text{O}$) values that suggest colder conditions and only one with markedly positive $\delta^{18}\text{O}$ values. Potential effect of diagenesis on the otolith records was also assessed through coupled laser ablation–inductively coupled plasma mass spectrometry (LA-ICP-MS) and by micro-X-Ray diffraction (μ -XRD). Results revealed the presence of only aragonite and no traces of calcite, providing no evidence of diagenesis that could significantly alter isotope results and lead to erroneous interpretations. The implications of these results are discussed and compared with data from other archeological sites, as well as data from micromorphology regarding continuity or interruption of site use and other faunal remains.

Keywords

Otoliths

Seasonality

Mesolithic
Shell middens
Argyrosomus regius
Diagenesis

Introduction

The understanding of lifestyles of hunter-gatherer communities, especially the so-called complex hunter-gatherers, has become central to contemporary archaeological investigations. This has resulted from the clarification of what defines complexity and from the review of new and old models of the emergence of complexity (Arnold, 1996).

These models focus on phenomena like changes in settlement patterns, mobility and the broadening in subsistence, and advances in technology that all influence each other (together with other influences like ideology, which also have an influence the). Mesolithic populations are a good template for what are considered to be complex hunter-gatherers, with the so-called complex characteristics becoming clearer in the archaeological context (Neeley and Clark, 1990; Spikins, 2009).

Understanding human subsistence, settlement, and mobility during the Mesolithic is one of the key research topics in the context of human behavior changes and adaptation during the onset of the Holocene climatic shifts. The Muge shell middens, from the Portuguese Iberian Atlantic coast, have been pivotal in the investigation of these topics and have provided a very rich set of relevant data through several excavations that started in the mid-nineteenth century and continued through to the more recent excavations with new high-resolution methods.

From these archaeological contexts, zooarchaeological investigations, particularly in the Cabeço da Amoreira shell midden site, have provided a key information of both terrestrial and aquatic resources exploited by the Mesolithic populations and have also provided important insights into their settlement and mobility. However, beyond such traditional taxonomic analysis, sclerochronological studies are beginning to provide additional robust environmental data (e.g., seasonality) that are proving fundamental to address human settlement and mobility. Nonetheless, these sclerochronological investigations, especially from the late Pleistocene/Early Holocene archaeological context, are still in their early infancy and require additional systematic investigations to validate such approaches.

The archaeological record of the Iberian Atlantic Ocean coast at the beginning of the early Holocene Atlantic period (c. 8–5 ka BP) is marked by a shift in both human settlement and—although only partially—subsistence and technology in the Portuguese territory (Bicho, 2009, Bicho, 1994; Bicho and Haws, 2008). By then, human occupation had clearly shifted from coastal areas to river estuaries, such as the Tagus and Sado, and a change is observed in the nature of sites in the Tagus river, which consist of very large shell middens with long and complex stratigraphy marked by a matrix of shell remains and includes habitational structures and burials (Bicho et al., 2009). Nevertheless, changes seem to have been more profound with respect to settlement and technology than with respect to species composition, since the terrestrial species used at the sites are generically the same (red deer, wild boar, aurochs, rabbit) (Detry, 2007) and the same can be said for shellfish (peppery furrow shell, common cockle, and grooved carpet shell) (Lentacker, 1986a, b). The novelty comes with the much more broad and intense utilization of fish (Dias et al., 2016, Dias et al., 2015) and the abandonment of species not so easily accessible in these new territories like the common mussel and goats (Bicho, 2009; Detry, 2007).

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Mesolithic subsistence in the shell midden complex of Muge, although using a broad spectrum of terrestrial and aquatic resources, involved a particularly strong component of aquatic resources as proven by both human stable isotope and oligoelement (carbon and nitrogen) results (Umbelino, 2006) and faunal remains (Detry, 2007; Dias et al., 2015; An Lentacker, 1986a, b; Lentacker, 1994). This included not only shellfish but also an important contribution of fish, comprising a wide variety of *taxa* (*vide* Table 1).

Table 1

Fish species distribution by layer from the Cabeço da Amoreira 2008–2015 excavations

Order AQ6	Family	Taxa	1	2	3	4	Comb1
			18	8	2	1	1
Pleurotremata		Pleurotremata	1	0	0	0	1
	Carcharhinidae	Carcharhinidae	23	13	1	1	0
		<i>Carcharhinus plumbeus</i>	1	5	1	0	0
		<i>Carcharhinus</i> sp.	2	1	0	0	0

Order AQ6	Family	Taxa	1	2	3	4	Comb1
			18	8	2	1	1
		cf. <i>Carcharhinus plumbeus</i>	0	1	0	0	0
	Lamnidae		2	0	1	0	0
		<i>Carcharodon carcharias</i>	0	1	0	0	0
	Odontaspidae		0	1	0	0	0
	Sphyrnidae	<i>Sphyrna cf. lewini</i>	0	1	0	0	0
			48	78	13	11	1
Hypotremata	Myliobatidae	<i>Myliobatis aquila</i>	84	119	20	4	1
		Myliobatidae	9	12	3	1	0
Perciformes			1	0	0	0	0
	Salmonidae	Salmonidae	0	1	0	0	0
	Moronidae	<i>Dicentrarchus labrax</i>	41	139	17	33	1
		<i>Dicentrarchus</i> sp.	3	0	0	0	0
		cf. <i>Dicentrarchus labrax</i>	9	25	3	6	0
	Clupeidae	Clupeidae	2	11	1	2	0
		<i>Alosa</i> sp.	15	50	7	26	2
		cf. <i>Alosa</i>	2	10	1	4	0
		<i>Sardina pilchardus</i>	0	0	0	0	0
	Anguillidae	<i>Anguilla anguilla</i>	0	0	0	0	0
	Sciaenidae	<i>Argyrosomus regius</i>	55	89	5	8	1
		cf. <i>Argyrosomus regius</i>	9	19	0	0	0
	Mugilidae	cf. Mugilidae	16	61	7	13	0

Order AQ6	Family	Taxa	1	2	3	4	Comb1
			18	8	2	1	1
		<i>Mugil cephalus</i>	9	9	2	5	0
	Mugilidae		197	618	74	115	7
		<i>Chelon labrosus</i>	9	11	3	4	0
		cf. <i>Chelon labrosus</i>	0	1	0	3	0
		cf. <i>Liza</i>	11	90	2	10	0
		cf. <i>Liza aurata</i>	0	2	1	0	0
		cf. <i>Mugil cephalus</i>	4	1	0	4	0
		<i>Liza aurata</i>	15	7	3	1	0
		<i>Liza</i> cf. <i>ramada</i>	1	0	0	0	0
		<i>Liza ramada</i>	30	8	1	0	0
		<i>Liza</i> sp.	377	1315	172	193	1
Cyprinidae	Cyprinidae		3	3	2	0	1
		cf. Cyprinidae	0	0	0	0	0
Pleuronectidae	cf. Pleuronectidae		0	2	0	0	0
		<i>Platichthys flesus</i>	0	1	0	0	0
		Pleuronectidae	3	5	1	1	1
		<i>Hippoglossus hippoglossus</i>	2	0	0	0	0
Soleidae	Soleidae		2	9	0	5	0
Serranidae	Serranidae		1	39	6	10	0
		cf. Serranidae	1	11	3	7	0
Sparidae	cf. Sparidae		2	9	2	0	0
		Sparidae	7	3	1	3	0
		<i>Sparus aurata</i>	14	47	9	3	0
		<i>Sparus</i> cf. <i>aurata</i>	0	4	1	1	0

Order AQ6	Family	Taxa	1	2	3	4	Comb1
			18	8	2	1	1
		<i>cf. Sparus aurata</i>	0	3	0	2	0
	Congridae	<i>Conger conger</i>	1	7	1	0	0
	Gadidae	Gadidae	1	0	0	0	0
			58	208	39	39	4
			1089	3058	405	516	22

AQ5

Otoliths are accretionary structures that are part of the orientation, motion, and auditory system of teleost fish—are important sources of information for archaeologists. Widely analyzed in modern fisheries investigations, they have been continuously growing in importance in archaeological and paleoenvironmental investigations, especially since the mid-twentieth century (Casteel, 1976; Disspain et al., 2011, 2015). Otoliths have been used as an accurate indicator of the age of fish since the late nineteenth century (Casteel, 2011, Casteel, 1976). The array of information they potentially record has been explored in the literature over the last decade and ranges from fish population structure and environmental reconstruction to very specific information about past occupation of a site, such as seasonality, subsistence, fishing strategies and methods, human adaptation to environmental change, and even trading routes (Andrus, 2011; Begg et al., 2005; Higham and Horn, 2000; Robson et al., 2012, 2015).

It has long been known that calcium carbonate (CaCO_3) is deposited on the surface of the otolith at seasonally variable rates, creating (in temperate regions) translucent and opaque zones representing fast (warm season) and slow growth (cold season) (Casteel, 1976), that could however be influenced by stress, gonad development, or starvation during spawning and therefore coupling otolith opacity and stable oxygen isotope composition can, and should, be used to estimate time of zone formation (Hoie and Folkvord, 2006). In fact, the regular deposition of increments makes them ideal for determination of fish age, growth, and diet (Radtke et al., 1996; Robson et al., 2012, 2015). Nevertheless, there have been few successful attempts to determine season of capture solely by the counting and interpretation of growth rings, especially the last increment (Higham and Horn, 2000; Van Neer et al., 1993). This lack of success has

several causes (a) growth rings that seem to be obvious in modern otoliths are not always as distinct in archaeological specimens because of a number of taphonomical issues, including the fact that the important area at the edge of the otolith is commonly damaged in these specimens; (b) the band counting technique is prone to error when carried out by an inexperienced researcher and the lack of archaeological reference material makes it difficult to obtain the necessary practice; (c) a large number of specimens are required; and (d) analysis becomes more difficult in longer lived animals since annuli tend to crowd towards the edge of the otolith. These difficulties are addressed by having multiple otolith readers (Beamish and McFarlane, 1983; Campana, 2001; Campana et al., 1995), the use of stable isotope geochemistry, and, to a lesser extent, the knowledge of ecology and biology of the species in question.

Otoliths are ideal structures for stable isotope analysis, being hard structures of somewhat long-lived organisms with marked growth rings (Hoie and Folkvord, 2006; Iacumin et al., 1992; Kalish, 1991; Radtke et al., 1996). Growth starts from before hatching to death through continuous deposition of CaCO_3 (Campana and Neilssra, 1985) that is metabolically inert and therefore independent of the subsequent physiological state of the fish since it cannot be remodeled or reabsorbed (Campana and Neilssra, 1985).

The measurement of oxygen isotope ratios ($\delta^{18}\text{O}$) in the CaCO_3 from the last growth increment of a shell or otolith has proven to be a successful approach for the assessment of temporal site occupation patterns in ancient communities (Monks, 1981; McKechnie and Moss, 2016; Shackleton, 1973; Wang et al., 2013). The use of this technique is based on the fact that the $\delta^{18}\text{O}$ of aquatic biogenic carbonate, in this case aragonite, is controlled by the $\delta^{18}\text{O}$ of the ambient water (a function of water temperature and salinity) from which the carbonate secretion occurs (Colonese et al., 2009; Iacumin et al., 1992; Radtke et al., 1996; Salgueiro et al., 2010; Thorrold et al., 1997; Wefer and Berger, 1991). CaCO_3 of otoliths accumulates in a continuous fashion over time. It is therefore possible to establish seasonal changes on oxygen isotopes and, although sometimes generically, the season of capture (Iacumin et al., 1992, p. 537).

AQ7

The use of carbon isotope ratio $\delta^{13}\text{C}$ in fossil otoliths, on the other hand, is more problematic due to its precipitation out of equilibrium with the total dissolved inorganic carbon species in sea water and variable “vital effect,” between different species and within the different individuals of the same species (Iacumin et al., 1992, p. 537). Some researches have pointed to the metabolic contribution of these values, since the long-term record of metabolic rates can produce important information on the evolutionary history of fish physiological

characteristics and their bioenergetic processes (Wurster and Patterson, 2003). $\delta^{13}\text{C}_{\text{max-min}}$ values have a significant negative covariation relation with $\delta^{18}\text{O}$ (CaCO_3) that suggest a more important change in metabolic rate through the fish's life in colder climates, characterized by shorter growth periods or a decrease of the summer-winter precipitation ratios. The magnitude of this variation is larger in the early ontogeny of the fish (Wurster and Patterson, 2003). In short, the isotopic composition of biomineral carbon varies according with average metabolic ratio and expresses the individual's metabolic response, consequence of the combination of physical and ecological setting. However, some researchers have advised against the use of this data for interpretation of metabolic rate when the composition of diet and dissolved inorganic carbon cannot be simultaneously determined (which is the case of this investigation) (Trueman et al., 2016). Therefore, these data was not used at this time.

The growing importance of stable isotopes methods comes from the emphasis given to economic assessments of prehistoric (and historic) populations and the necessity of having knowledge of temporal patterns of exploitation of certain resources in order to determine mobility and site use patterns associated with either seasonal or permanent (all year round) occupations. Likewise, the necessity for baseline isotopic data to reconstruct human diet has led to increasingly frequent isotope analysis (Richards and Hedges, 1999; van der Merwe et al., 2003). Significant developments in the area have come chiefly from fisheries research focused mainly on paleoenvironmental reconstructions and age determination for fish conservation and stock assessments (Assis, 2000; Griffiths and Hecht, 1995; Prista et al., 2009; Surge and Walker, 2005; Wang et al., 2011; Wurster and Patterson, 2001). Notwithstanding, some significant developments have been made in the application of the techniques in archaeological specimens for paleoenvironmental and seasonality investigations in shellfish (Colonese et al., 2012, Butler et al., 2009; Jones et al., 2008; Kennett and Voorhies, 1996; Mannino et al., 2003; Mannino et al., 2007; Quitmyer et al., 1997; Wang et al., 2013) and fish (Brewer, 1987; Davis et al., 2003; Higham and Horn, 2000; Robson et al., 2016; Wang et al., 2013; Wurster and Patterson, 2003) and **this "and" should be erased and a comma should be put in its place** size estimation in fish (Gabriel et al., 2012), and the importance of fish remains for reconstruction of environment and subsistence has now long been recognized (Hufthammer et al., 2010; McKechnie et al., 2014).

AQ8

Quantitative and qualitative data for fish in Mesolithic Portuguese sites is available, especially for the Sado's shell middens (Gabriel, 2015, Gabriel, 2011, 2007; Moreno García, 2010) and those in Muge (Dias et al., 2015; Lentacker, 1986a, b; Lentacker, 1994), as well as some assessments of subsistence

strategies, namely about size reconstructions of *Argyrosomus regius* specimens in Mesolithic and Epipaleolithic sites (Gabriel et al., 2012). However, data about temporal exploitation patterns in Muge is still scarce and mostly based on terrestrial faunal remains (Detry, 2008, Detry, 2007, 2000; Dias et al., 2015).

AQ9

AQ10

Here we examine seasonality of the last Mesolithic occupation of the Cabeço da Amoreira shell midden through the study of *A. regius* otoliths. This will provide a new insight to improve the reconstruction of human occupation patterns (season of capture) during the Mesolithic of the Iberian Atlantic Ocean coast.

We also ascertain the validity of isotopic data through testing diagenesis with Raman and laser ablation–inductively coupled plasma mass spectrometry (LA-ICP-MS) analysis that have showed that no significant alterations occurred that could change isotopic values in a significant way, leading to erroneous interpretations.

Archaeological context

The Cabeço da Amoreira Shell midden is one of several shell middens in the Muge complex, known and excavated since the nineteenth Century. The middens are characterized by very large accumulations of shells in layers sometimes as large as 60/70 m in diameter and up to 4 m in height and containing more than 300 human skeletons (Athayde, 1940; Cunha and Umbelino, 1995; Umbelino, 2006). That being said, pieces of information about some of these middens are from the first excavations and a number of these have subsequently been destroyed or lost (Bicho et al., 2011, Bicho et al., 2010). Their characteristics initially lead to the assumption that these sites could be the consequence of extensive residential occupations prolonged in time, with possible seasonal rotations between them (Arnaud, 1993, Arnaud, 1987, Arnaud, 1986).

Additionally, Rolão (1999) argued that there were two types of midden: larger middens (like Cabeço da Amoreira) with residential characteristics and smaller middens with logistic functions.

AQ11

AQ12

AQ13

Cabeço da Amoreira is one of the largest shell middens with c. 60 m in diameter and c. 2.5 m to c. 3.5 m, located in one of the Tagus subsidiaries, the Muge River (Fig. 1). Starting with the 2008 project, a large (72 m²) excavation area was opened on the southwest quadrant (Fig. 2), along with profile cleaning of the old

excavations for a better vision of the stratigraphy and AMS dating (Table 2) that dated the beginning of occupations to c. 7.9 Ka BP (Bicho et al., 2011). Also, microstratigraphy studies revealed an intricate and laterally variable stratigraphic succession of layers and lenses that allowed new insights into site formation and anthropogenic activities at the site, suggesting periods of abandonment through nondepositional hiatuses and discrete geogenic sedimentation (Aldeias and Bicho 2016).

Fig. 1

Location of the Cabeço da Amoreira shell midden. Map with the Mesolithic shell middens in the Muge region. 1. Fonte da Moça I; 2. Fonte da Moça II; 3. Fonte do Padre Pedro; 4. Flor da Beira; 5. Cabeço da Arruda; 6. Moita do Sebastião; 7. Cabeço da Amoreira; 8. Cova da Onça; 9. Monte dos Ossos; 10. Magos de Cima; 11. Cabeço da Barragem; 12. Cabeço dos Morros; 13. Magos de Baixo adapted from André and Bicho (2016) and Bicho et al. (2013)

AQ14

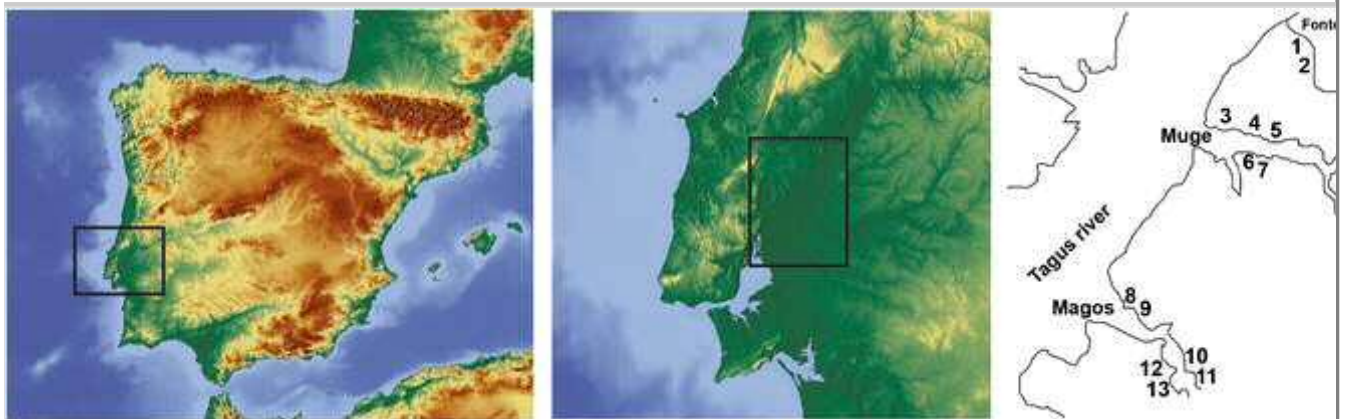


Fig. 2

Plan view of the excavation and testing in Cabeço da Amoreira adapted from Aldeias and Bicho (2016) and Bicho et al. (2013)

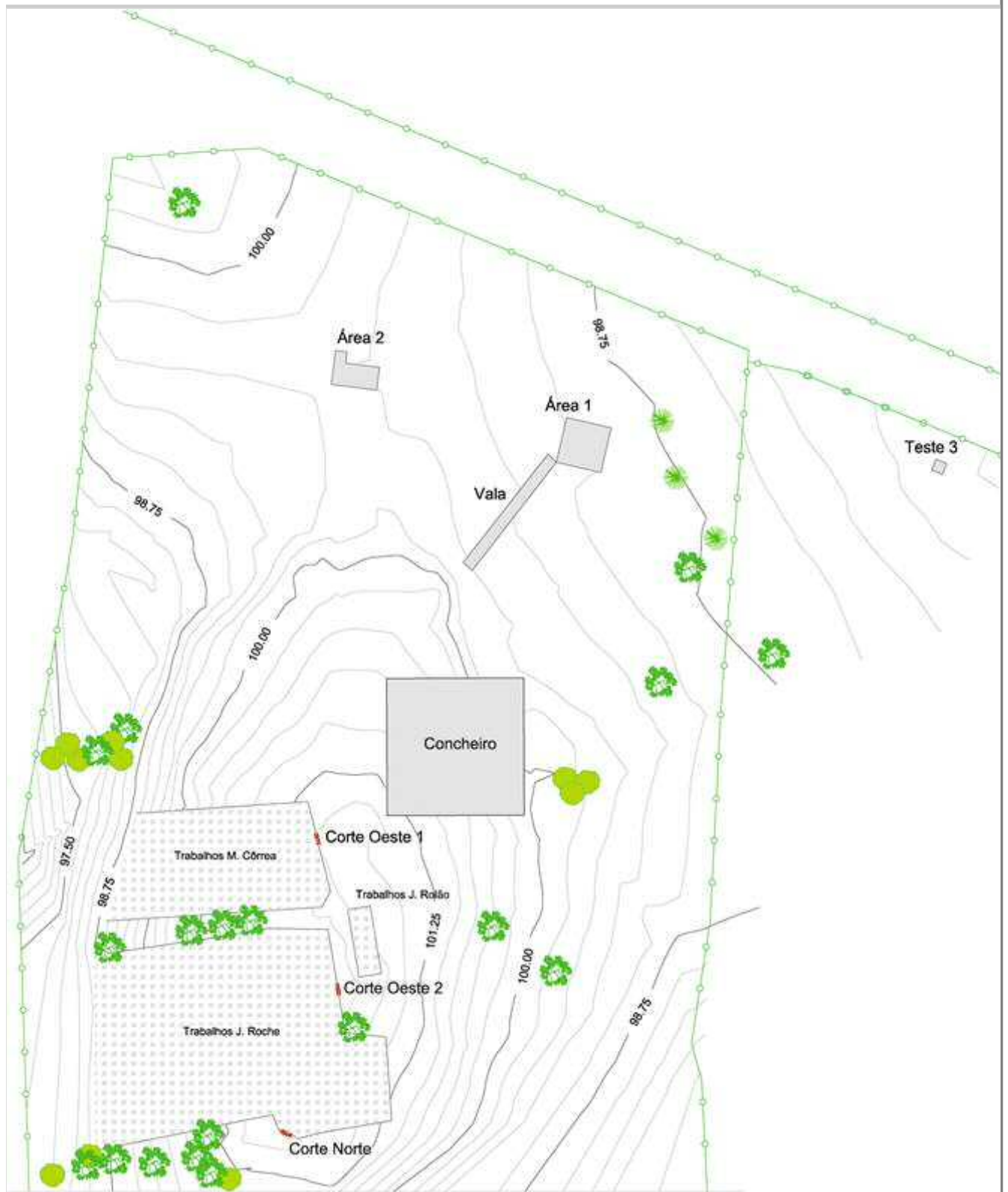


Table 2

AMS determinations from Cabeço da Amoreira according to Bicho et al., 2013

AQ15

Provenience	Context	Lab code	Material	Date BP	Cal BP 2σ	Posterior probability cal BP 2σ	F

Provenience	Context	Lab code	Material	Date BP	Cal BP 2σ	Posterior probability cal BP 2σ	F
Multiple burial CAM-01-01	Cairn	WK-26796	<i>Homo sapiens</i> , bones	6329 ± 40	7145–6860	6329 ± 40	B (2)
Multiple burial CAM-01-01	Cairn	TO-10225	<i>Homo sapiens</i> , bones	6550 ± 70	7570–7325	6550 ± 70	R (2) A
Multiple burial CAM-01-01	Cairn	TO-10218	<i>Homo sapiens</i> , bones	6630 ± 60	7435–7185	6630 ± 60	R (2) A
Camada 3 (=camada 1b)	Cairn	Sac-2079	Shell	7050 ± 45	7545–7300	7050 ± 45	M al A
Camada 3 (=camada 1b)	Cairn	Sac-2080	Shell	7080 ± 80	7595–7275	7080 ± 80	M al A
Camada 1b	Cairn	WK-26798	<i>C. edule</i>	7145 ± 37	7590–7410	7145 ± 37	B (2)
Camada 3 (=camada 1b)	Cairn	Sac-2023	Shell	7260 ± 60	7740–7460	—*	M al A
Camada 1b	Cairn	WK-26797	<i>C. edule</i>	7291 ± 35	7510–7730	—*	B (2)
Burial 2011.2—layer 2b	Shell layers	Wk-32142	<i>Homo sapiens</i> , rib	6910 ± 40	7565–7430	7555–7455	B (2)
?	Shell layers	Beta-127450	<i>Homo sapiens</i> , bone	6850 ± 40	7565–7430	7575–7475	M et (2) A
Camada 22	Shell layers	Wk-30674	<i>Scrobicularia plana</i>	7356 ± 33	7800–7575	7685–7600	B (2)
Camada 22	Shell layers	Wk-30672	<i>Scrobicularia plana</i>	7360 ± 34	7805–7575	7685–7600	B (2)
Camada B	Shell layers	Wk-28050	<i>Scrobicularia plana</i>	7377 ± 33	7820–7590	7685–7600	B (2)
Camada C	Shell layers	Wk-28049	<i>Scrobicularia plana</i>	7193 ± 33	7900–7660	—*	B (2)

*Calibration with IntCal09 and Marine09 curves (Reimer, et al., 2009) using OxCal 4.1 (Ramsey, 1995). ΔR value used in the Homo samples of 140±40 from Martins et al., 2008

**Outlier results

Provenience	Context	Lab code	Material	Date BP	Cal BP 2 σ	Posterior probability cal BP 2 σ	F
Camada D	Shell layers	Wk-28048	<i>Scrobicularia plana</i>	7445 ± 33	7630–7435 AQ22	—*	B (2)
Camada F	Shell layers	Wk-28047	<i>Scrobicularia plana</i>	7376 ± 34	7820–7590	7685–7615	B (2)
Camada G	Shell layers	Wk-28046	<i>Scrobicularia plana</i>	7368 ± 39	7815–7580	7685–7620	B (2)
Camada H	Shell layers	Wk-28045	<i>Scrobicularia plana</i>	7315 ± 35	7765–7550	7690–7620	B (2)
Camada H	Shell layers	Wk-28044	<i>Scrobicularia plana</i>	7311 ± 34	7760–7545	7690–7625	B (2)
Camada I	Shell layers	Wk-28043	<i>Scrobicularia plana</i>	7273 ± 34	7710–7500	7695–7630	B (2)
Camada J	Shell layers	Wk-28042	<i>Scrobicularia plana</i>	7323 ± 48	7795–7535	7695–7635	B (2)
Camada K	Shell layers	Wk-28041	<i>Scrobicularia plana</i>	7384 ± 48	7845–7580	7700–7640	B (2)
Camada K	Shell layers	Wk-28040	<i>Scrobicularia plana</i>	7305 ± 48	7770–7515	7705–7645	B (2)
Camada L	Shell layers	Wk-28039	<i>Scrobicularia plana</i>	7395 ± 48	7855–7585	7710–7650	B (2)
Camada M	Shell layers	Wk-28038	<i>Scrobicularia plana</i>	7365 ± 49	7825–7570	7715–7650	B (2)
Camada N	Shell layers	Wk-28037	<i>Scrobicularia plana</i>	7307 ± 48	7775–7515	7720–7655	B (2)
Camada O	Shell layers	Wk-28036	<i>Scrobicularia plana</i>	7251 ± 48	7706–7465	7725–7655	B (2)
Camada O	Shell layers	Wk-28035	<i>Scrobicularia plana</i>	7395 ± 48	7855–7585	7730–7655	B (2)
Camada P	Shell layers	Wk-28034	<i>Scrobicularia plana</i>	7370 ± 48	7830–7570	7745–7660	B (2)
Camada 2	Shell layers	Wk-30671	<i>Scrobicularia plana</i>	7417 ± 34	7855–7615	7745–7660	B (2)
Camada 2	Shell layers	Wk-30673	<i>Scrobicularia plana</i>	7406 ± 32	7855–7615	7745–7650	B (2)

*Calibration with IntCal09 and Marine09 curves (Reimer, et al., 2009) using OxCal 4.1 (Ramsey, 1995). ΔR value used in the Homo samples of 140 ± 40 from Martins et al., 2008

**Outlier results

Provenience	Context	Lab code	Material	Date BP	Cal BP 2 σ	Posterior probability cal BP 2 σ	R
Camada Q	Bellow shell layers	Wk-28033	<i>Scrobicularia plana</i>	7479 \pm 48	7930–7670	7810–7695	B (2)
Burial 2011.1	Bellow shell layers	Wk-32143	<i>Homo sapiens</i> , tibia	7132 \pm 41	7785–7595	7775–7675	B (2)
Burial CAM-00-01	Bellow shell layers	TO-11819-R	<i>Homo sapiens</i> , bone	7300 \pm 80	8015–7695	7840–7710	R (2) A
Hearth 1	Bellow shell layers	UGAMS-7196	Charcoal	6990 \pm 30	7930–7735	7865–7725	B (2)
Pit. 1	Bellow shell layers	UGAMS-7197	<i>Scrobicularia plana</i>	7450 \pm 30	7900–7665	7900–7735	B (2)

*Calibration with IntCal09 and Marine09 curves (Reimer, et al., 2009) using OxCal 4.1 (Ramsey, 1995). ΔR value used in the Homo samples of 140 ± 40 from Martins et al., 2008

**Outlier results

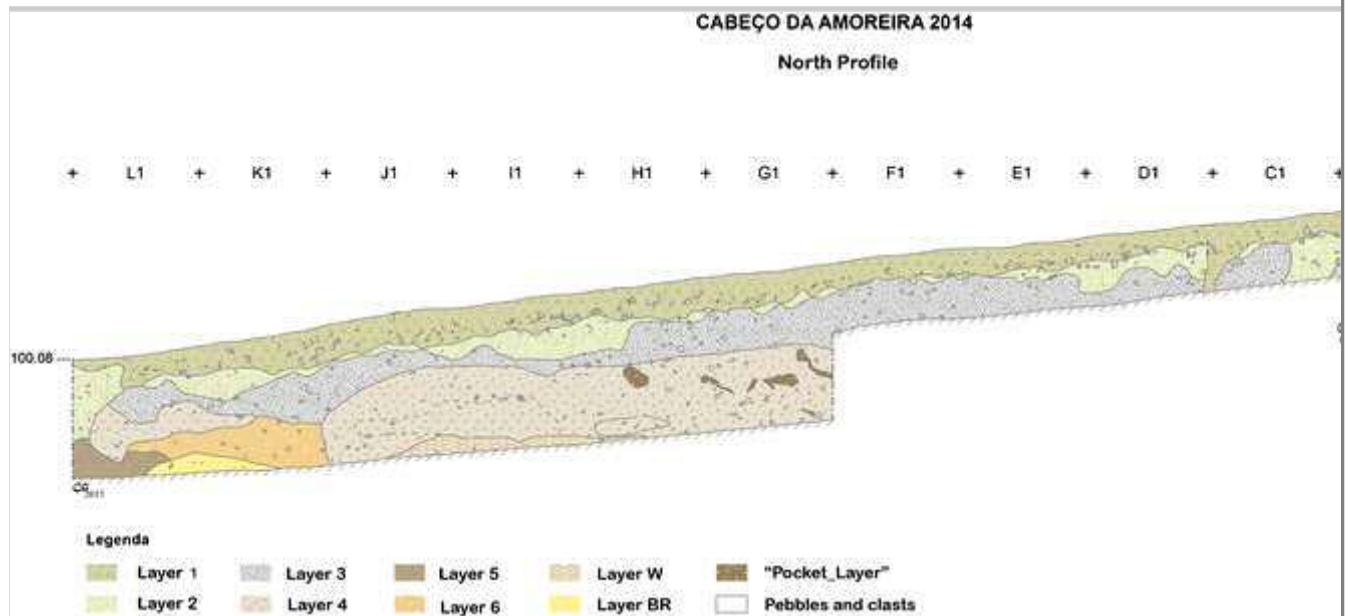


Thus far, four main layers have been identified, with lateral sedimentary variations being attributed an alphabetic letter and sequential number for each 5-cm spit (Fig. 3). In addition, several test trenches were opened around the main excavation area, one of them 12-m long (Fig. 2) (Aldeias and Bicho, 2016; Bicho et al., 2010). The layers in the main area seem to have accumulated quite rapidly from c. 7900 to 7500 cal BP (Bicho et al., 2013). Also, other archaeological horizons with several layers were uncovered to the south of the main area, with no shell or faunal remains.

Fig. 3

Stratigraphic section of the north profile of the CAM excavation area in 2014 adapted from André and Bicho (2016) and Bicho et al. (2013)

AQ24



There are occasional pottery sherds in the mound surface, as well as Neolithic human burials, with one individual whose diet isotopic signal, mainly from terrestrial origin, contrasted with all other individuals in the older layers whose diet signal showed around 50% aquatic origin (Table 3) (Bicho et al., 2013; Umbelino, 2006). AMS dating for the skeletons is consistent with other Neolithic sites in the region which probably accounts for a Neolithic use of the site, after 7.4 Ka BP, sometimes intruding in the cairn layer.

Table 3

Percent of marine diet in human skeletons of Cabeço da Amoreira in Bicho et al. (2013)

Context	Lab code	Posterior probability (cal BP- 2σ)	$\Delta^{13}\text{V}\text{C}$ (‰)	$\Delta^{15}\text{N}$ (‰)	% Marine diet
Burial 2011.2-Camada 2b	Wk-32142	7560–7455	– 15.78	12.82	58

Dates calibrated with OxCal 4.1 (Bronk Ramsey, 1995) based on the IntCal09 (Reimer et al. 2009). ΔR value of 140 ± 40 from Martins et al. (2008)

AQ25
AQ26
AQ27
AQ28

Context	Lab code	Posterior probability (cal BP- 2σ)	ΔC (‰)	ΔN (‰)	% Marine diet
Burial 2011.1. base Jean Roche	Wk-32143	7780–7675	– 15.97	13.86	56
Multiple burial CAM-01-01	Wk-26796	7160–6910	– 16.9	12.3	46

Bellow shell midden	TO11819-R	7840–7710	– 16.3	–	50
Skeleton 7	Beta-127450	7575–7475	– 16.5	11.9	48
Multiple burial CAM-01-01	TO-10218	7425–7185	– 17.1	–	42
Multiple burial CAM-01-01	TO-10225	7475–7315	– 20.1 – 21.8	8.2 4.9	0 0
Skeleton 4	–	–	– 15.7	12.7	59
Skeleton 6	–	–	– 14.8	12.5	69
Skeleton 8	–	–	– 15.6	12	60
<p>Dates calibrated with OxCal 4.1 (Bronk Ramsey, 1995) based on the IntCal09 (Reimer et al. 2009). ΔR value of 140 ± 40 from Martins et al. (2008)</p> <p>AQ25 AQ26 AQ27 AQ28</p>					

The layer pertinent to this study is layer 1. It was excavated in an area of 12×12 m in 2008 to 12×9 in 2009 and 12×6 in 2010 and 2011 and mainly consists of abundant quartzite pebbles, some of them altered by fire, incipient soil formation (Aldeias and Bicho, 2016; Bicho et al., 2011, 2010), some shells, and 1089 fish remains (especially vertebrae) (Tables 1 and 4).

Table 4

Anatomical elements by species in layer 1

Taxa	Pectoral fin	Spine (neural/haemal)	Basioccipital	Cranium	Dentary
	0	0	0	0	0
<i>Pleurotremata</i>	0	0	0	0	0
<i>Carcharhinidae</i>	0	0	0	0	0
<i>Carcharhinus</i> sp.	0	0	0	0	0
cf. <i>Carcharhinus plumbeus</i>	0	0	0	0	0
Hypotremata	0	0	0	0	0
<i>Myliobatis aquila</i>	0	0	0	0	0
Myliobatidae	0	0	0	0	0

Taxa	Pectoral fin	Spine (neural/haemal)	Basioccipital	Cranium	Dentary
Perciformes	0	0	0	0	0
<i>Dicentrarchus labrax</i>	0	0	1	0	0
<i>Dicentrarchus</i> sp.	0	0	0	0	0
cf. <i>Dicentrarchus labrax</i>	0	0	0	0	0
Clupeidae	0	0	0	0	0
<i>Alosa</i> sp.	0	0	0	0	0
cf. <i>Alosa</i>	0	0	0	0	0
<i>Argyrosomus regius</i>	0	0	1	0	0
cf. <i>Argyrosomus regius</i>	0	0	0	0	0
cf. Mugilidae	0	0	0	0	0
<i>Mugil cephalus</i>	0	0	0	0	0
Mugilidae	0	0	9	0	0
<i>Chelon labrosus</i>	0	0	0	0	0
cf. <i>Liza</i>	0	0	0	0	0
cf. <i>Mugil cephalus</i>	0	0	0	0	0
<i>Liza aurata</i>	0	0	0	0	0
<i>Liza</i> cf. <i>ramada</i>	0	0	0	0	0
<i>Liza ramada</i>	0	0	2	0	0
<i>Liza</i> sp.	0	0	0	0	0
Cyprinidae	0	0	0	0	0
Pleuronectidae	0	0	0	0	0
<i>Hippoglossus hippoglossus</i>	0	0	0	0	0
Soleidae	0	0	0	0	0
Serranidae	0	0	0	0	0

Taxa	Pectoral fin	Spine (neural/haemal)	Basioccipital	Cranium	Dentary
cf. Serranidae	0	0	0	0	0
cf. Sparidae	0	0	0	0	0
Sparidae	0	0	0	0	1
<i>Sparus aurata</i>	0	0	0	0	0
<i>Conger conger</i>	0	0	0	0	0
Gadidae	0	0	0	0	0
Unknown	1	2	1	1	0
	1	2	14	1	1

The quantity of these so-called thermoclasts ($N = > 80,000$) and its dispersion, all over the midden, the fact that there was no obvious mixing or intrusions, dip, and strike of artifacts was random, and the impossibility of them being naturally deposited lead to the conclusion that this could be a cairn, intentionally made to protect the shell midden (Bicho et al., 2010, 2013, in press; Bicho and Gonçalves, 2016.).

Environmental/climatic and hydrological setting

Until fairly recently, the Holocene epoch was believed to be a climatically stable period (McManus et al., 1999). However, recent high-resolution paleoenvironmental studies (e.g., high-resolution sediment paleoproxies, ice cores, dust records) have revealed a substantial climate at a range of timescales relevant to societal development within the last 11 ka BP (Rodrigues et al., 2009).

The development of Mesolithic technology and settlement systems on the Iberian Atlantic Ocean coast has been linked with climate variability within the Holocene, including (1) the appearance of a great estuarine lake in the Tagus valley during the Atlantic period as a consequence of increasing sea level, (2) subsequent geomorphological and coastal environment alterations, (3) the decrease in upwelling along the Atlantic Portuguese coast (Haws, 2003; Monge Soares, 2006, Monge Soares, 2005), (4) warm sea surface temperature levels for the Iberian Atlantic coast (Rodrigues et al., 2009), and finally (5) the 8.2-ka BP cold event (Bicho, 2009; Bicho et al., 2010).

Information on the paleoenvironmental conditions of the Atlantic Portuguese coast has been acquired through a series of terrestrial cores (Cearreta et al., 2003; da Conceição Freitas et al., 2003; Fletcher, 2005; Fletcher et al., 2010; Santos and Fernanda Sánchez Goñi, 2003; Schriek, van der Schriek, 2004; Schriek et al., Van Der Schriek et al., 2007; van der Knaap and van Leeuwen, 1997) and deep-sea cores (Bard et al., 1987; Fletcher et al., 2010; Park, 1998; Penaud et al., 2010; Roucoux et al., 2001; Schönfeld and Zahn, 2000; Turon, 2003; VOELKER et al., 2006; Zahn et al., 1997), macro- and microbotanical remains (Araújo and Almeida, 2013; Figueiral, 1993; Figueiral and Carcaillet, 2005; Figueiral and Terral, 2002; Haws, 2003; Monteiro, 2012; Van Leeuwaarden and Queiróz, 2003; Zambujo and Lourenço, 2002; Zilhão et al., 1996), and both macro- and microfaunal remains (Bicho, 2003; Bicho et al., 2011; Cardoso and Rolão, 1999; Dias et al., 2016; Haws and Valente, 2006; A. Lentacker, 1986a, b; An Lentacker, 1986a, b; Moreno-García, 2011; Póvoas, 2001),

Lentacker (1986a, b) concluded that the microfaunal assemblage of the Muge's shell middens was similar to species found in Pleistocene contexts with the presence of *Arvicola sapidus*, *Pytimis duodecimcostatus*, *Microtus agrestis*, *Apodemus sylvaticus*, and *Eliomys quercinus*. Data from the microfaunal assemblage of coeval sites in the Iberian Peninsula are consistent with this panorama (Araújo and Almeida, 2013, Andrus, 2011, Gabriel 2010, Moreno García, 2010, Póvoas, 2001).

AQ29

AQ30

Vegetation evolution seems to suggest a transition from a continental/dry climate to an oceanic/moist climate indicated by a mitigation of seasonal contrast (Fletcher et al., 2010). The Atlantic woods continued their evolution during the Boreal (9–8 ka BP), following the temperature and humidity rise and climatic stability characteristic of the Atlantic period. Nevertheless, there is always a higher presence of mixed complex forests in coastal areas like Guadiana and Sado (Costa Tenorio et al., 2005). Microfaunal data show that the identified species are consistent with temperate forest or Supra/Meso-Mediterranean forested environments.

AQ31

AQ32

During the Holocene, the Tagus hydrological regime was influenced by lithology regional tectonic climate (Julião et al., 1998). At present, the Tagus hydrographic basin ($\sim 80,629 \text{ Km}^2$) has a great variety of physiographic aspects, ranging from mountainous regions to low marginal areas, like the estuary (Costa,

1999). Floods are a common phenomenon in the Tagus basin partially because its geomorphological characteristics lead to surface runoff from rainwater resulting in water erosion processes that increase flood potential with variations in outflow throughout the year (Julião et al., 1998; Ramos and Reis, 2001) and also because of the climatic characteristics of the basin, with higher precipitation on the north edge (higher altitude). Precipitation is very irregular inter-annually and even monthly.

The interaction between the fluvial system and the ocean is influenced by both climatic variability and sea level variations (dos Santos, 2010).

Geological investigations (e.g., van der Schriek, 2004; van der Schriek et al., 2007; van der Schriek et al., 2008) and Soares on the ocean reservoir effect (Soares, 2005) in the Muge valley and by Rodrigues et al. (2009) and Santos in her PhD dissertation (dos Santos, 2010) have provided important information about the influence of brackish water and chemical salt water in the estuary. Rodrigues et al. (2009) and dos Santos (2010) evaluate this influence by presence of marine diatoms or marine affinity diatoms and C_{37} alkenones. Works of van der Schriek and Soares, on the other hand, established the beginning of the brackish water fill of the Muge valley between 8.3 and 8.0 ka BP (Bicho et al., 2010; van der Schriek et al., 2008) essentially coinciding with the shell middens' first occupation (Bicho, 2009).

AQ33

The decrease of the upwelling along the Portuguese coast certainly affected marine productivity in the Estremadura coast at the onset of the Holocene (Bicho, 2009; Bicho et al., 2010; Bicho and Haws, 2008). Although a seasonal phenomenon today, it was not a constant in both the late Pleistocene and the beginning of the Holocene, with a decrease in the *upwelling* starting at ~ 20.0 ka BP (when it was at its maximum) but still three to seven times greater than present day (Rodrigues et al., 2009; Salgueiro et al., 2010; dos Santos, 2010). The Pre-Boreal and the Boreal periods saw further decreases, corresponding to the bond 6 (9.4 ka BP) and bond 5 (8.2 ka BP) events, up to the beginning of the Atlantic period when values were lower than today's (Rodrigues et al., 2009; Salgueiro et al., 2010; dos Santos, 2010).

Holocene river water temperature records from several cores and particularly one at the superior area of the estuary, at Vila Franca de Xira (VFX—38° 56' 24" N, 8° 56' 19" W), have shown a decreasing temperature trend from the beginning of the Holocene, in agreement with records from other cores in the Tagus prodelta, like D13882 (38° 38.07' N, 9° 27.25' W) (Rodrigues et al., 2009),

showing that water coming from the river is generally colder than water in the Atlantic coast were the river drains to (Rodrigues et al., 2009; dos Santos, 2010).

Subtle variations in water surface temperature in Vila Franca de Xira (around 1–2 °C) at around 11.3–11.1, 10.5–10, 7.8, 6.6, 5.8, 5, 4.5, and 1.4 to 0.8 ka BP coinciding with the so-called Bond cool events in North Atlantic (Bond, 1997; dos Santos, 2010 along with decreased water surface temperatures led to dryer atmospheric conditions (Cacho et al., 1999; Frigola et al., 2007; McDermott et al., 2001; Rasmussen et al., 2006). Notwithstanding, when comparing water surface temperature values in the Tagus with the maximums of terrigenous contributions, it is possible to find five coincident events: 10.5, 7.8–7.7, 6.7–6.6, 5.9–5.8, and 5–4.5 ka BP, parallel to cold humid periods and flooding or at least increased activity periods in the Tagus (Benito et al., 2008, Benito et al., 2003; dos Santos, 2010, p. ii; Vis et al., 2010), making the Tagus an ecological niche, with a great concentration of food resources both aquatic and terrestrial (Bicho, 2009; Bicho et al., 2010).

Materials and methods

Sample

The majority of fish remains ($N = 5410$ from layers 1 to 4 until the 2014 excavations) consists of vertebral remains, with very few cranial elements ($N = 22$; Table 4) including otoliths from only one species: *A. regius* (Meagre) ($N = 35$). The majority of the otoliths were recovered from layer 1 (1 and 1B, $N = 26$), although they were also present in some of the other layers (layer 2 = 7, layer 4 = 1). This is probably due to *A. regius* otolith characteristics, being generically more robust than those of other species (Assis, 2000; Gabriel et al., 2012; Prista et al., 2009) and therefore better equipped for resisting varied post-depositional conditions. Other *A. regius* bones are also common in layer 1, but they are present in some other layers in larger numbers (e.g., layer 2 with $N = 89$ remains vs. $N = 55$ remains in layer 1 and a total of $N = 163$ through all layers).

We examined 15 *A. regius sagittae* otoliths recovered from layer 1. They were collected both by sieving of the 10-l buckets recovered in the field and as piece plotted elements (> 2 cm) with a total station.

They were stored in plastic zip lock bags and washed with cold tap water following transportation to the laboratory.

Otoliths were, as previously mentioned, chosen by their chronostratigraphic context from layer 1, the last Mesolithic occupation of Cabeço da Amoreira. The majority of the otoliths were juveniles, with two exceptions (2072 and 1982).

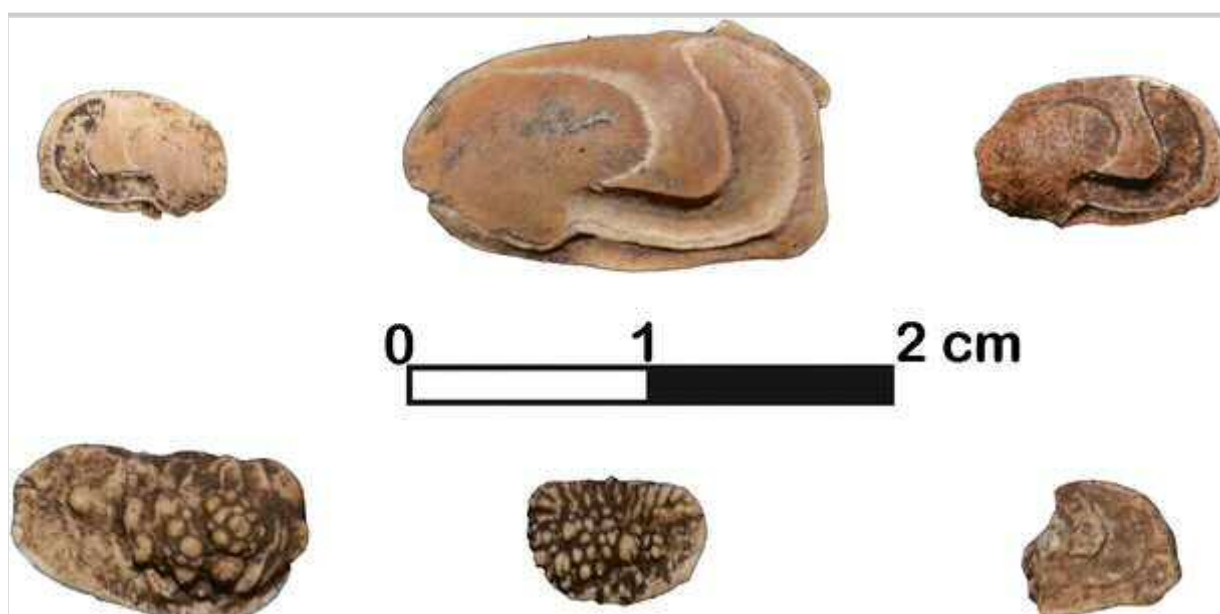
The two larger ones were sampled respectively 52 and 58 times along annual growth increments to ensure that there was a proxy/reference pattern for isotope values for the time frame and area that would allow a clearer interpretation of results from the smaller ones, sometimes less than 1-year-old.

Sampling preparation

The fact that these are archaeological samples, not always in pristine conditions (i.e., surface damage, edge damage etc.), made it necessary to adapt known methodologies (Prista et al., 2009) (Fig. 4).

Fig. 4

Examples of otolith conservation at Cabeço da Amoreira



Firstly, *A. regius* otoliths are too thick to directly use age determination with entire otoliths. Secondly, the need to sample for isotope analysis led to the necessity of establishing an ideal methodology for sectioning them. That decision was made based on a few parameters: (1) which plane (i.e., ventral-dorsal vs anterior-posterior) offered the best reading possibility, that is, a bigger portion of preserved edge (this being essential for seasonal interpretations)?; (2) which plane offered a larger area for sampling?

The next stage was embedding in polyester casting resin. We decided that complete embedding was a better method than partially embedding in clear adhesive on to standard microscope slides (Prista et al., 2009) since it would make the otoliths more stable and robust for sectioning and drilling and the required softening of the solid adhesive sticks over a hot plate before embedding

would risk further damage to the otoliths. Embedded samples were allowed 48 h to dry before sectioning.

Sectioning was accomplished with a low speed saw equipped with a fine-grit diamond-impregnated grinding wheel (3 mm thickness) and aimed to cut as close to the center of the otolith as possible.

Grinding and polishing were executed to remove coarse scratches that could make reading of the *annuli* more difficult. The embedded otoliths were ground using a sequence of coarser to finer carborundum paper on a rotor with running water and were finally polished with a 3- μm diamond paste, also on a rotor equipped with a velvet surface (Butler et al., 2009; Scourse et al., 2006).

The blocks that resulted from sectioning were then observed both under a stereomicroscope and a metallographic microscope (under transmitted and/or reflected light) to determine the readability of the samples for future sampling and ascertain the necessity of making a thin section as an image guide for drilling and estimate of age. Estimate of age (although not a primary goal of this investigation) was made under both transmitted and reflected light and, at this point, had only one reader and tree readings in separate days and aleatory order of sample reading. Also with this purpose, an acetate peel replicate from two otoliths was produced after etching with 0.1 M HCl for 15 and 30 min. It was concluded that thin sectioning would not improve or help significantly the reading/interpretation of annuli and would instead make it more time consuming and expensive. These otoliths were then re-polished prior to extracting the powder for isotope analysis.

AQ34

Sample powder (minimum mass of $\sim 50 \mu\text{g}$) was then milled with a New Wave microdrill using a carbide burr (300 μm). Rotation was kept at 8% (equivalent to $\sim 2967 \text{ rpm}$) to keep the aragonite to calcite conversion to a minimum and the samples were stored in low absorption weighting paper to avoid loss due to electrostatic charge (Foster et al., 2008).

AQ35

Isotope analysis

Stable isotope composition of the sample powder was measured on a Thermo MAT253 dual inlet mass spectrometer coupled to a Kiel IV carbonate preparation device at Cardiff University. Results are reported per mil (‰) notation on the Vienna-Pee Dee Belemnite (VPDB) scale. The 1-sigma external precision of an in-house carbonate standard was better than $\pm 0.05\text{‰}$ for $\delta^{18}\text{O}$ during the period that the samples were analyzed. Sample weight allowed

running a number of duplicates in some of the samples, generally with good reproducibility due to their quality (i.e., powder was homogeneous). Precision of replicate samples is $\pm 0.08\%$ for $\delta^{18}\text{O}$.

Diagenesis analysis

DAnalysis was conducted on the otoliths embedded in epoxy to assess the preservation vs alteration of the otolith samples, in order to confirm whether or not the $\delta^{18}\text{O}$ data were reliable for reconstructing a seasonality pattern during Mesolithic in Central Portugal. Testing diagenesis has been done in other occasions and the potential of a multitude of techniques as discussed (Cook et al., 2015; Disspain et al., 2011; Dufour et al., 2000).

1. Raman spectroscopy (microspectrometry)

μ -Raman analysis was performed to identify the mineralogical composition of the otoliths and especially to distinguish aragonitic (well-preserved) from calcitic (diagenetically altered) otoliths. Analysis was conducted using a Horiba-Jobin Yvon XploRA confocal Raman spectrometer coupled to an Olympus BX41 microscope equipped with a digital camera. A pure silicon standard was used to calibrate the wavelength scale prior to any analysis. Raman spectra were acquired using LabSpec software, using the following experimental conditions: laser source $\lambda = 785$ nm; laser objective of $\times 50$, filter 50%; 1200 T; 699.88 cm^{-1} ; and acquisition, 1, 5, and 20. Spectra deconvolution was performed using LabSpec (V5.78). The identification of compounds was made in good agreement with the literature (White, 2009), spectral ID, and our own reference spectra (Kremer).

AQ36

Two to three sample spots were analyzed per sample. The determination of aragonite versus calcite was based on the following vibrational bands: aragonite ($155, 207, 704, 1085\text{ cm}^{-1}$) and calcite ($158, 282, 713, 1086\text{ cm}^{-1}$) (White, 2009). Therefore, the presence of a peak at 207 cm^{-1} especially was checked in order to assess for the preservation of the aragonite.

2. Laser ablation inductively coupled plasma mass spectrometry

AQ37

Trace elemental composition of one sample (2072) was analyzed by LA-ICP-MS, using an Agilent 8800 ICP-MS Triple Quad coupled to a CETAC LSX-213 G^{2+} laser ablation system. Operating conditions are described in Table 5. The equipment was calibrated and tuned prior to the analysis with the glass certified reference material NIST 612. Elemental fractionation was monitored and

optimized using $^{238}\text{U}/^{232}\text{Th}$ ratio ($\cong 101\%$) and oxide formation was evaluated using $^{248}\text{ThO}/^{232}\text{Th}$ ratio ($< 0.3\%$). Li, Na, Mg, Ca, Mn, Fe, Co, Cu, Zn, Sr, Y, Ba, La, Ce, Nd, Sm, Yb, Pb, Th, and U were screened and analyzed.

Table 5

Operating conditions for the LA-ICP-MS analyses

Acquisition mode	TRA (time resolved analysis)
Scan type and tune mode	MS/MS in no gas mode
Plasma parameters	
RF power	1550 W
RF matching	1.3 V
Sample depth	4 mm
Dilution gas (Ar)	0.65 L/min
Plasma gas (Ar)	15 L/min
Dwell time and measure isotopes	
2 ms	^{44}Ca
5 ms	^{43}Ca
10 ms	^7Li , ^{23}Na , ^{24}Mg , ^{39}K , ^{55}Mn , ^{63}Cu , ^{66}Zn , ^{88}Sr
20 ms	$^{56,57}\text{Fe}$, ^{59}Co , ^{89}Y , ^{137}Ba , ^{139}La , ^{140}Ce , ^{146}Nd , ^{147}Sm , ^{172}Yb , ^{208}Pb , ^{232}Th , ^{238}U

Prior to the analysis by LA-ICP-MS, a pre-ablation of the sample was performed in order to clean the sample surface and thus avoid possible handling contaminations.

Mapping the distribution of these elements within the otolith sample was done ablating 30 lines (around 5000 μm length), 20 μm between lines (to prevent contamination between lines). Conversion of intensities in cps to images was done using iQuant2 software, developed by Institute of Technology of Tokyo and University of Kyoto.

Quantification analysis was done by spot analysis. Ninety-four spots of 50 μm were ablated along the cross section of the otolith. Total sample ablation time was set to 40 s, including 10 s of blank acquisition and 10 s of wash out. Cps intensities were converted to part per million concentrations with Glitter® Software, using CaO as internal standard and previously converted from Ca

concentrations obtained by SEM-EDS. A synthetic calcium carbonate (USGS MACS3) was used as an external standard in order to correct for instrumental drifts. All the laser ablation operational conditions are summarized in Table 6.

Table 6

Laser ablation conditions

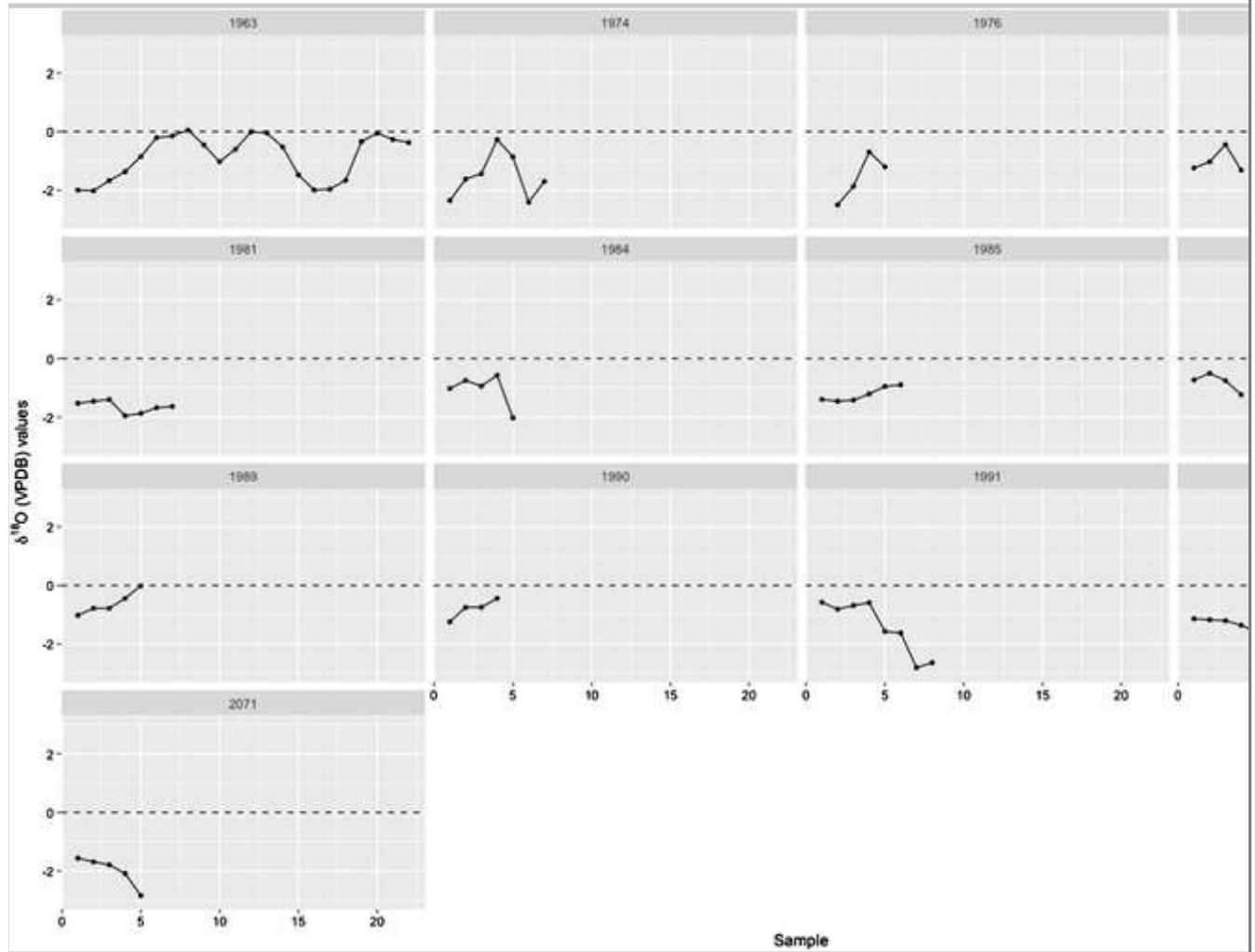
	Mapping conditions	Quantification conditions
Laser energy	65%	65%
Spot size	50 μm	50 μm
Repetition rate	10 Hz	20 Hz
Method selection	Line scan (30 lines \cong 5 mm)	Single point (94 spots)
Space between lines	20 μm	—
Scan rate	20 $\mu\text{m/s}$	—
Burst count	—	400
He carrier flow	1 L/min	1 L/min

Results

All the examined otoliths showed clearly marked banding patterns, even though the majority were juvenile specimens. Two of the observed otoliths were from fairly large and older animals (2072 = + 33 and 1982 = + 12 see Table 6), although far from the maximum registered in modern specimens (i.e., ~ 2 m and 50 kg of weight) (Chao and Trewavas, 1990; Pollard et al., 2015) (see Table 6 for age and seasonal estimate).

A clear annual temperature cycle was visible in all otoliths, especially those older than 1 year (Fig. 5) and the last increment provided estimated seasonality of death through $\delta^{18}\text{O}$ values (Figs. 6 and 7); in other words, negative—lighter—values tend to represent higher temperatures, common from spring to late summer or autumn in analyzed latitudes, and positive values, heavier, tend to represent lower temperatures, common through autumn and winter. The variation in the $\delta^{18}\text{O}$ values throughout the fish's life, as represented in the graphics, shows the temperature variations expected with changing seasons and the last value could therefore provide an estimation of season of capture, which in general seems to indicate the majority of captures occurred between spring and late summer/autumn.

Fig. 5

$\delta^{18}\text{O}$ seasonal variations in analyzed specimens**Fig. 6**

$\delta^{18}\text{O}$ results for sample 1982 used has proxy (the last increment was not sampled in both cases has its conservation was not completely clear)

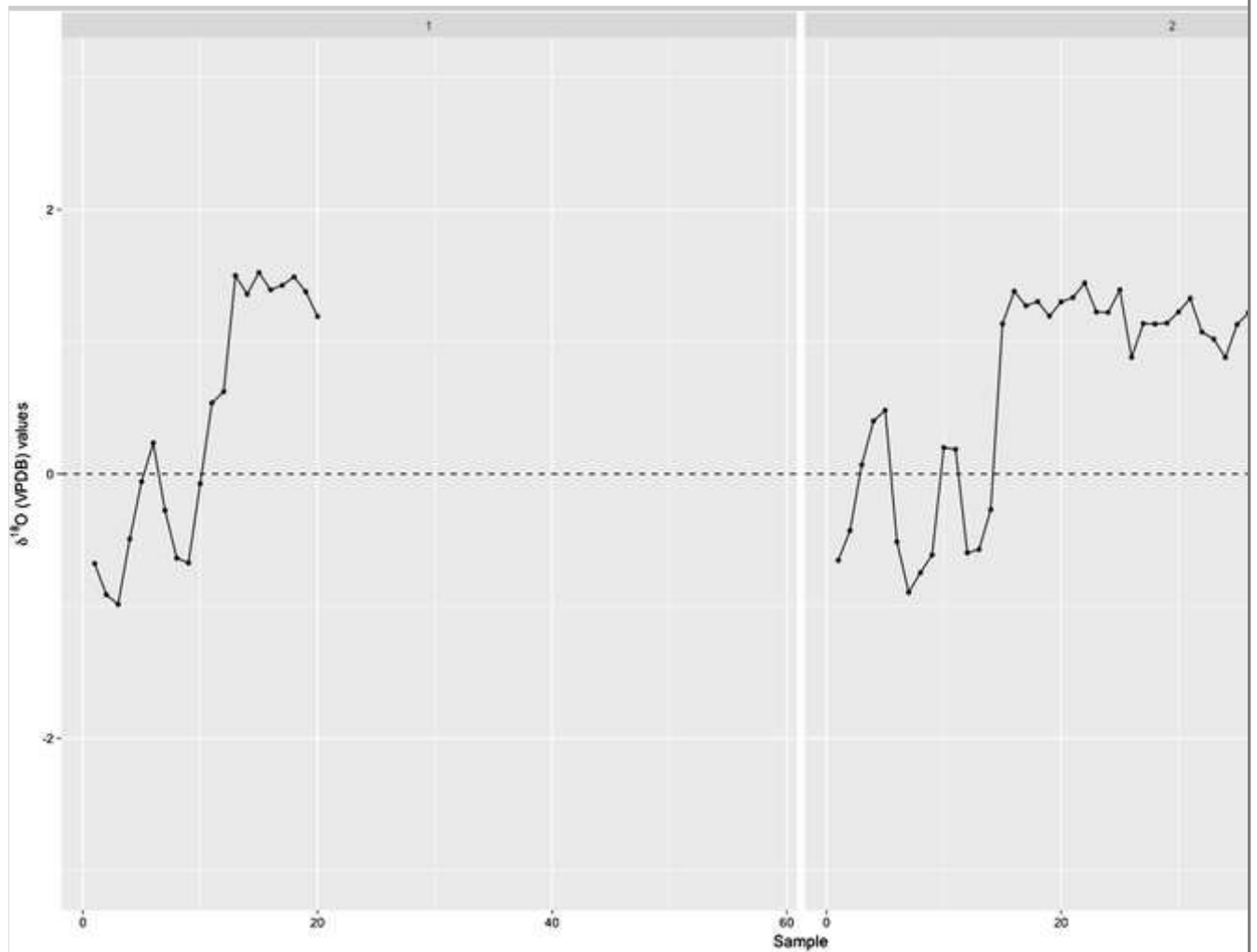
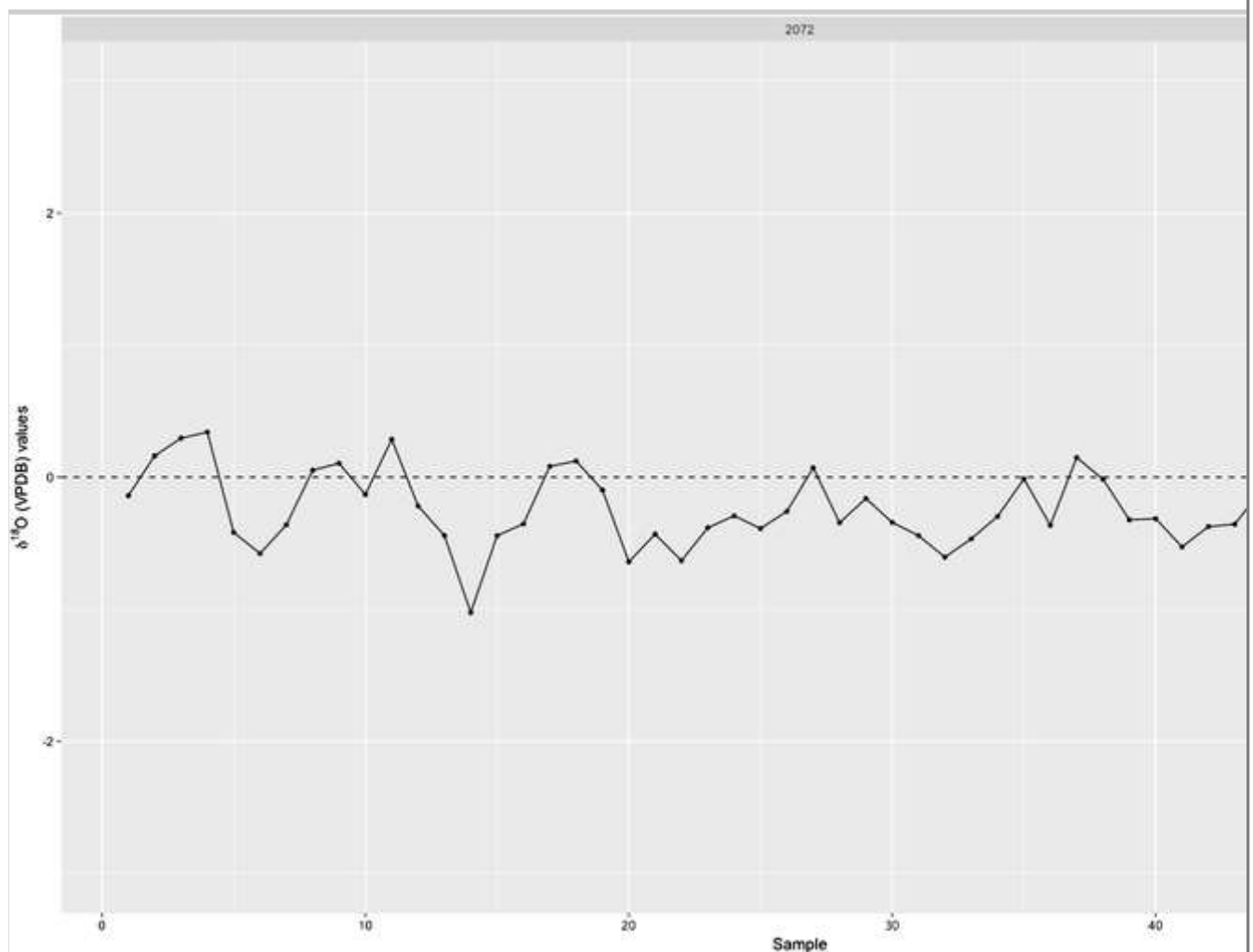


Fig. 7

$\delta^{18}\text{O}$ results for the two samples of otolith 2072 (both halves) used as proxy (the last increment was not sampled in both cases as its conservation was not completely clear)



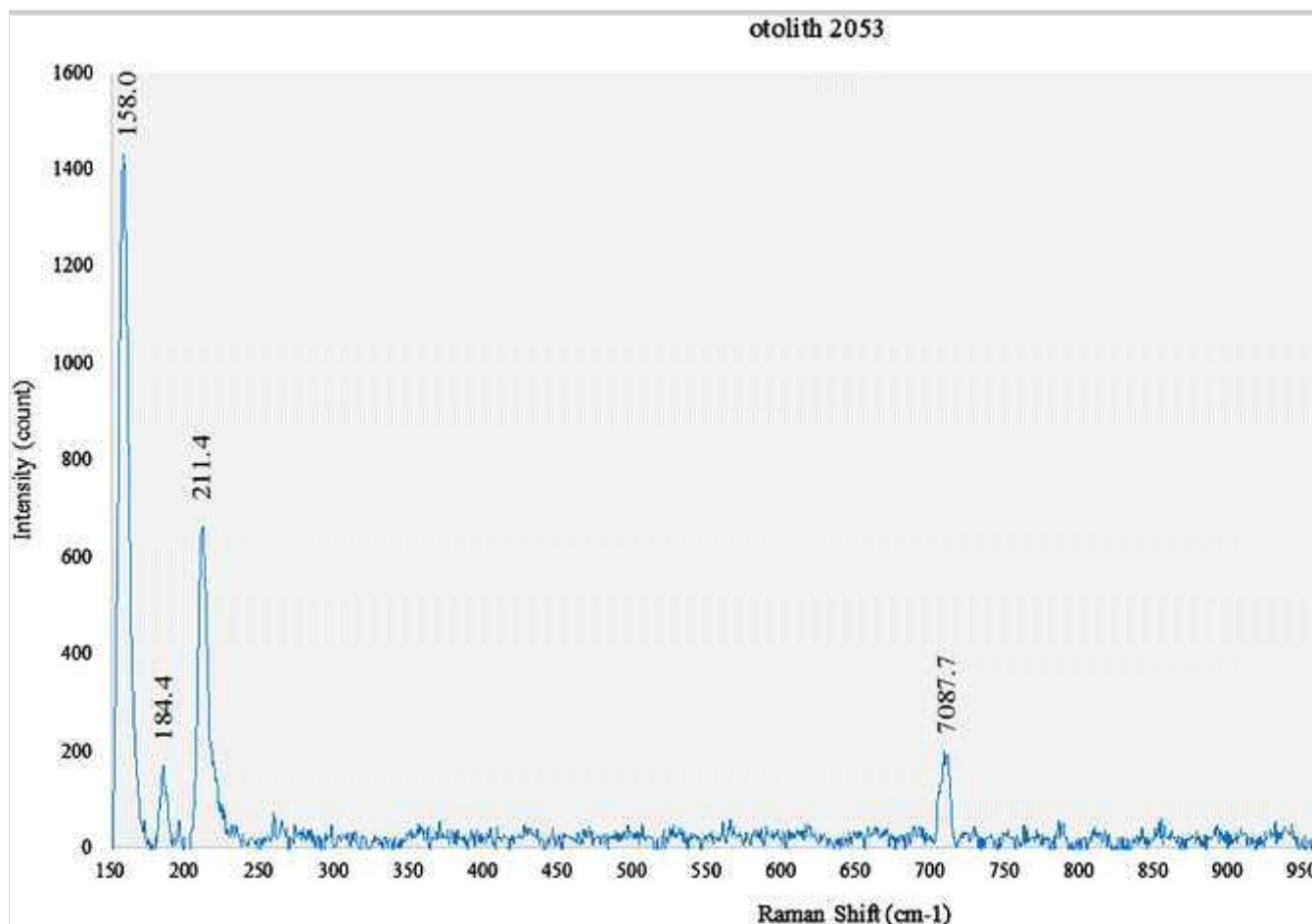
1. Raman spectroscopy—mineralogical composition of the otoliths

AQ38

The spectra obtained using μ -Raman, for all 15 otolith samples analyzed, show a consistent pattern with presence of aragonite and no indication of calcite (Fig. 8).

Fig. 8

μ -Raman spectra obtained for otolith sample 2072. Aragonite-vibrational bands at 155, 207, 704, and 1085 cm^{-1} . Calcite vibrational bands at 158, 282, 713, and 1086 cm^{-1} not detected



2. Laser ablation ICP-MS—trace elemental composition of the otoliths

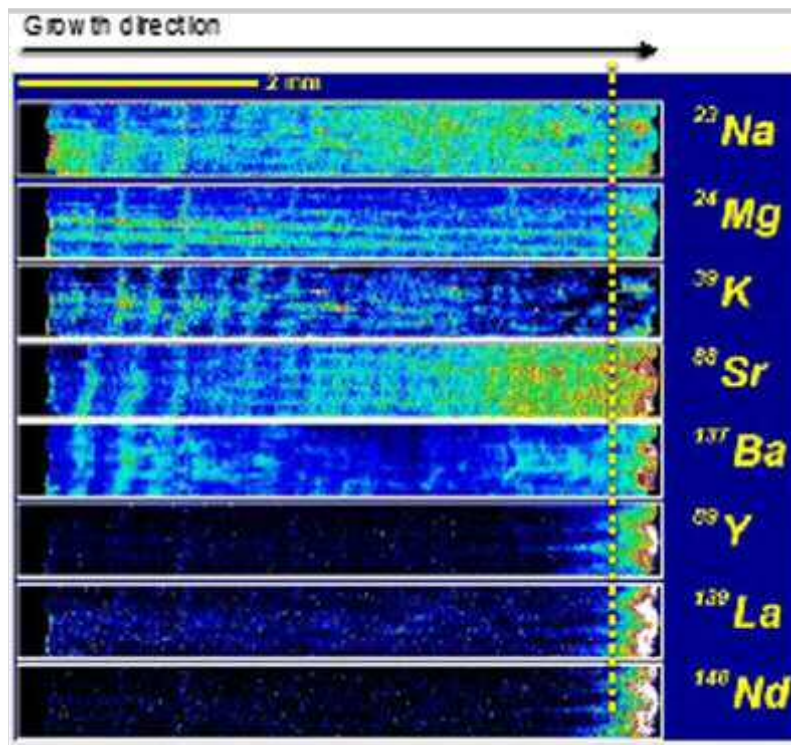
The elemental mapping and quantification of trace elemental content of the sample 2072, performed by LA-ICP-MS, suggests that the outer edge yields higher concentrations of rare earth elements (Y, La, Ce, Nd, Sm, and Yb) compared to its internal part where the concentrations of these elements do not exceed 18 ppbs (Fig. 7 and Table X). This slight enrichment does not alter the $\delta^{18}\text{O}$ values.

AQ39

Other elements such as Na, Mg, K, Sr, and Ba seemed to reflect the growth incremental pattern of the otoliths (Fig. 9).

Fig. 9

Elemental distribution of some selected elements performed by LA-ICP-MS

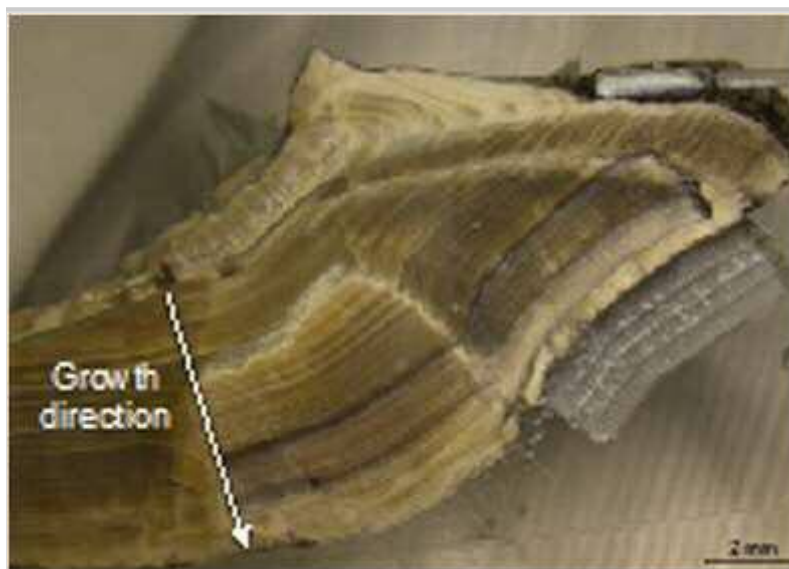


Discussion and conclusions

Discussion of whether or not occupation dynamics are dependent on the diversity and/or availability of animal resources is not new (Dupont, 2016) but the seasonality and duration of these particular occupations are rarely discussed in detail (i.e., 19% of published papers on European shell middens. See Dupont, 2016, p. 4), contrary to the permanence of this occupation, which seems to be more frequently discussed (e.g., like Cabeço da Amoreira, used for over c. 200 years, although maybe not permanently throughout the year) Fig.10.

Fig. 10

Lasered otolith (2072)



AQ40

In some sites, human occupation does not necessarily coincide with periods of accessibility to food resources (Dupont, 2016). That does not seem to be the case with Cabeço da Amoreira. There is not enough evidence at this point to be confident about the existence of specialized activity camps, since further investigation of the other surviving shell middens is required.

Although $\delta^{18}\text{O}$ results seem to generically frame the date of collection/death (Fig. 5 and Table 7), it is still difficult to investigate the occupation mode (i.e., duration and type of occupation) and seasonality because many factors are involved in what is available to construct seasonal use of resources' and spaces' models (e.g., Dupont 2016 or Bicho, 2009, Bicho et al., 2011, 2013). These questions have still not been systematically investigated in the Atlantic shell middens (with some exceptions where researchers used up to 12 different criteria to ascertain season of occupation) (Dupont, 2016). Nevertheless, the large array of shell midden investigations, in the majority of cases, contributed to hinder a clear notion of how/when these populations were occupying these shell middens—assuming there is a pattern—either locally/regionally or more generically. The best approach seems to be to first start at the scale of an archaeological site and only then compare between them as suggested by Dupont (2016) Table 8.

Table 7

Cabeço da Amoreira otolith data

Otolith I.D.	Provenance	Age at death	Inferred season at death** AQ42
2072	1B.2	+ 33	— ^a
1982	1.3	+ 12	— ^a
1963	2.4	~ 2	Spring
1976	1.2	< 1	Spring/late summer?
1974	1.?	2	Spring
1984	1B.3	~ 1	Summer

Seasons were distributed as follows: March–April–May (spring), June–July–August–September (summer), October–November (autumn), and December–January–February (winter)

These otoliths were used as proxies to frame juvenile data and were not sampled to the last ring. 2072's last ring was not completely preserved not allowing a sufficient sample

Otolith I.D.	Provenance	Age at death	Inferred season at death** AQ42
1978	1.3	< 1	Late spring/summer
1990	1.2	< 1	Autumn
1991	1B.1	< 1	Summer
1981	1.1	~ 1	Late summer
1986	1.2	< 1	Late spring/summer
1985	1B.2	< 1	Late summer/autumn
2071	1B.3	< 1	Summer
1989	1.2	< 1	Autumn
2051	1B.3	~ 1	Summer
Seasons were distributed as follows: March–April–May (spring), June–July–August–September (summer), October–November (autumn), and December–January–February (winter)			
^a These otoliths were used as proxies to frame juvenile data and were not sampled to the last ring. 2072's last ring was not completely preserved not allowing a sufficient sample			

Table 8

Ranges of concentrations in the different preserved areas of the otolith sample 2072

	Diagenetically altered	Preserved
%		
Ca	37–42	37–46
Ppm		
Na	915–1130	922–1939
Mg	18–34	10–26
Mn	0.228–8.25	0.043–4.84
Fe	70–92	34–69
Sr	2503–3253	1080–2981
Ba	9–22	0.7–37
Ppb		
Co	30–268	13–92
Cu	157–469	65–1710

	Diagenetically altered	Preserved
Zn	137–407	76–1480
Y	117–1690	4–46
La	12–1490	0.3–13
Ce	12–575	0.2–12
Nd	13–1380	1.7–13
Sm	8–266	3–8
Yb	13–72	2–18
Pb	10–37	12–120
Th	2–50	0.8–7
U	18–36	1.4–136
Li	<detection limit	<detection limit

AQ41

In order to address seasonality, in this paper, we used the case study of the archaeological site of Cabeço da Amoreira. The Cabeço da Amoreira shell midden has been the object of a number of studies that are relevant to the investigation of seasonality: (1) sclerochronology and isotope analysis (this study and Dias et al., unpublished on rays and sharks sclerochronology); (2) microstratigraphy records (Aldeias and Bicho, 2016); spatial analysis and site formation (Bicho et al., 2010, in press; Bicho and Gonçalves, 2016.; Conyers et al., n.d.; Dias et al., 2012; Gonçalves, 2009); (3) faunal remains analysis (Detry, 2007; Dias et al., 2016, Dias et al., 2015; Dupont, 2016; Dupont et al., 2009); (4) lithics analysis (Jesus et al., 2010; Marreiros et al., 2015, n.d.); (5) chronology (Bicho et al., 2013); (6) biological anthropology (Cunha et al., 2003; Cunha and Umbelino, 1995); (7) archaeology of death (Figueiredo, in press; in press(a), 2015; Peyroteu- Stjerna, 2016); (8) human remains isotopes (Umbelino, 2006); and (9) paleobotanical research (Monteiro, 2011; Monteiro and Zapata, n.d.).

AQ43

AQ44

Data supports the suggestion of a prolonged human occupation of the site but not necessarily a permanent or continuous one. Several suggestions have been made that these shell middens (both in Muge and Sado) indicate a sedentary or semi-sedentary occupation (Gutiérrez-Zugasti et al., 2011; Rolão, 1999). However, micromorphology data shows evidence of a likely geogenic hiatuses in the accumulation of the shellmound occupations, compatible with site abandonment

periods (Aldeias and Bicho, 2016, p. 546) which (based on Bayesian modeling of ^{14}C dates) lasted no longer than 50 to 100 years in Muge (Bicho et al., 2013). It is important to add that the micromorphology data are derived from sample areas rather than from the full site and a more thorough micromorphological study is required to confirm these conclusions for the whole site.

Data from bird remains, contrary to the data from fish remains, seem to suggest winter occupation of the site, indicated especially by the presence of several wintering species, although also including *H. himantopus*, which is only present from spring to autumn (see Detry, 2007 for the list of wintering species in the Muge shell middens).

The $\delta^{18}\text{O}$ values for otoliths from Cabeço da Amoreira clearly show seasonal changes (Fig. 9), ranging from heavier values during the winter to lighter values in summer. However, as *A. regius* may move seasonally between areas with different freshwater content (they may enter estuaries in spring or prey on schools of mugilids all year round), the seasonal variability observed may also to some extent reflect changes in salinity of the waters the fish inhabit and as such both temperature and salinity could increase the seasonal $\delta^{18}\text{O}$ range (i.e., warm/fresher vs cold/salty). This could lead to complications in the interpretation of the seasonal temperature signal, especially since even when *A. regius* do not enter estuaries, they live in waters with variable freshwater influence (Hufthammer et al., 2010), migrating northward and southward along the Portuguese Atlantic coast (Prista, 2013).

However, although isotopic variability should be expected in areas directly affected by inflow of river water, $\delta^{18}\text{O}(\text{H}_2\text{O})$ seawater can generally be assumed to range from c. + 0.2 to 0.8‰ in surface or intermediate water and c. + 0.9 to c. + 1.4‰ in deep waters (Iacumin et al., 1992), so that $\delta^{18}\text{O}$ values should be lighter for periods in which *A. regius* are in estuaries, which in this case coincides with negative values as a consequence of temperature.

AQ45

In terms of possible alteration of results due to taphonomical processes, mineralogical analysis of the otolith samples performed using μ -Raman showed that both analyzed otoliths preserved their pristine aragonitic composition. However, this does not preclude any other diagenetic process that might have modified the biological composition of the otolith. In order to assess the preservation of the geochemical composition of the otolith, LA-ICP-MS analysis was used for mapping intensities of Ca, Sr, Ba, Pb, Fe, Cu, Zn, Co, U, Th, Nd, Ce, La, and Y and seeing whether their distribution pattern might relate to post-burial transformations. Although the distribution pattern of Y, La, and Nd

obtained using LA-ICP-MS analysis showed that the outer edge of the sample might have become slightly enriched in these elements during burial, the part of the otolith sampled for measuring oxygen and carbon isotopes seems not to have been subject to taphonomic contamination. Therefore, the combination of mineralogical and geochemical data suggest a preservation of the otolith against diagenetic processes and therefore confirm that the isotopic composition should reflect the *in vivo* composition and can be used for reconstructing fish exploitation strategies adopted by the Mesolithic population of Cabeço da Amoreira.

Seasonality results from Cabeço da Amoreira's layer 1 *A. regius* otoliths are in agreement with seasonal abundance of this species in the Tagus estuary. Modern fisheries investigations indicate that *A. regius* concentrates in the estuary from May through September, migrating along Portuguese coastal areas in the autumn-winter months (Prista, 2013). The targeting of this species in the Muge area (or more generically the Tagus estuary) was probably determined by the species regular annual migration cycle, from the estuary to the coast and vice versa as it is in the present day.

In addition, the stratigraphic distribution of the species, with the greatest concentration being on layer 1, could indicate either a species targeting/preference or a consequence of taphonomic degradation with less well-preserved otolith remains in other layers.

All these data might suggest a seasonal pattern of gathering activities. Although still heavily dependent on terrestrial resources (especially rabbits and red deer *vide* Detry, 2007 and Dias et al., 2016 and Umbelino, 2006 for isotope results in human remains), this human group(s) have based their subsistence on a seasonal round of activities with a transient population, in family groups (for further discussion on this see Gonçalves et al., 2014), involved in seasonal gathering and fishing/hunting. These activities could (and must have been) dependent on the seasonal abundance of resources in the region.

AQ46

Further seasonality investigations must be carried out to validate and further contribute to these results. These preliminary results and the method here established for the analysis of these types of archaeological remains and sites, however, show the potential of these investigations, suggesting that an investment should be made in creating both archaeological and modern reference collections.

The quantity of shell remains at this site and their differential preservation indicate great potential of the site as an example of the usefulness of archaeological isotopic analysis which, although increasingly frequent, is still far from ideal. Analysis of age of death in vertebral and other fish remains can also make a significant contribution to seasonality studies, although this is severely limited by taphonomical issues; in particular, the preservation state of the last increment needs to be taken into account. This method can also be limited by subjectivity (Casselman, 1983; Wheeler and Jones, 2009).

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