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MARIA JOÃO VALENTE
CLEIA DETRY
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(eds.)

EZI 2017

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BIOSTRATINOMIC STUDY OF BONES IMMERSED IN MARINE WATER: PRELIMINARY RESULTS AND INFERENCES FROM THE DELTA PROJECT BONE ASSEMBLAGES

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Abstract
In this paper, we present the preliminary results of a biostratinomic study of bones submerged in sea water with the aim to identify patterns that may help us to understand the diagenetic processes that took place in the assemblages of two historical shipwrecks (16th and 17th centuries) located in the Bay of Cádiz (Spain). The faunal assemblage of these shipwrecks was composed by 122 NISP of cattle, caprine, suid, cat, chicken and probably cetaceous species that were recovered with different taphonomical characteristics (cut marks, stains, erosion and fouling) and, probably, different origins (refuse from the ships or from the slaughterhouses located around the bay). In order to understand the taphonomic characteristics and to attempt to reconstruct the origin of the shipwreck bone assemblages we collaborated with the ARQUEOMONITOR Project during its final year.
The main objective of this project was to document the alteration of different submerged materials at two sites within the Bay of Cádiz. The reference bone materials used in the experiment were cattle tibia and femur, half of them boiled before immersion to remove the collagen. These samples were then retrieved at different times over a one-year period in order to study the macroscopic taphonomy produced by the marine sediments and biota. The preliminary results enable us to define differences between boiled and raw bones during the first 6 months of the trial, although after this time alterations are similar in both samples. Furthermore, we are able to understand the fouling of the archaeological bones from the shipwrecks as modern bioerosion. We also present the initial results of microanalysis using SEM (scanning electron microscopy) and EDX (energy dispersive X-ray spectroscopy) for the study of microerosion and chemical changes in bone structure.

Keywords
Biostratinomy, bioerosion, Taphonomy, shipwreck, fouling.

Introduction

In the Modern Age, Cádiz was an important city and was one of the main ports that connected Spain and America in the 17th and 18th centuries (Cervera 2007). According to Navarro (2007), around 1000 ships were anchored here in the 18th century. This important trade by sea is probably one of the reasons for the number of shipwrecks along the Cádiz coast (García and Alzaga 2008). Some have been excavated by the Underwater Archaeology Centre of the Instituto Andaluz del Patrimonio Histórico (IAPH) (Ridela et al. 2017) and the faunal assemblages have been studied by the Laboratory of Palaeobiology of the IAPH (Bernáldez-Sánchez et al. 2014a, 2014b). In particular, the Delta Project, conducted by Tanit Gestión

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Arqueológica S.C. and the Underwater Archaeology Centre, excavated two shipwrecks named Delta I and Delta II (Higueras-Milena et al. 2014). Delta I was dated in the 17th century and Delta II in the 16th century (Ridela et al. 2017).

The faunal assemblages from Delta I and II studied by Bernáldez-Sánchez et al. (2014a) were constituted by 122 NISP with a total weight of 11207.3 g from seven species: *Bos taurus*, *Capra hircus*/*Ovis aries*, *Sus scrofa* and *Felis catus*, also an undetermined cetaceous and one *Gallus gallus domesticus* (Tables 1 and 2). The majority of these species were terrestrial animals that were probably consumed by humans. In addition, it was observed that 46% of the bones displayed butchery marks. Some of these bones and perhaps all of the horns of the assemblage may be linked to the slaughterhouses located in the same area (Caballero et al. 2006; Márquez 2008), but other bones could be part of the refuse from daily activities, possibly on board the ships. However, it was difficult to establish the origins of the bones assemblages and to confirm that they were rubbish derived from the daily life of the sailors on board these vessels. Fouling was observed on 15% of the bones, probably related to a recent colonization (Bernáldez-Sánchez et al. 2014a), but no reference was available for the time of colonization of the different species in the Cádiz Gulf in order to certify this hypothesis.

In order to address these outsatnding questions we collaborated with researchers of the ARQUEOMONITOR Project during its final year and we submerged animal bones at two different sites on the Cádiz coast (Bethencourt et al. 2014). Unfortunately, we could only retrieve and analyse the samples located around the S.M.I. Bucentaure shipwreck in the Bay of Cádiz (Bethencourt et al. 2014, 2018), as the other samples were moved after tidal waves. We are aware of the limitations of the preliminary study, due to the small sample size and the short period of sampling time, however the experiment has provided useful results to explain some of the taphonomic aspects of underwater taphocoenosis in marine environments (González-Duarte et al. 2018).

The analysis of biostratinomic and diagenetic processes in current ecosystems is useful to understand taphocoenosis (Andrews 1995; Bernáldez-Sánchez et al. 2017; Denys 2002; Domínguez-Rodrigo 1998; Lyman 1994). There are a number of papers about the diagenesis of bones (Behrensmeyer 1978; Bernáldez-Sánchez 2011; Domínguez-Solera and Domínguez-Rodrigo 2009; Yravedra 2006), some of them related to aquatic ecosystems (Anderson and Bell 2014; Arnaud et al. 1978; Ascenzi and

Delta I	Anatomical part	NISP	Bioerosion & fouling	Erosion	Stains	Cut marks
Bos taurus	Skull	5	1 bone with fouling 1 bone with ostreids	-	1	5
	Humerus	2		-	-	1
	Radius	1		1	1	-
	Metacarpus	1		-	-	-
	Femur	3	1 bone with serpulids & bryozoans	1	1	3
Ovis aries / Capra hircus	Skull	3		-	-	1
	Maxilla	1		-	-	-
	Pelvis	1		-	1	1
	Tibia	1		-	-	-
Sus sp.	Vertebra	1		-	-	1
	Scapula	1		-	-	1
Macrovertebrate	Pelvis	1	Ostreids & serpulids	-	1	-
Gallus domesticus	Tibia	1		-	-	-
Total		22	4 bones with fouling	2	5	13

Table 1 - NISP determined in the Delta I bone assemblage.

Silvestrini 1984; Fernández-Jalvo and Andrews 2003; Pokines and Higgs 2015). In aquatic environments the decay of soft tissue usually takes longer than in terrestrial sites (Anderson and Bell 2014; Haglund 1993; Pinheiro 2006), for instance the decay of the soft tissue of one red deer (*Cervus elaphus*) that died in a lake took three times longer than other red deer carcasses located on terrestrial biotopes in the Sierra Norte of Seville (García-Viñas and Bernáldez-Sánchez 2018).

Delta II	**Anatomical part**	**NISP**	**Bioerosion & fouling**	**Erosion**	**Stains**	**Cut marks**
Bos taurus	Skull	3	1 fouling	-	-	2
	Mandible	5		1	-	1
	Vertebra	9	2 fouling 1 bone with ostreids & bryozoans 1 bone with fouling & algae 1 algae	1	-	6
	Rib	2		-	-	1
	Sternum	1		-	-	-
	Radius	3	1 bone with balanus, bivalves, bryozoans & serpulids	-	-	1
	Pelvis	1		-	-	-
	Femur	4	2 bone with fouling 1 bone with ostreids & algae 1 bone with ostreids & serpulids	-	-	2
	Tibia	1		-	-	-
	Humerus	5		-	-	4
	Metacarpus	1		-	-	1
	Metapodium	1		1	-	-
	Phalange	2		1	-	-
Ovis aries / Capra hircus	Skull	4	1 bone with fouling	-	1	3
	Mandible	1		-	-	1
	Femur	3	Gnawing marks of a rodent	-	-	2
	Vertebra	2		-	-	2
	Metacarpus	1		-	-	-
Sus sp.	Mandible	2		-	-	-
	Vertebra	1		-	-	-
	Humerus	4		-	-	1
	Pelvis	1	1 bone with ostreids, bryozoans & serpulids	-	-	-
	Tibia	1		-	-	-
	Tarsal	1		-	1	-
Felis catus	Talus	1		-	-	-
Macrovertebrate	Rib	7	1 bone with ostreids, bryiozoans & serpulids	-	-	3
	Vertebra	2		-	-	1
	Scapula	1		-	-	-
	Long bone	2		2	1	-
Mesovertebrate	Mandible	1		-	-	-
	Rib	10		-	-	3
	Vertebra	3		-	-	1
	Sternum	1		-	-	-
	Femur	1		-	-	-
Indeterminate*		7		2	-	-
Gallus domesticus	Tibia	5		-	-	1
Total		100	14 bones with fouling	8	3	36

Table 2 - NISP determined in the Delta II bone assemblage. *two of these fragments belong to Cetaceous.

Taphonomic analysis of faunal adhesions (fouling) makes it possible to distinguish bones from marine and freshwater environments (Pokines and Higgs 2015), although in the microscopic analysis the bones from both ecosystems show the same erosion patterns (De Battista et al. 2013). The estimation of the time that carcasses were immersed in an aquatic environment has been addressed forensic anthropologists, although the focus has mainly been placed on the study of changes in soft tissue (Pinheiro 2006). Some specialists have, however, carried out studies in order to estimate the date of bone remains (Castellano et al. 1984; Yoshino et al. 1991), although mainly centering on the date of death. In this case, we study the processes undergone by submerged bones, boiled and raw, in order to obtain some useful keys for the interpretation of the faunal assemblages from historical shipwrecks, focusing on the first months before burial in sediment.

Study area

The specific environmental and biological conditions of each study area are important in order to understand the taphonomic processes observed in archaeological bones (Gregory 2009; Pinheiro 2006). The experimental submersion site used for this study is located next to the Bucentaure shipwreck, in the outer Bay of Cádiz (Figure 1; Bethencourt et al. 2018), on a mixed rocky-sandy seabed at a depth of 12 m. The water temperature varies throughout the year from 13°C in February to 20°C in September. The salinity also shows seasonal differences: 36.7% in September, due to the increase of rainfall and decrease of evaporation in summer, and 35.7% in April.

Figure 1 - Study area.The Bucentaure shipwreck is located in the Bay of Cadiz (south-western Spain). Images from d-maps.com (copyright Daniel Daley).

The community of this biotope is adapted to these environmental factors. According to González-Duarte et al. (2018), the sessile community identified in wood samples located in the Bucentaure area after 15 months was composed by a number of different species: Algae (*Ceramium diaphanum+Scagelia* sp., *Dictyota dichotoma*), Mollusca (*Anomia ephipium*, *Teredo navalis*), Bryozoa (*Cellepora pumicosa*, *Plagioecia patina*), Polychaeta (*Filograna implexa*, *Spirobranchus triqueter*), Crustacea (*Balanus* sp.) and Tunicata (*Ecteinascidia turbinate*).

Other environmental parameters of interest in this study are the characteristics of the sediment and the velocity of the waves. Sediments can be classified as moderately sorted coarse sand (D50 = 1.095 mm), making their transport in suspension mode difficult (Bethencourt et al. 2018). The wave velocity range is between 0.25 m/s and 0.6 m/s.

Methodology

The biostratinomic analysis was carried out using the diaphysis of a tibia and a femur from the same individual of *Bos taurus*, since cattle bones are large enough to carry out all of the analyses and even to repeat some if necessary. According to Gruppe (1988), compact bones are chemically more stable than cancellous bones. We used raw and boiled bone samples to test if the bioerosion processes were different, in order to distinguish bones from the slaughterhouses and from daily refuse (that could have been cooked) in the taphocoenosis collected under the marine water. We were not able to observe all of the traces produced by abrasion (Aslan and Behrensmeyer 1996; Fernández-Jalvo and Andrews 2003) because the samples were fixed to a panel (Figure 2), but it is possible to see the erosion produced by sand impacts due to the energetic water movements that characterise coastal marine environments (Pokines and Higgs 2015). Ten samples (five of them boiled) were submerged on the 18th of July, 2014, and were collected at four intervals over a one-year trial period:

- First sampling. October 25th, 2014
- Second sampling. January 11th, 2015
- Third sampling. May 7th, 2015
- Final sampling. July 20th, 2015

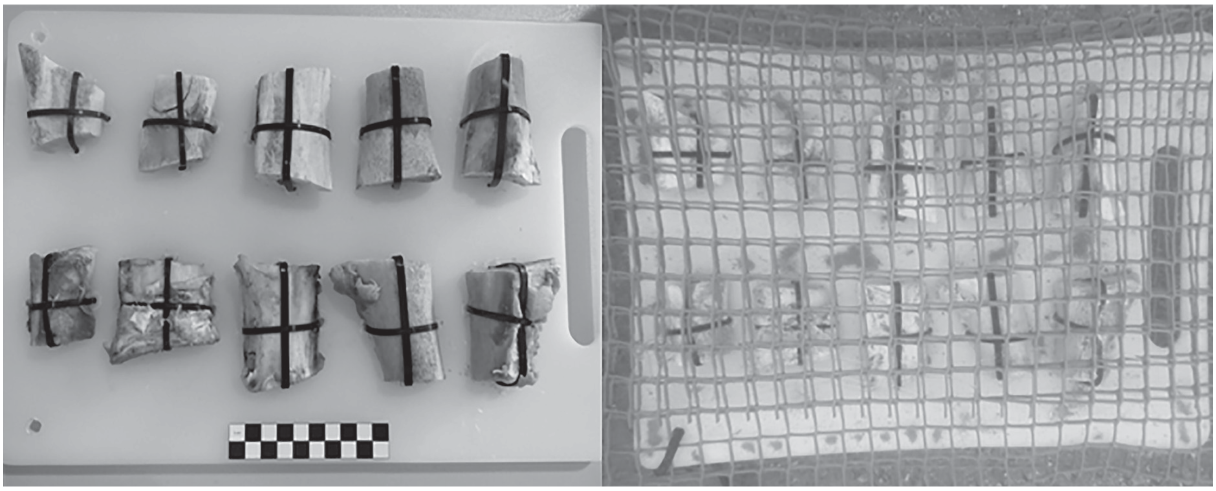

Figure 2 - Experimented bone samples. The upper row are boiled samples, the bottom row are raw bones (soft tissue is visible in the latter).

The macroscopic analysis focused on the observation of adhesions of sediment and taxa (molluscs, algae, bryozoans, etc.), the conservation of soft tissue (adipocere), erosion and changes in coloration. We are currently following up the microscopic analysis using SEM-EDX (Scanning Electron Microscopy - Energy Dispersive X-ray spectroscopy) in order to study in greater detail the micro-morphological alterations and changes in the chemical composition of the samples.

Results and discussion

Macroscopic taphonomy

The results of the macroscopic analysis are presented here following the time-line of the sampling in order to understand some of the main aspects of the biostratinomic processes suffered by the bones immersed in marine ecosystems. We have included observations of both of the faces of the shaft bone (internal and external). Although the internal face was better preserved from the water movements (Figure 1), fouling is present on both faces.

First sampling. October 25th, 2014

After three mounts under water, two samples of femur were collected (Figure 2) and kept in the fridge (all of the samples recovered throughout the experiment were treated in the same conditions). The boiled bone showed some algae filaments and gastropods. The raw bone did not display bioerosion (Figure 3), although some sediment was present in the cancellous bone. The gastropods were determined as an *Anomia ephippium* (LM= 14.13 mm) and some serpulids, that are known to be common in materials recovered after a time under marine water (Bernáldez-Sánchez and García-Viñas 2012; Cámara et al. 2017). The determination of vermetids is very difficult due to the similarities in shape with other invertebrates such as serpulids (Gofas et al. 2011), but Cámara et al. (2017) have identified these forms as serpulids at the same location.

In contrast to terrestrial conditions, in which the absence of organic material produces the disinterest of scavengers (Thompson and Lee-Gorishti 2007), in this case the soft tissue conserved in the raw bone preserved the bone from biological colonization.

Also we determined adipocere (saponification) in the cancellous bone of both samples, although more intense in the raw bone (Pokines and Higgs 2015). This adipocere is a soap like substance composed of hydrated body fats (Figure 4; Kahana et al. 1999; O'Brien et al. 2007; Ubelaker et al. 2011) that gradually disappears in parallel to other degradation processes. Adipocere formation depends on water temperature, oxygen concentration and is also promoted by the bacterium *Clostridium* (O'Brien et al. 2007; Ubelaker et al. 2011).

The bones appear well preserved in the macroscopic analysis, but blanching and some white dust was noted when they were touched. However, no rounding of the exposed margins was observed, a common taphonomic characteristic produced by battering and abrasion in water environments.

Second sampling. January 11th, 2015

Two samples of femur were collected after six months of immersion. The raw bone continued without any great bioerosion, presenting a good state of preservation with only a barnacle (*Balanus* sp.) and a serpulid. The invertebrate colonies were larger in the boiled sample: serpulids, bryozoans (*Escharoides coccinea*) and one mollusc (Figure 3) were identified. It is interesting to note that bryozoan colonies may

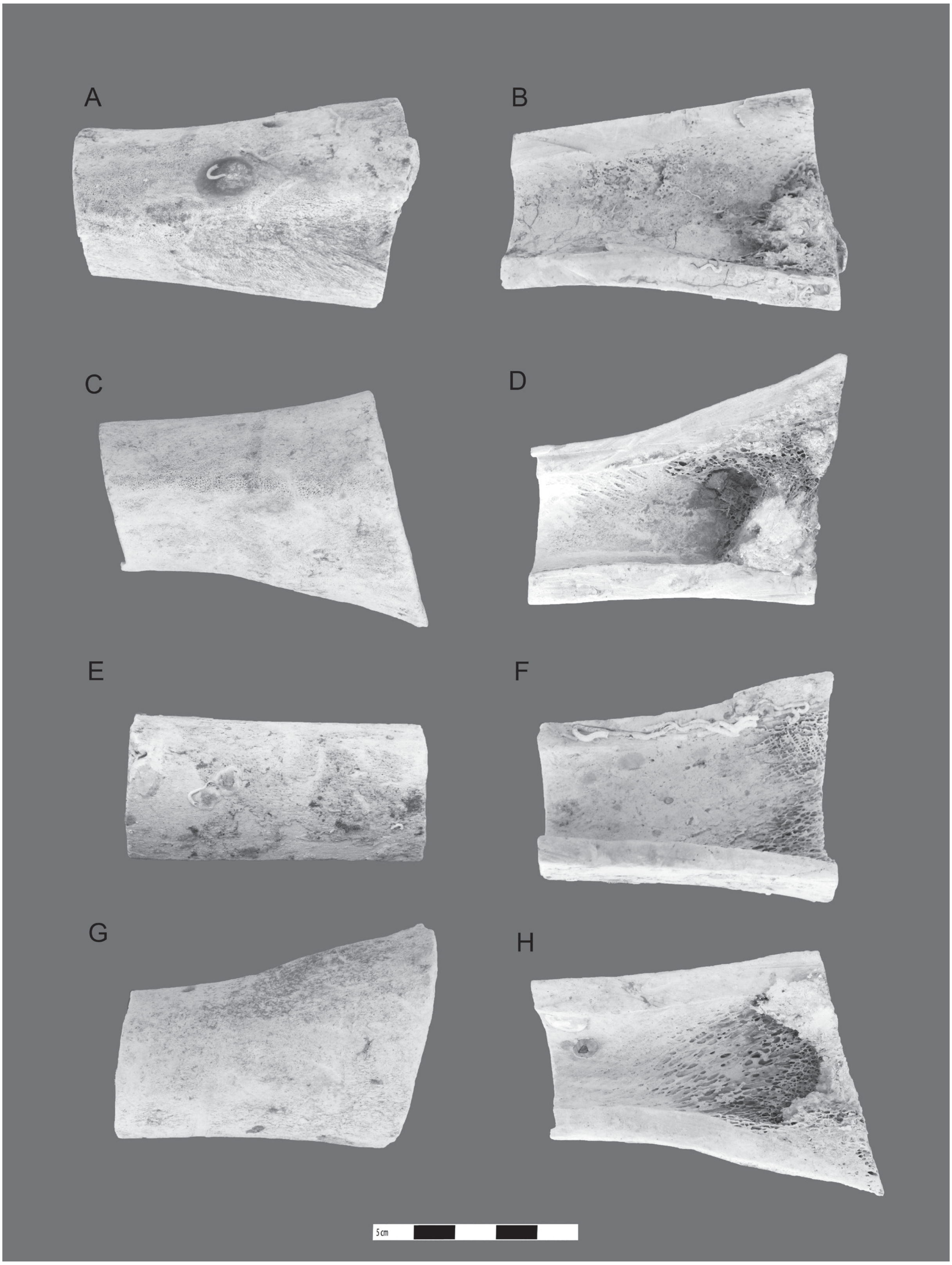

Figure 3 - Samples collected after three (A-D) and six months (E-H) in marine water. A-B, E-F. Boiled bone; C-D, G-H. Raw bone. After three months, it is possible to observe adipocere in the cancellous bone of B and D. After six months, the adhesion of biota in more intense in the boiled samples than in the raw bones. It is also possible to observe adipocere in the cancellous bone in H. Although the internal face was better preserved from the water movements, fouling is observed on both faces..

take only minutes to form (Pokines and Higgs 2015). In the historical faunal assemblages from the Delta shipwrecks 15% of the bones displayed fouling, some of them with live bryozoans, probably linked to a recent colony during the archaeological activity (Bernáldez et al. 2014a).

Only in the raw sample was is possible to observe adipocere in the cancellous bone. At this moment, it is possible to distinguish raw bone from boiled bone immersed at the same time in a marine environment through the analysis of macroscopical characteristics.

In addition the surface of the bone was eroded and it was possible to observe grooves, produced by bioerosion and/or the collision of marine water carrying sand and sediment. The bones were bleached and it was possible to observe the partial rounding of the margins.

Third sampling. May 7th, 2015

After 10 months under water, both samples of tibia (raw and boiled) had been colonized by invertebrates. However, the bioalteration from serpulids recorded on the boiled bone was relatively higher than that of the raw sample (Figure 4). The boiled sample was colonized by one *Anomia ephippium* (LM= 13.46 mm), serpulids, bryozoans, while an ostreid and a serpulid were adhered to the raw sample.

It is interesting to note that after ten months there was no adipocere left in the bones, nor in the rest of the samples that remained immersed, although adipocere is known to persist for long periods in the environment in enclosed spaces (Pokines and Higgs 2015). This observation could be useful to establish the relative time during which a raw bone is immersed, although in this study we have an insufficient number of samples to confirm this hypothesis.

On the external surface of the raw sample it was possible to observe exposed cancellous bone due to the battering of sand and other sediments. The bones were bleached and their margins were possibly rounded.

Final sampling. July 20th, 2015

Four samples of tibia were collected 12 months after the initial immersion, two raw bones and two boiled samples (Figure 4). All of these materials displayed adhesions of sediments and organisms: algae filaments, serpulids, molluscs. The bones were bleached, taking the appearance of a carbonate, and the partial rounding of the margins was noted. At this moment it is very difficult to distinguish boiled bones from raw samples.

Inferences from the Delta Project bone assemblages

During the Delta Project, archaeologists excavated two shipwrecks in the Bay of Cádiz. The faunal assemblage was composed by seven species. Although the biostratinomic and diagenetic processes affect bones with different size and density in different ways (Lyman 1994; Yravedra 2006; García-Viñas and Bernáldez 2018), in this study 80% of the NISP belonged to ungulates. For this reason the experimental biostratinomic results obtained from cattle bones may be used as a reference for the interpretation of the bone assemblages of the Delta Project.

In the historical bone assemblage of Delta I (17th century), composed by 22 NISP, 60% of NISP displayed cut marks and 18% fouling (Table 1). This fouling was produced by bryozoans, ostreids, balanus, vermetids/serpulids and algae. In Delta II (16th century) there were 100 NISP, 36% of which displayed butchery marks and 14% fouling produced by the same organisms observed in Delta I (Table 2). According to our experimental results, this kind of fouling could have been produced in less than two months, and

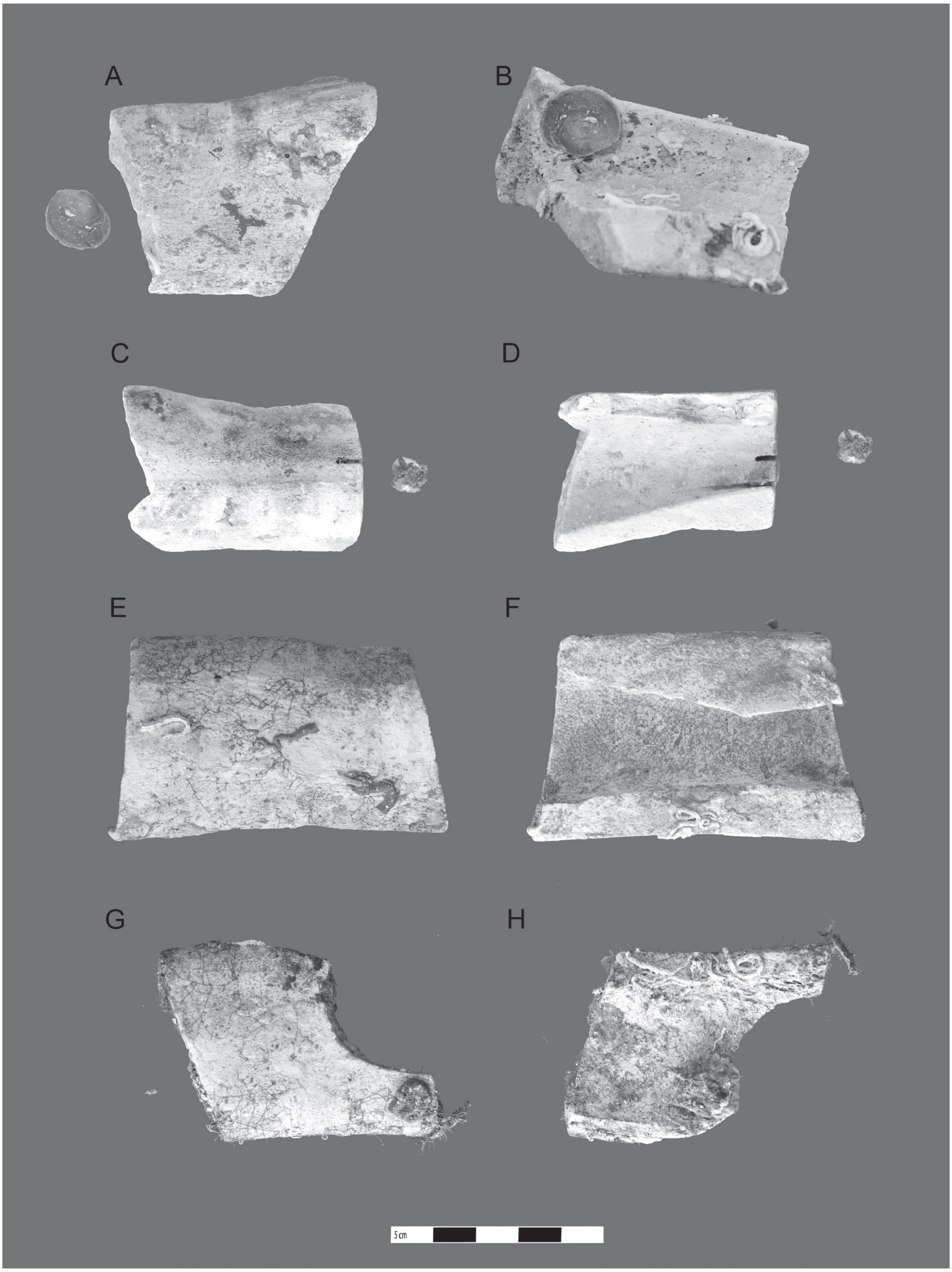

Figure 4 - Samples collected after ten (A-D) and twelve months (E-H) in marine water. A-B, E-F. Boiled bone; C-D, G-H. Raw bone. After 10 months under water, both boiled and raw samples were colonized by invertebrates, although this colonization is higher in the boiled bone (A-B). After twelve months, it is no longer possible to distinguish boiled and raw samples based on fouling. Although the internal face was better preserved from the water movements, fouling is observed on both faces.

we therefore suggest that the fouling observed on the historical bones probably took place during the archaeological excavation. In addition, the small size of the fouling indicates the recent formation of these colonisations. Fouling characteristics cannot, therefore, be used to determine the origin of these archaeological bone assemblages.

It is interesting to note that 8 bones from the Delta Project excavations showed dark stains, although the majority of the NISP were dark coloured. These stains and dark coloration may have been produced by different factors (Yravedra 2006), but in the experimental biostratinomic study no staining was observed, despite the presence of metals and other material in the same location as the submerged samples. The microscopic analysis with SEM-EDX of some of the historical bones showed that these stains were produced by inlays of silt and clays during the diagenesis. Attending to the absence of ancient fouling and the presence of this kind of inlays, it was probably that the archaeological bones were rapidly buried in sediment. In addition, the historical bones from the Delta Project showed a good state of preservation. Evidence of erosion was identified on only 10 bones, while other organic materials such as leather, fibres, wood, etc. were also well preserved at these shipwrecks (Bernáldez et al. 2014a). In contrast, in the experimental biostratinomic study it is possible to detect partial rounding of the margins of the bone samples, even though the samples were fixed and impossible to roll. This difference between the historical and experimental bones supports the idea of a rapid burial of the archaeological materials. The conditions of preservation could be related to the sediment, since the conservation of bones is good, generally, in marshy environments without oxygen (Yravedra 2006).

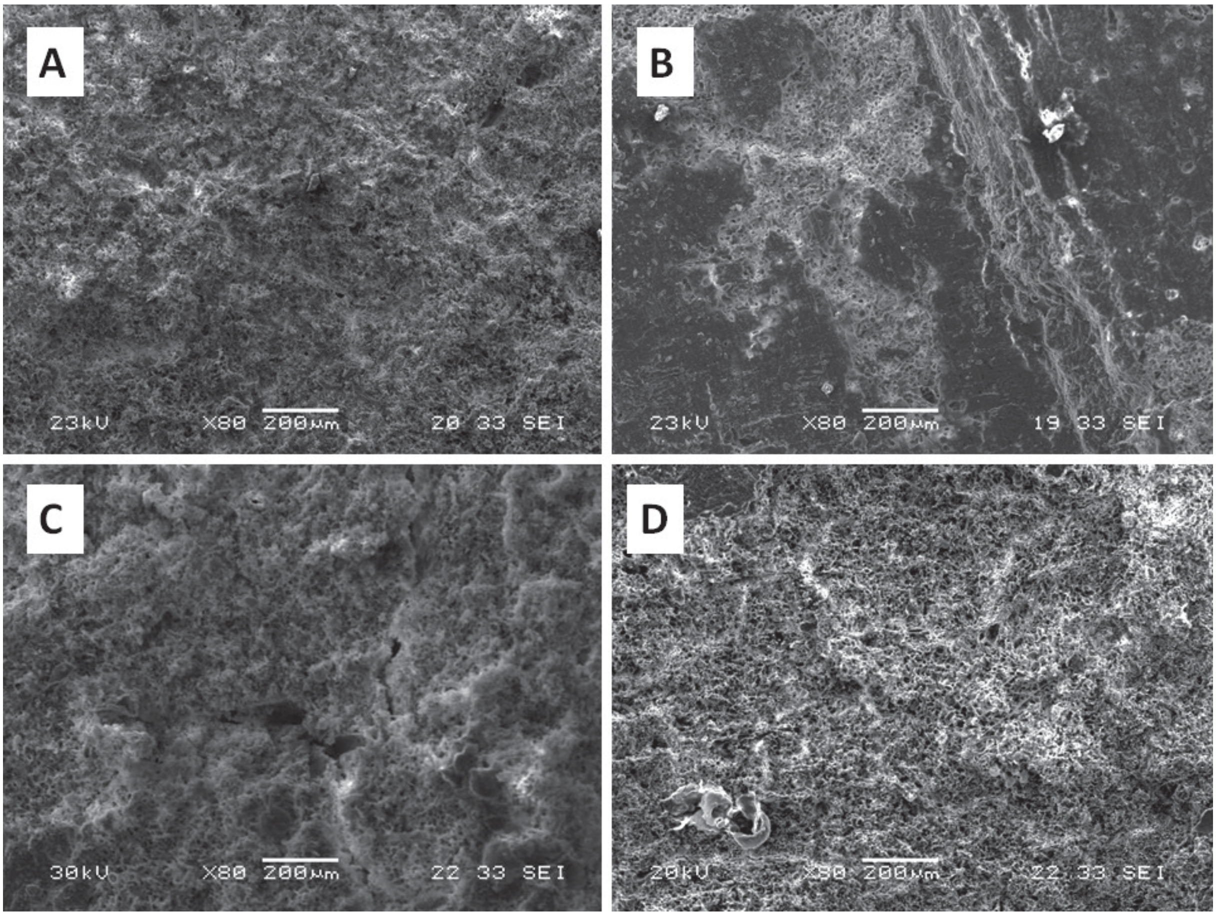

Figure 5 - The preservation of raw bones was worse than that of boiled samples after three (A. Raw bone, B. Boiled bone) and six months under water (C. Raw bone, D. Boiled bone).

Finally, we detected some mineralization of pyrite and barite in the SEM-EDX analysis of the historical bones. During the fossilization process in marine environments pyrite is commonly generated in the pores and fissures of the bones (Pfretzschner 2004; Raiswell et al. 1993; Sawlowicz and Kaye 2006). This kind of mineral was also identified in the bones of the 18th century Fougueux shipwreck located in the Bay of Cádiz (Bernáldez-Sánchez et al. 2014b). Moreover, pyrite was identified in some samples of the experimental biostratinomic analysis, but at present there is insufficient data in order to calculate the speed or conditions of formation. Another aspect analysed by SEM is the microscopic structure of the bone samples. The analysis of the trial samples is currently ongoing, but our first results indicate a higher erosion of raw samples after three and six months submerged (Figure 5). The evolution of this trend in samples submerged for a period of one year is currently under study.

Conclusions

Despite the limitations of the experimental biostratinomic analysis, due to the small number of samples and the reduced time of sampling, we have nonetheless obtained results that may contribute insights to the interpretation of the historical shipwreck bone assemblages.

We found differences in the erosion of raw and boiled bones, almost from the outset of the biostratinomic process. The bioerosion of bones with soft tissue is slower than that of boiled samples, perhaps because the soft tissue protects the bone during the first six months. There was a little fouling in the first three months of immersion and it is possible to observe adipocere in raw samples for as long as six months. At this time it is still possible distinguish raw from boiled bone. However, after 10 months submerged this is no longer possible due to the absence of adipocere in raw bones and the similarities in fouling between the two samples.

The biostratinomic results confirmed that the fouling observed in the historical Delta Project bones may in fact have been the result of a recent colonization. In addition, the absence of ancient fouling and the preservation of the margins of the shipwreck bones support the hypothesis of a quick burial in a muddy sediment. The microscopic analysis with SEM-EDX enabled us to link staining to the incorporation of mud into the bone structure. Further microscopic and chemical analysis is currently in progress. The first results from the experimental samples show that raw bones display worse preservation than boiled samples after three and six months under water. We have also confirmed the presence of pyrite in some samples, a common mineral in bones fossilized in underwater environments, also present in the historical Delta Project bones.

Finally, we may note, as mentioned above, that this biostratinomic analysis is not yet concluded and the sampling is limited, particularly with regards to the exposure time of the bones. The results presented here should be taken only as trends, since the fossilization process of the bones takes much longer than the duration of this experiment. However, it is known that both biostratinomic and diagenetic processes present great differences depending on the environmental conditions, and we did not want to miss the opportunity to carry out an experimental trial, albeit of small scale, at the same site from which we have previously studied the historical bone assemblages of the Delta Project in collaboration with the ARQUEOMONITOR team.

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In recent decades, there has been a great evolution in the study of past human-animal interactions, clearly demonstrated by the influx of researchers to the Encontro de Zooarqueologia Ibérica (EZI, or Encontro de Zooarqueologia Ibérica), held in April 2017 in Faro, Portugal, and organized by University of the Algarve.

This volume shows the diversity of zooarchaeological themes treated therein, from different perspectives and approaches, undoubtedly one of the greatest valences of the EZI conferences. We hope it will be a valuable contribution to the knowledge of zooarchaeology in the Iberian Peninsula.

Nas últimas décadas, tem havido uma grande evolução no estudo das interações entre humanos e animais no passado, claramente demonstrada pela afluência de investigadores ao Encontro de Zooarqueologia Ibérica (EZI), realizado em abril de 2017 em Faro, Portugal, e organizado pela Universidade do Algarve.

Este volume mostra a diversidade dos temas zooarqueológicos nele tratados, com diferentes perspetivas e abordagens, sem dúvida uma das maiores valências das conferências do EZI. Esperamos que seja um valioso contributo para o conhecimento da zooarqueologia na Península Ibérica.