Master in Forensic Sciences

University of Porto

Timing of blunt force injuries in long bones:

The effects of the environment, PMI length and human surrogate model

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Porto, 2012

ORIGINAL ARTICLE

A Dissertation presented for the
Master in Forensic Sciences Degree

**Timing of blunt force injuries in long bones:
The effects of the environment, PMI length and human surrogate model**

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Acknowledgments

Words can not express my gratitude to all who contributed to this study

To those who shared my concerns and uncertainties

To those who supported and accompanied me in spite of all the difficulties

To those who endured all for me

Thank you

To my Adviser, for his guidance and suggestions

To Faculty of Medicine of Porto University, for partially funding this study

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ABSTRACT

The timing of blunt force trauma in human skeletal material is a critical issue in forensic pathology and anthropology. However, there is still limited knowledge as to how fracture morphology is influenced by specific environmental conditions, postmortem interval (PMI), different bone types and animal models. This study aims at evaluating the influence of the type and duration of the postmortem environment in interpreting blunt skeletal trauma as perimortem or postmortem, based on comparisons of fracture morphology from long bones with different postmortem intervals and decomposition environments while simultaneously assessing variations in fracture characteristics between different bone types and species. Fresh limb segments from pig and goat were used in this study and were sequentially left to decompose, under three different environmental circumstances (ground surface, buried and submerged), during a total period of 196 days, after which all sets of limb segments (each with different PMI) were fractured together with a fresh set. Fractured bones (total n = 325; pig tibia = 110; pig fibula = 110; goat metatarsals = 105) were assessed macroscopically and classified according to the Fracture Freshness Index (FFI). Climatic data for the location of the experiment was collected. Statistical analysis included descriptive statistics, correlation analysis between FFI and PMI, Man-Whitney U tests for comparisons of FFI medians for different PMI's and linear regression analysis using PMI, mean pluviosity and mean temperature as predictors for FFI. Surface samples presented increasing FFI values for each PMI increment, with positive correlation for all studied bone types, the same observed in submerged samples, except for pig tibia. Median FFI values for surface samples with PMI = 0 could be statistically differentiated from PMI = 56 days or above. Buried samples presented no significant correlation between FFI and PMI as well as no statistically significant linear regression models. Linear regression analysis of surface and submerged samples suggested differences in FFI variation with PMI between bone types, although it failed to show statistical significance. When adding climatic data to the surface regression models, PMI was no longer a predictor of FFI. When comparing different animal models, linear regressions seemed to

suggest greater increases in FFI with increasing postmortem period in pig samples compared to goat samples, in both surface and submerged environments, but they failed to reach statistical significance. No differences were found between environments except for buried *versus* submerged metatarsal goat samples and surface *versus* buried or submerged tibia pig samples. FFI seems to have a weak association with PMI, possibly due to the slow-rate fracture morphology changes with increasing PMI, and it seems to be affected by various factors, such as different bone types, decomposition environment and climatic factors. Nonetheless, it does show some discriminating power in fracture morphology during the early postmortem period. The apparent variation between bone types can reveal a serious handicap of current experimental studies, as extrapolations to the human species can be challenged. The present study demonstrates the potential of the FFI in reflecting fracture morphology changes over time, however, peri or postmortem fracture diagnosis based on the FFI seems to be extremely difficult.

Keywords: Bone trauma, fracture morphology, postmortem interval, postmortem environment, Fracture Freshness Index,

INTRODUCTION

Recognizing perimortem trauma in human skeletal material is a critical issue in forensic pathology and anthropology since it may have serious legal implications [1]. For that purpose, forensic pathologists frequently rely on the expertise of the forensic anthropologist, who analyses a variety of morphological features in bone in order to determine when the injury occurred relative to the time of death, therefore tentatively distinguishing between perimortem and postmortem skeletal fractures [1-4]. Similarly, archaeologists and paleoanthropologists are frequently faced with the same issue, when interpreting bone fractures from prehistoric or historic animal and human skeletal remains.

Fracture characteristics indicative of timing of trauma are dependent on the condition of the bone prior to fracture, with bone moisture content having a very significant influence on fracture morphology [1,5,6]. As the amount of moisture content that is preserved after death varies considerably, the perimortem interval is often viewed as an ambiguous and elastic interval of unspecified duration, depending on the postmortem conditions of decomposition [7]. Contrary to the forensic pathology's conception, in which "perimortem" and "postmortem" are defined in terms of time intervals relative to the actual moment of death, in forensic anthropology the divisions are based instead on qualities of the bone tissue [2], namely whether the bone is "fresh" or "dry".

Previous research on bone fracture morphology has sought to identify fracture characteristics associated with either perimortem or postmortem trauma. However, several recent studies show that some types of bone fractures typically associated with perimortem trauma can also be seen when fractures occur postmortemly [3,7,8]. Nonetheless, forensic case reviews have shown that the analysis of fracture patterning in combination with fractured edge characteristics may be the most useful approach for the assessment of perimortem skeletal trauma [9]. These same characteristics have also been used in the analysis of archaeological bone, where Outram [5,10,11] has developed the Fracture Freshness Index (FFI), as a means of differentiating fractures produced in fresh and dry

bone. This index was created by combining the three main morphological criteria used in determining bone fracture timing - fracture angle, fracture surface and fracture outline [5,10,11].

In blunt force trauma, fresh bone tends to fail along a spiral or helical path and leave a fracture surface that is smooth and at an acute or obtuse angle to the bone's cortical surface, whereas dry bone tends to fracture in straight lines, with the fracture surface at right-angles to the bone's cortical surface and rougher as a result of micro-cracks [5]. However, taphonomic factors can have a significant effect on bone decomposition [12], particularly on the amount of moisture in bone after death, which may change the bone response to mechanical loading. The rate at which bone loses its moisture and fibrous content and becomes dry and brittle depends on the postmortem microenvironment. This means that the timing at which bone may exhibit specific fracture properties ("fresh" or "dry") varies greatly depending upon the postmortem environmental conditions to which the bone is itself subjected to [1,13]. Change in fracture properties is not only a function of time since death, but also of the postmortem conditions. Consequently, the observation and accounting of taphonomic processes becomes fundamental in order to accurately determine the timing of skeletal trauma [1,3]. Unfortunately, there is still limited knowledge on how fracture properties are influenced by different environmental conditions.

Recent experimental studies on bone fracture timing have used various types of animal models, such as pig [4,12], deer [6,7], horse [13] and cattle [13], because research using human bone material is limited by difficulties in obtaining large samples for experiments that can be controlled and replicated [6]. Pig bone, in particular, is frequently used as it is considered an effective analogy for human bone due to compositional similarities [12]. In addition to different animal models, experimental studies have also used different bone types, such as humeri, radio-ulnae, metacarpals, femora, tibiae, fibulae, patellae and metatarsals [6,7,12,13]. However, possible variations between bone types and species have not been investigated in detail.

The main purpose of this study is to assess the influence of the type and duration of the postmortem environment on bone fracture properties that can be used to distinguish blunt force trauma as perimortem (“fresh”) or postmortem (“dry”), based on comparisons of fracture morphology from long bones with different postmortem intervals (PMI) and exposed to different decomposition environments, using the Fracture Freshness Index (FFI) to quantify changes in bone properties, to determine what are the best morphological criteria for differentiating perimortem from postmortem bone trauma, as well as to establish a minimum PMI from which fracture morphology variations are detectable. Simultaneously, this study wishes to assess variations in fracture characteristics between different bone types and between different animal models.

MATERIALS AND METHODS

Fresh limb segments (less than 2 days postmortem) from pig (knee to hoof) and goat (ankle to hoof), obtained at a local meat store, were used in this study. During a total period of 196 days (from April to October 2011), seven sets of ten limb segments (five from pig and five from goat) were sequentially left to decompose, in a rural area of Central Portugal, under three different environmental circumstances (Figure 1): A) on the ground surface; B) buried; and C) submerged (total n = 210; total pig = 105; total goat = 105).

Figure 1 – Limb segments decomposing under three different environmental circumstances (A - ground surface; B - buried; C - submerged).

At the start of the study, one set of ten limb segments was placed in each environment (surface, buried, submerged), and then another set was placed every 28 days, until 196 days were reached and seven sets had been placed, reflecting seven different postmortem intervals (PMI = 28, 56, 84, 112, 140, 168 and 196 days) (Figure 2).

Samples placed on the surface were scattered over a 180 m² area in an approximately grid disposition. Each set or PMI station occupied 2 square meters and was placed at a distance of 1 meter apart from other stations. In order to minimize scavenging and specimen loss, each station was individually protected with a wire net, placed over a frame 50 cm high. Surface soil pH was recorded at each PMI interval.

Buried samples were treated individually, that is, specimens of each set were buried individually 1 meter deep, a least 1 meter apart from other specimens of different sets. In

order to minimize specimen loss and facilitate recovery, one end of a cable was tied to each specimen and the other end was left visible on the surface. Initial soil pH values were recorded at the bottom of each pit.

Figure 2 – Scheme illustrating the procedure adopted in this study for obtaining sets (1 to 7) of bones with different postmortem intervals, at the time of the experimental bone fracture (see text for details) (d: days).

Submerged samples were placed approximately 1.5 meters deep in a small river bay. Each set was submerged together inside a 50x50 cm wire cage, which was attached to a nearby boulder by a steel cable, in order to prevent specimen loss. Water temperature and pH was recorded at each PMI interval. Limb segments were placed in each environmental setting in a fresh state without prior removal of soft tissues, in order to better simulate natural decomposition conditions. Each limb specimen was given a unique number.

Immediately after the 196th day, all sets of limb segments were removed from their setting and, together with a fresh set (PMI = 0), were fractured using a custom-made apparatus consisting of a drop weight and a wooden frame (Figure 3). Rather than placing all seven sets of limb segments, under different environmental circumstances, at the start of the study and then remove a set every 28 days, where the bones would be fractured, an inverse scheme was adopted (the first set laid in the field had a PMI of 196 days and the last set a PMI of 28 days – Figure 2) to ensure that all bones from all sets were fractured almost simultaneously. At the end of the field experiment only the tibia and fibula from the pig limbs and the metatarsals from the goat limbs were retained and fractured. The apparatus used for fracturing consisted of a weight (5,9 Kg), which was dropped from a height of 80 cm,

indirectly impacting the mid anterior diaphysis of each bone, over a wood rod, to allow a better control over the point of impact and obtain a standardized impact area across samples. While being fractured, the bone was only supported at the epiphyses, so that a complete diaphyseal fracture could be obtained (Figure 3).

Figure 3 – Scheme illustrating the custom apparatus used for bone fracturing (1 – drop weight; 2 – wooden rod; 3 – bone specimen; 4 – wooden support frame) (see text for details).

After fracture, bone fragments from each specimen were recovered and stored in a net bag, after which they were macerated with the aid of an enzymatic agent (Tergazyme®, Alconox.Inc) and were allowed to dry.

Each individual specimen was assessed macroscopically in the lab and classified according to the Fracture Freshness Index (FFI) described by Outram [5,10,11]. This scoring system allows the expert to classify the type of fracture according to the 1) angle, 2) surface texture, and 3) outline: for fracture angle, a score of 0 is given if no more than 10% of the fracture surface is perpendicular to the cortical surface, 1 is given if between 10% and 50% is perpendicular and 2 is awarded if more than half the fracture surface is at right angles; for fracture surface texture, 0 is scored if the surface is entirely smooth apart from stress relief features, 1 is scored if there is some roughness but the texture is mainly smooth and 2 denotes a fragment with largely rough edges; for fracture outline, 0 means that there are only helical breaks, 1 denotes a mixture of fracture outlines and 2 means an absence of helical outline [11]. With this classification, bones obtain a total score between 0 (fresh) and 6 (dry) (Figure 4).

Figure 4 – (A) Bone fracture with helical outline (A1), smooth surface (A2) at an acute/obtuse angle with the cortical surface (A3), corresponding to an FFI value of 0; (B) Bone fracture with absence of helical outline (B1), rough surface (B2) perpendicular to the cortical surface (B3), corresponding to an FFI value of 6.

Climatic data was also collected from the National Meteorology Institute website (www.meteo.pt), relative to the weather station closest to the field study area and for the duration of the experiment. Namely, daily pluviosity (mm/day) and daily maximum and minimum temperature (degrees centigrade) were obtained.

For each environment setting (surface, buried, submerged) descriptive statistics were calculated for PMI stations/bone sets and bone type. Spearman's correlation coefficient was used to test the association between PMI and FFI, as well as FFI's components. Man-Whitney U tests were used to compare median FFI values between PMI stations. Linear regression models were also obtained for each bone type, with FFI as the dependent variable and PMI as the predictor, after which both constant and slope coefficients were compared between regression formulae for each of the bone types and species. In addition, for surface samples, a multiple linear regression model was calculated where pluviosity and temperature were added as predictor variables, in order to determine their contribution to changes in FFI values. Finally, regression coefficients for each bone were compared across the different environmental setting. All calculations were done using IBM SPSS version 20, and an alpha level of 0.05 was used as a general guide for statistical analysis and interpretation.

RESULTS

Surface samples

During fieldwork, pH monitoring showed a neutral soil (pH 6-7) with no significant variations between the different PMI stations. Regular observations of decomposing specimens, showed a larger insect activity on pig compared to goat specimens. Additionally, goat specimens seemed less prone to show bone exposure, as skin and fur were more resistant to degradation, contrary to pig specimens, where one could see exposed bone after only 28 days.

Table 1 shows descriptive statistics for FFI broken down by PMI and bone type, which suggests that FFI increases with PMI. This increase is confirmed by correlation analysis which shows significant positive correlation between PMI and FFI for all bone types (Table 2). Additionally, correlation between PMI and the FFI scoring components (Fracture angle, Surface texture and Fracture outline) showed that each bone type has a better correlation with different individual FFI scoring criteria. Namely, for goat metatarsals, FFI is more correlated with fracture angle and texture; for pig fibula, FFI is more correlated with fracture outline; and for pig tibia, FFI is more correlated with fracture angle.

Comparisons of FFI medians between PMI stations for each bone type are presented in Table 3. Considering pig samples, FFI medians for PMI=0 were significantly different from almost every other PMI station (for fibula: $0 \neq 56, 84, 112, 140$ and 196 ; for tibia: $0 \neq 84, 112, 168$ and 196). Comparatively, goat's FFI medians for PMI=0 are found not to be statistically different from any other PMI, although FFI values for PMI=28 differed from PMI=56, 112, 140 and 196.

Using linear regression models with PMI as a predictor of FFI (Table 4), statistically significant coefficients were obtained for all bone types. Results show that, for each increase in PMI unit, a greater increase in FFI is seen for pig tibia, compared to pig fibula and goat metatarsals. When comparing the different regression models, no significant difference was found between pig fibula and tibia (constant: $t=-1,931$; $p=0,057$; PMI coefficient: $t=0,155$;

p=0,877) and between goat metatarsals and pig fibula (constant: $t=-1,130$; $p=0,262$; PMI coefficient: $t=-0,007$; $p=0,317$), although between goat metatarsals and pig tibia, a significant difference was found, but only for the regression constant (constant: $t=-3,728$; $p=0,000356$; PMI coefficient: $t=-0,694$; $p=0,490$). An additional linear regression model for pig specimens was created combining the fibula and tibia (Table 4), with statistically significant coefficient estimates. When comparing the new pig model to the goat model, a significant difference was found, but only for the regression constant (constant: $t=-2,610$; $p=0,010$; PMI coefficient: $t=-0,584$; $p=0,561$).

Using climatic data retrieved from the National Metereology Institute website, a multiple regression model was calculated for goat and pig surface samples (Table 4), results showing non-significant coefficients estimates for goat samples. For pig samples, PMI was no longer a significant predictor of FFI, with pluviosity taking its place.

Buried samples

During fieldwork, pH monitoring showed a neutral soil (pH 6-7) with no significant variations between sample pits at the time of burial. Specimens with PMI>140 days showed complete skeletonization. All but three buried specimens (1 goat specimen for PMI=168 and 2 goat specimens for PMI=196) were recovered and analyzed.

Descriptive statistics for FFI broken down by PMI and bone type are presented in Table 1. Data suggest that FFI does not vary with PMI, which is confirmed by no significant correlations observed between PMI and FFI or any of the individual FFI scoring components (Fracture angle, Surface texture and Fracture outline) for all bone types (Table 2).

Significance values for comparisons of FFI medians between different PMI's, using the Mann-Whitney U Test, are shown in Table 3. For pig fibula, PMI=0 was significantly different from PMI=56, 84, 140 and 196; as for pig tibia, PMI=0 was different from PMI=28, 56 and 84. Comparatively, no significant differences between FFI medians were found between goat metatarsal samples. Using linear regression models with PMI as predictor for FFI, no significant PMI coefficient estimations were obtained for any bone type (Table 4).

PMI (days)	FFI								
	Goat metatarsals			Pig fibula			Pig tibia		
	Med.	Mean	S.d.	Med.	Mean	S.d.	Med.	Mean	S.d.
Control samples (n=15)									
0	1,0	1,00	1,000	1,0	0,80	0,447	1,0	1,60	0,894
Surface samples (n=105)									
28	0,0	0,40	0,548	2,0	2,00	1,871	3,0	2,60	1,673
56	1,0	1,20	0,447	2,0	2,40	1,140	3,0	3,00	1,225
84	1,0	1,20	0,837	3,0	3,20	1,304	5,0	4,20	1,304
112	1,0	1,60	1,342	2,0	2,00	0,707	3,0	3,60	0,894
140	2,0	2,00	0,707	2,0	2,00	0,000	3,0	2,40	0,894
168	1,0	1,40	1,517	2,0	2,20	1,789	3,0	3,80	1,304
196	2,0	2,00	0,707	4,0	3,80	1,789	5,0	4,00	1,414
Buried samples (n=102)									
28	1,0	1,20	0,837	1,0	2,00	1,732	3,0	3,60	0,894
56	2,0	2,00	1,581	2,0	2,40	0,894	4,0	3,60	1,517
84	1,0	1,20	0,447	2,0	2,80	1,643	4,0	4,20	0,837
112	1,0	1,20	0,837	2,0	1,40	0,894	2,0	2,20	1,304
140	2,0	1,60	0,548	3,0	2,80	0,837	3,0	3,00	1,581
168	1,0	1,00	0,816	1,0	1,20	1,095	3,0	2,80	1,304
196	2,0	1,67	0,577	2,0	2,20	0,837	2,0	2,00	1,581
Submerged samples (n=103)									
28	1,0	0,80	0,447	0,0	0,60	1,342	2,0	1,80	1,483
56	1,0	1,00	1,000	0,0	0,40	0,548	3,0	2,80	0,837
84	3,0	2,20	1,304	1,0	0,80	0,837	2,0	3,20	1,643
112	1,0	0,80	0,837	1,0	1,00	1,225	2,0	1,60	1,140
140	4,0	3,20	1,095	1,0	1,40	0,548	2,0	2,00	1,000
168	2,5	2,00	1,414	2,0	2,20	2,280	1,0	1,20	0,837
196	2,0	2,50	1,915	1,0	2,40	1,949	2,0	2,40	2,074

Table 1 – Descriptive statistics for FFI broken down by environment, PMI and bone type (n=325)
(Med.: Median; S.d.: Standard deviation).

	PMI					
	Goat metatarsals		Pig fibula		Pig tibia	
	r	p	r	p	r	p
Surface samples (n=120)						
FFI	0,413	0,008	0,399	0,011	0,383	0,015
Angle	0,324	0,042	0,258	0,108	0,484	0,002
Texture	0,379	0,016	0,267	0,096	0,211	0,192
Outline	0,056	0,733	0,329	0,038	0,228	0,157
Buried samples (n=117)						
FFI	0,095	0,575	0,213	0,186	-0,084	0,605
Angle	-0,035	0,837	0,309	0,052	-0,121	0,456
Texture	0,233	0,164	-0,031	0,848	-0,077	0,636
Outline	-0,112	0,509	-0,024	0,884	0,022	0,893
Submerged samples (n=118)						
FFI	0,416	0,009	0,448	0,004	-0,084	0,606
Angle	0,399	0,013	0,288	0,072	-0,172	0,288
Texture	0,103	0,538	0,491	0,001	-0,060	0,712
Outline	0,466	0,003	0,259	0,106	0,194	0,229

Table 2 – Spearman's correlation coefficient for PMI vs. FFI and each of the components of FFI, broken down by environment and bone type (n=355).

PMI (days)	Surface samples (n=120)							Buried samples (n=117)							Submerged samples (n=118)						
	PMI							PMI							PMI						
	0	28	56	84	112	140	168	0	28	56	84	112	140	168	0	28	56	84	112	140	168
Goat metatarsals																					
28	0,307	0,042						0,740	0,390						0,734						
56	0,734	0,118	0,905					0,283	0,905	0,371					1,000	0,734					
84	0,740	0,042	0,881	0,906				0,734	0,905		0,905				0,104	0,080	0,104				
112	0,575	0,042	0,881	0,906				0,740	1,000	0,390					0,740	0,905	0,740	0,085			
140	0,116	0,013	0,065	0,142	0,214			0,307	0,419	0,662	0,221	0,419			0,021	0,006	0,021	0,233	0,013		
168	0,663	0,307	0,911	0,914	0,575	0,518		1,000	0,694	0,315	0,661	0,694	0,227		0,203	0,156	0,203	0,786	0,165	0,203	
196	0,116	0,013	0,065	0,142	0,214	1,000	0,518	0,334	0,414	0,759	0,217	0,414	0,860	0,252	0,209	0,081	0,209	0,898	0,123	0,451	0,766
Pig fibula																					
28	0,178							0,095							0,204						
56	0,019	0,517						0,005	0,214						0,221	0,699					
84	0,007	0,195	0,329					0,019	0,324	0,813					0,905	0,408	0,419				
112	0,018	0,655	0,575	0,118				0,174	0,911	0,095	0,180				0,905	0,343	0,419	0,911			
140	0,004	0,521	0,521	0,054	1,000			0,007	0,163	0,343	0,828	0,034			0,093	0,100	0,031	0,212	0,313		
168	0,080	0,736	0,738	0,264	1,000	1,000		0,606	0,347	0,063	0,084	0,584	0,041		0,178	0,146	0,080	0,233	0,334	0,656	
196	0,018	0,202	0,159	0,517	0,086	0,090	0,239	0,018	0,381	0,906	0,664	0,178	0,316	0,125	0,095	0,038	0,032	0,146	0,146	0,637	1,000
Pig tibia																					
28	0,240							0,016							0,827						
56	0,065	0,914						0,049	0,584						0,065	0,193					
84	0,018	0,086	0,189					0,010	0,268	0,572					0,080	0,146	0,911				
112	0,016	0,382	0,262	0,324				0,432	0,104	0,126	0,033				0,913	0,914	0,101	0,146			
140	0,180	0,588	0,572	0,053	0,060			0,129	0,515	0,515	0,197	0,393			0,496	0,747	0,228	0,277	0,588		
168	0,016	0,382	0,219	0,519	0,906	0,060		0,128	0,382	0,264	0,083	0,451	0,831		0,572	0,511	0,023	0,033	0,511	0,228	
196	0,024	0,165	0,268	0,906	0,577	0,100	0,827	0,664	0,105	0,105	0,033	0,831	0,341	0,393	0,572	0,743	0,233	0,313	0,743	0,913	0,316

Table 3 – Mann-Whitney U Test *p*-values for comparisons of FFI medians between PMI stations, broken down by environment and bone type (*n*=355).

Bone		B	Std. Error	t	p
Surface samples					
Goat metatarsals (n=40)^a					
	(Constant)	0,733	0,267	2,743	0,009
	PMI	0,006	0,002	2,757	0,009
Pig (tibia+fibula) (n=80)^b					
	(Constant)	1,892	0,283	6,680	<0,001
	PMI	0,009	0,002	3,517	0,001
Goat – climate data (n=40)^c					
	(Constant)	34,147	19,920	1,714	0,096
	PMI	-0,002	0,008	-,250	0,804
	Pluviosity	-1,413	1,215	-1,163	0,254
	Temperature	-1,510	0,895	-1,687	0,102
Pig – climate data (n=80)^d					
	(Constant)	-17,828	21,225	-0,840	0,404
	PMI	0,001	0,008	0,172	0,864
	Pluviosity	3,321	1,295	2,565	0,013
	Temperature	0,908	0,954	0,952	0,345
Buried Samples					
Goat metatarsals (n=47)^e					
	(Constant)	1,263	0,264	4,774	<0,001
	PMI	0,001	0,002	0,405	0,688
Pig fibula (n=40)^f					
	(Constant)	1,717	0,360	4,774	<0,001
	PMI	0,002	0,003	0,776	0,443
Pig tibia (n=40)^g					
	(Constant)	3,083	0,418	7,378	<0,001
	PMI	-0,002	0,004	-0,596	0,555
Submerged samples					
Goat metatarsals (n=38)^h					
	(Constant)	0,773	0,361	2,142	0,039
	PMI	0,009	0,003	2,951	0,006
Pig fibula (n=40)ⁱ					
	(Constant)	0,267	0,361	0,738	0,465
	PMI	0,010	0,003	3,089	0,004
Pig tibia (n=40)^j					
	(Constant)	2,133	0,394	5,417	<0,001
	PMI	-0,001	0,003	-0,177	0,860

^a R = 0,408; R² = 0,167; Adj. R² = 0,145; Std. error of estimate = 0,926

^c R = 0,507; R² = 0,257; Adj. R² = 0,185; Std. error of estimate = 0,908

^e R = 0,068; R² = 0,005; Adj. R² = -0,024; Std. error of estimate = 0,899

^g R = 0,096; R² = 0,009; Adj. R² = -0,017; Std. error of estimate = 1,448

ⁱ R = 0,448; R² = 0,201; Adj. R² = 0,180; Std. error of estimate = 1,251

^b R = 0,370; R² = 0,137; Adj. R² = 0,126; Std. error of estimate = 1,387

^d R = 0,359; R² = 0,129; Adj. R² = 0,089; Std. error of estimate = 1,368

^f R = 0,125; R² = 0,016; Adj. R² = -0,010; Std. error of estimate = 1,246

^h R = 0,441; R² = 0,195; Adj. R² = 0,172; Std. error of estimate = 1,238

^j R = 0,029; R² = 0,001; Adj. R² = -0,025; Std. error of estimate = 1,364

Table 4 – Linear regression models for different environments and bone types (dependent variable: FFI; predictors: PMI, Mean pluviosity, Mean temperature).

Submerged samples

During fieldwork, water pH monitoring showed neutral values (pH 6-7), with measured temperature values varying between 7 and 16 (mean=11) degrees centigrade. Regular observations showed relative preservation of soft tissue in the specimens, with pig limbs showing partial saponification after 140 days. Goat specimens seemed less prone to preservation, showing no signs of saponification, with complete skeletonization by day 168 postmortem. All but two submerged specimens (1 goat specimen for PMI=168 and 1 goat specimen for PMI=196) were recovered and analyzed.

Descriptive statistics for FFI broken down by PMI and bone type are presented in Table 1. Values in this table suggest that FFI increases with PMI, which is confirmed by correlation analysis, showing significant positive correlation between PMI and FFI for all but pig tibia. As for FFI scoring components, a positive correlation coefficient was observed between PMI and fracture angle and outline for goat metatarsals, and fracture texture for pig fibula (Table 2).

Comparisons of FFI medians between PMI stations for each bone type are presented in Table 3. For goat samples, PMI=0 was only found to be significantly different from PMI=140, with no differences observed between PMI=0 and the remainder PMI stations, for pig samples.

Using linear regression models with PMI as predictor for FFI, statistically significant coefficients were obtained for all but pig tibia (Table 4), with pig fibula presenting a greater PMI coefficient than goat metatarsals, similar to that observed on surface samples, although when comparing the regression models, no significant difference was found between goat metatarsals and pig fibula (constant: $t=1,614$; $p=0,111$; PMI coefficient: $t=-0,453$; $p=0,652$), with both differing from pig tibia (for pig fibula vs. tibia : constant: $t=-3,378$; $p=0,001$; PMI coefficient: $t=2,005$; $p=0,048$; for goat metatarsals vs. pig tibia: constant: $t=-2,824$; $p=0,006$; PMI coefficient: $t=2,337$; $p=0,022$).

For each bone type, regression models from all environment conditions were compared. When considering goat metatarsals, no significant differences in the coefficient for PMI were found between buried and surface samples ($t=-1,597$; $p=0,115$) or submerged and surface samples ($t=0,813$; $p=0,419$), with buried and submerged samples showing significant differences ($t=-2,096$; $p=0,040$). Conversely, pig tibia presented no differences between buried and submerged samples ($t=-0,312$; $p=0,756$), while showing differences between buried and surface samples ($t=-2,248$; $p=0,027$) as well as between submerged and surface samples ($t=-1,992$; $p=0,050$). For pig fibula, no significant differences were found between regression models for any of the environment conditions (buried vs. surface: $t=-1,306$; $p=0,195$; submerged vs. surface: $t=0,261$; $p=0,795$; buried vs. submerged: $t=-1,642$; $p=0,105$).

DISCUSSION

The Fracture Freshness Index (FFI) has been considered useful as an archaeological tool for differentiating fresh and dry bone fractures [5,10,11] but its forensic significance has yet to be demonstrated. In this study, the efficiency of the Fracture Freshness Index (FFI) was assessed on human surrogate skeletal material exposed to different postmortem environments and for different periods of time, which was subsequently fractured experimentally. FFI only increased with an increasing postmortem interval on surface samples, and to a lesser degree on the submerged samples. On the other hand, this increase was not significant for goat metatarsals in the surface samples and for pig tibia in the submerged samples. FFI in the buried samples did not show any significant changes with increasing postmortem interval as well. Linear regressions seemed to suggest greater increases in FFI with increasing postmortem period in pig tibia and/or fibula compared to goat metatarsals, in both the surface and submerged samples, but they failed to reach statistical significance.

Although these differences could not be statistically confirmed, results may indicate the existence of between and within species variation, possibly due to intrinsic bone characteristics and their response to different postmortem environments, as well as a consequence of observed varying rates and levels of soft tissue decomposition between pig and goat samples. For example, submerged pig samples presented a greater degree of preservation, mainly due to soft tissue saponification, when compared to goat samples, which suffered complete skeletonization. This is justifiable by the greater degree of fat tissue present in pig samples, which exposed to a moist environment results in adipocere formation [14]. These species differences may raise an important concern, as to whether animal bone studies can be easily extrapolated to the human context, since different animal models can provide different results. This apparent variation, neglected in previous studies, may result in erroneous generalizations and thus must be taken into account when interpreting

experimental results. However, further studies are needed in order to fully address intra and inter species variations.

Although the FFI shows some change in response to an increasing postmortem interval, none of its components seems to change more than the others. Different components had different contributions to the change in the FFI in different bone types in different environments. This suggests that no particular component is more useful than the other in distinguishing fractures in fresh from dry bone and that the FFI may be a tool best applied as a whole. These differences in the component's contribution to FFI increase are probably a consequence of differences in intrinsic properties of the bones analyzed, such as different bone dimensions, different cortical structure and thickness, resulting in different levels of fracture morphology variation. They can also result from the difficulty in observing those component characteristics on bones with varied dimensions, as in this study.

Previous recent research on the use of fracture characteristics for estimating the timing of bone fracture has showed that bones do not consistently manifest postmortem-associated or dry bone characteristics until 141 days postmortem [4]. Comparatively, in the surface samples of this study, the Fracture Freshness Index (FFI) was able to distinguish perimortem fractures (0 days postmortem) from fractures produced in a PMI as small as 56 days. This suggests that the FFI can be advantageous for estimating fracture timing in forensic context, compared to previously described features, as the FFI may allow the detection of earlier changes in fracture morphology.

Because bone degradation over time is a dynamic process that varies considerably depending upon the ambient conditions to which bones are exposed [13], this study also wished to understand the effects of different environments on bone fracture morphology. When comparing the three environments no differences were found except for buried *versus* submerged metatarsal goat samples and surface *versus* buried or submerged pig tibia samples. Buried samples are distinct from other samples because no association was found between FFI and PMI. Comparatively, this association was stronger in submerged samples, compared to surface samples, which may indicate faster bone degradation on submerged

samples. This observation challenges the general assumption that decomposition in an aquatic environment occurs at a rate roughly half that of decomposition in air [15]. In fact, there are conflicting observations within the current literature of how moisture affects skeletal decomposition [12], such that water in which bodies are totally or partially submerged may accelerate or retard decomposition, depending on its chemical characteristics [16]. However, the effects of high variation in FFI values for each PMI found in the submerged samples cannot be ruled out as a factor behind the greater slope coefficients in these samples, compared to that of the surface ones.

Although differences in bone degradation were not quantified, changes in soft tissue can provide an idea of the differential impact of the environment on pig and goat samples. Contrary to observations in surface samples, submerged pig samples presented a greater degree of preservation, mainly due to soft tissue saponification, when compared to goat samples, which suffered complete skeletonization. Furthermore, the effects of different environments may be dependent on intrinsic bone characteristics, with each bone type being more or less prone to reflect the influence of a specific environmental setting. Compared to either surface or submerged samples, buried samples may reflect a slower bone degradation rate with consequent slower fracture morphology changes over time and is consistent with other authors' claims that buried remains seem to decompose at a rate of eight times slower than that of surface remains in the same environment [12] [15].

Fracture morphology seems to show considerable variation depending upon the conditions to which bones are subjected, and this is particularly noteworthy on surface samples. When climatic variables were included on the regression models, PMI lost its prediction capability and fracture morphology became, instead, significantly predicted by mean pluviosity. These results support the idea that what is important in determining bone changes is not time itself, but the cumulative effects of environmental factors over time. Therefore, as pointed out by other authors [2-4], the use of the terms perimortem and postmortem for describing fracture morphology seems to be inadequate, as this distinction is

really based on the effect of environment conditions on bone structure and only indirectly reflects the elapsed time since death.

This study adopted an experimental design which was meant to address two issues. By “inverting” the sequence by which samples are laid on the field and then fractured, this design wished to produce all bone fractures on the same day, virtually eliminating the possibility of gross variation in the fracture procedure. On the other hand, the sequential use of small sets of fresh specimens at a time did not require specimen conservation prior to laying the specimens in the field. Previous studies required specimen preservation (namely freezing) between the time when they were picked up at the meat store and the analysis, until all specimens were collected [4,12]. It was assumed that bones can be kept frozen for up to 2 weeks and thawed without alterations [12]. However, freezing is a bone drying process which will have different degrees of effect dependent on temperature and time [5]. Recent research has shown that fresh fracture properties of bones frozen for one week are more pronounced than in fresh bones and that, although freezing is a slow process, bones do degrade over time while frozen, with consequent changes in fracture properties of bones [13]. These changes can be further potentiated by thawing [13,17]. This methodology allowed circumventing the issue of specimen conservation, by assuring that all specimens were used immediately after acquisition.

While attempting to avoid some methodological problems, the experimental design created a potential limitation. Since progressively exposing bone specimens inevitably leads to each sample set being exposed to different initial climatic conditions, one can not be sure how these different conditions translate in terms of fracture morphology.

When developing an experimental design for a taphonomic study, it is virtually impossible to control all variables and bias. Although the choice of protecting specimens with wire net reduced the possibility of animal scavenging, other methodological factors may have affected the results of this study. Namely, limb segments were obtained from animals approximately the same age, but small variations in weight and dimensions were found, that can induce differences in fracture behaviour. Additionally, a large number of environmental

variables could not be controlled nor monitored, such as solar exposure, soil composition and soil drainage, water composition and water fauna, just to name a few. Obviously, all these factors play a role in the decomposition process and should be taken in consideration when interpreting fracture morphology modification over time. Finally, bone samples were macroscopically and qualitatively assessed. Although there are clear advantages in using this methodology, as it is non-destructive, repeatable and can be performed with limited resources and training, the inherent subjective nature of such observations cannot be ignored [12]. Moreover, due to its nature, the FFI can really only be applied to dense diaphysis bone and, although showing promising features, it cannot provide all the information needed to make a final interpretation [11].

In spite of the limitations highlighted above, the present study demonstrates the potential of FFI in reflecting fracture morphology changes over time in hypothetical forensic scenarios. However, distinguishing peri from postmortem fractures, or more correctly, bone fractures occurred in a fresh from a dry state, in the early postmortem period, based on FFI, seems to be extremely difficult. Further studies are needed in order to evaluate the practical applicability of this tool in a real context. Although the results show significant variation in fracture morphology between different environments and different bone types, the full extent of those differences could not be fully understood. Future studies with a larger time-span and with a tighter control over several variables may help to clarify this issue.

CONCLUSIONS

By combining three major fracture morphology criteria, the Fracture Freshness Index seems to have a weak ability to discriminate fresh from dry bone fractures in the early postmortem period. This discrimination was only possible for surface samples and a PMI of at least 56 days postmortem. FFI seems to have a weak positive correlation with PMI, possibly due to the slow-rate fracture morphology changes with increasing PMI, and it is strongly affected by various factors, such as different bone types, decomposition environment and climatic factors. The observed variation between bone types and species can reveal a serious handicap of current experimental studies, as extrapolations to the human species can be challenged.

Investigation on this topic has yet to identify a fracture characteristic truly unique to either perimortem or postmortem trauma. In fact, it is extremely difficult to determine if any fracture characteristic appears solely in bones broken around the time of death, because of the inherent variation of the so-called perimortem interval. Nonetheless, new methodological tools are needed to allow a better assessment of forensic cases, which frequently involve serious legal consequences.

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