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MicroCT analysis of archaeozoological remains: a non-destructive approach to the age estimation

L'applicazione della microtomografia allo studio dei resti archeozoologici: un metodo non distruttivo per determinare l'età degli individui

Summary - Several recent studies are based on microCT analyses of the bone structure at micro- and meso-scales. Most of them are carried out in the field of biomedical research (particularly for bioengineering applications) and, increasingly, in palaeoanthropology and palaeontology.
The potentiality of microCT structural analysis of archaeozoological remains is here assessed with the aim to evaluate the applications of such a non-destructive approach to the age estimation of animal remains through the analysis of the bone's internal structure.
More specifically, the possible age-related trends observed in the micro-morphology of some bones of modern foxes (such as connectivity, trabecular thickness, ratio between bone volume and total volume, cortical bone fraction and its porosity) of different ages, from a few months old to adult specimens, are discussed.
The analysis was carried out using a computed X-ray microtomography system recently built by the *Abdus Salam* International Centre of Theoretical Physics in collaboration with Sincrotrone Trieste S.C.p.A. (Trieste, Italy) in the framework of the EXACT (Elemental X-ray Analysis and Computed Tomography) project, funded by the Regione Friuli - Venezia Giulia.
Preliminary results show an increase in bone volume and in cortical bone fraction as well as a reduction of cortical bone porosity after the first few months of life.

Riassunto - Recentemente le analisi microtomografiche applicate allo studio della struttura ossea si sono notevolmente diffuse. La maggior parte degli studi sono stati eseguiti in campo medico (in particolare per la bioingegneria) e sempre di più in campo paleoantropologico e paleontologico. Vengono qui discusse le potenzialità che tale tipo di ricerca può offrire in campo archeozoologico. Lo scopo è valutare la possibilità di stimare l'età degli individui, analizzando la struttura interna dell'osso attraverso analisi non distruttive.
Si è deciso di eseguire le tomografie di alcune ossa di volpi attuali di diverse età e di analizzare la variazione di alcune caratteristiche dell'osso (per esempio il numero delle trabecole, lo spessore delle stesse, la quantità dei vuoti, lo spessore della porzione corticale e la sua porosità) a partire da pochi mesi di vita fino allo stadio adulto. Le analisi sono state eseguite usando un microtomografo computerizzato a raggi X messo a punto presso l'Abdus Salam International Centre of Theoretical Physics in collaborazione con Sincrotrone Trieste S.C.p.A. (Trieste) nell'ambito del progetto EXACT (Elemental X-ray Analysis and Computed Tomography) finanziato dalla Regione Friuli - Venezia Giulia.
In via preliminare si osservano l'aumento del volume di tessuto osseo, l'ispessimento della porzione corticale e la diminuzione della sua porosità nei primi mesi di vita.

Keywords: MicroCT analysis, age at death estimation, zooarchaeology
Parole chiave: Analisi microtomografiche, determinazione dell'età, archeozoologia

INTRODUCTION

The estimation of the age at death of the individuals is crucial in the zooarchaeological research to better understand the animal management by past hunters or herders. For mammal remains, mortality data are commonly provided by the analysis of dental eruption and wear, and epiphyseal fusion (rev. in O'Connor 2006). Osteohistological studies are less widespread but have also provided methods of determining age-at-death of individuals, quantifying osteons and growth rings through the observation of thin slides (rev. in Dammers 2006).

The application of such destructive technique on archaeological specimens is problematic.
The computed microtomography (microCT) represents non invasive alternative in the analysis of bone microstructure.
In the last years its applications are being carried out in biomedicine paleoanthropology and palaeontology, thanks to their non-destructive character (e.g. Müller *et al.* 1998; Tanck *et al.* 2001; Macho *et al.* 2005; DeSilva, Devlin 2012).

Some authors pointed out the correlation of particular features of the bone structure related to ontogeny (Tanck *et al*. 2001; Ruimerman *et al*. 2005).
The aim of this paper is to test the potentiality of microCT analyses to estimate age at death using skeletal elements without epiphysis, such as for instance carpal or tarsal bones.
In mammal *taxa* characterised by a rapid ontogeny (for instance carnivores), some bones do not show significant size differences during life whilst there are important changes in the bone structure.
Moreover there are taxa showing similar skeletal morphology but a very different body size at the adult stage.
For example, specimens belonging to young wolves from prehistoric sites could be identified as adult dogs, or specimens belonging to medium-sized adult dogs could be identified as young wolves that did not reach the adult body size.
A more reliable taxonomic identification should be of great interest to better understand the process of dog domestication throughout time.

Individuals	**253**	**254**	**TS JUV**	**329**	**313**	**479**	**160**	**49**	**299**	**101**	**47**	**178**	**338**
Premolars	P1+/-	P1+/-	P2-3 -	+	+	+	+	?	+	+	?	+	+
Molars	-	-	M3 -	M3+	M3+	M3+	M3+	?	M3+	M3+	?	M3+	M3+
Canines	-	-	+/-	+	+	+	+	?	+	+	?	+	+
Atlas	-	-	+	+	+	+	?	?	+	+	+	+	+
Ribs	-	-	-	-	-	+	?	?	+	+	+	?	+
Scapula	-	-	+	+	+	+	+	?	+	+	+	+	+
Humerus prox.	-	-	-	-	-	-	+	+	+	+	+	?	+
Humerus dist.	-	-	+	+	+	+	+	+	+	+	+	?	+
Radius prox.	-	-	-	-	+/-	+	+	+	+	+	+	?	+
Radius dist.	-	-	-	-	-	+	+	+	+	+	+	?	+
Ulna prox.	-	-	+/-	+	+	+	+	+	+	+	+	+	+
Ulna dist.	-	-	-	-	-	-	+	+	+	+	+	+	+
Pubic symphysis	-	-	-	-	-	-	-	?	+	+	+	+	+
Acetabulum	-	-	+	+	+	+	+	?	+	+	+	+	+
Femur prox.	-	-	-	-	-	+/-	+	+	+	+	+	?	+
Femur dist.	-	-	-	-	-	-	+	+	+	+	+	?	+
Tibia prox.	-	-	-	-	-	-	+	+	+	+	+	+	+
Tibia dist.	-	-	-	-	-	+	+	+	+	+	+	+	+
Fibula prox.	-	-	-	-	-	+/-	+	+	+	+	+	+	+
Fibula dist.	-	-	-	-	-	+/-	+	+	+	+	+	+	+
Metapodials dist.	-	-	-	-	+	+	+	+	+	+	+	+	+
Pisiform	-	-	+	+	+	+	+	+	+	+	+	+	+
Calcaneum	-	-	+	+	+	+	+	+	+	+	+	+	+
First phalanx	-	-	+/-	+/-	+	+	+	+	+	+	+	+	+
Second phalanx	-	-	+/-	+	+	+	+	+	+	+	+	+	+
Age (months)	2	2	4 to 5	5 to 6	6 to 8	6 to 8	8 to 12	>8	>12	>12	>12	>12	>12

Tab. 1. *Vulpes vulpes*: epyphiseal fusion and tooth eruption of the individuals. Symbols and abbreviations: prox.: proximal; dist.: distal; +: fused/erupted; -: unfused/unerupted; +/-: in fusion/in eruption; ?: unknown. Age at death data from Habermehl (1986).

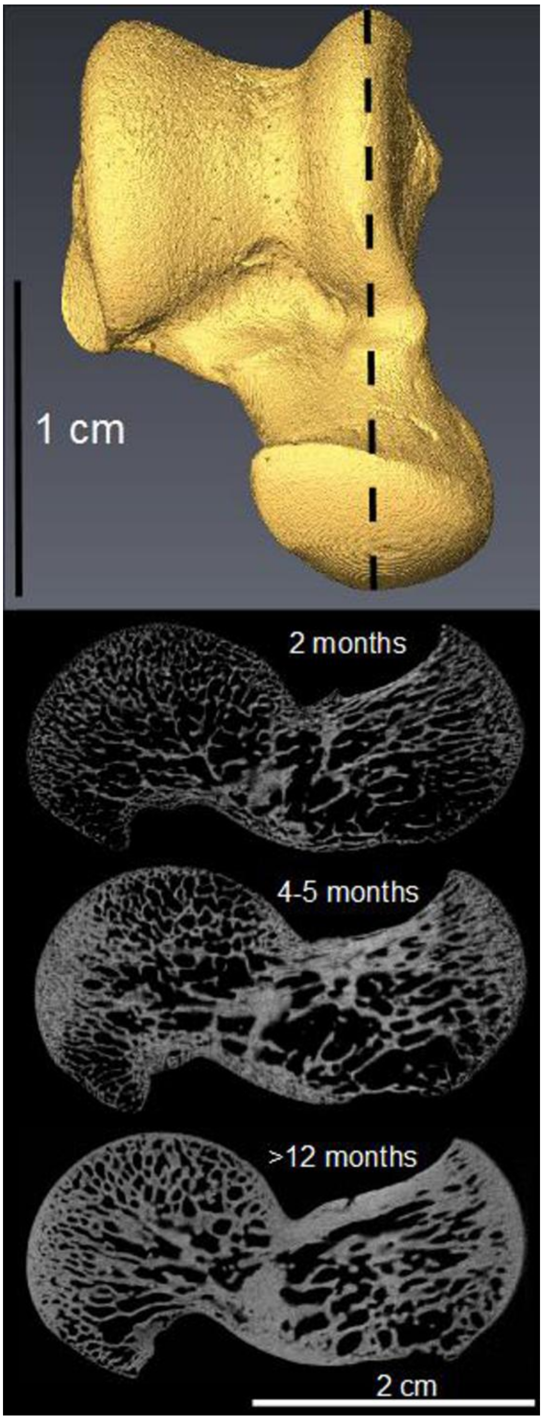

Fig. 1. Position of the virtual longitudinal cross-section performed on the specimens and cross-sections of three individuals of different age.

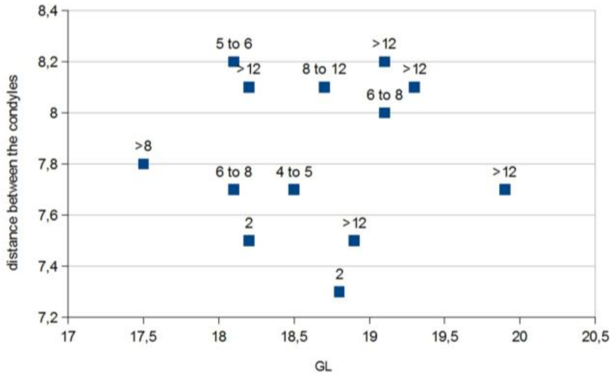

Fig. 2. Size of the specimens according to the estimated age (in months).

Materials and Methods

The *tali* of 13 modern red foxes (*Vulpes vulpes*) were considered here to observe possible age-related trends in the bone micro-morphology.

The skeletons are part of the osteological comparative collection of the Research Unit of Prehistoric Ecology of the University of Siena (Italy) and belong to individuals of different ages, from few month-old to adult stage. The age at death of the individuals (Tab. 1) was estimated observing teeth eruption and epiphyseal fusion as proposed by Habermehl (1986). Greatest length (following von den Driesch 1976) and the width of the trochlea at the apex of each condyle were measured for all specimens (Fig. 2).

The microCT acquisitions were realized at the Multidisciplinary Laboratory of the 'Abdus Salam' International Centre for Theoretical Physics of Trieste, using the following parameters: 110 kV voltage; 90 µA current; 2400 projections. The slices were reconstructed using Cobra v.7.4 (Exxim), obtaining an isotropic voxel size of 17.42 µm (Tuniz *et al.* 2013). Using Amira v.5.3 (Visualization Sciences Group Inc.), a semi-automatic threshold-based segmentation was carried out in order to separate the bone tissue from the interstitial air between the trabeculae.

The ratio between the bone volume and the total volume was counted for each specimen.

All specimens were oriented in the same direction in order to extract two homologous cubic subvolumes of trabecular tissue with a side length of 1742 µm, one located in the lateral condylus of the proximal trochlea (subvolume 1) and the other one in the neck (subvolume 2).

This method is commonly used to analyse bone microstructure (e.g. Ding, Hvid 2000; Tanck *et al*. 2001; Mulder *et al.* 2005). Characteristics of the cancellous bone were measured using the software BoneJ (Doube *et al*. 2010) (Tab. 2).

A longitudinal virtual cross-section of each bone was taken using a tangent plane to the apex of the medial condyle of the trochlea (Fig. 1).

Cortical bone fraction and porosity of the cortical bone were counted using the software ImageJ (Schneider *et al*. 2012).

Results

The size of the bones does not allow separating between individuals of different ages (Fig. 2). Conversely, the two youngest individuals (about 2 months old) are characterized by a significantly lower density of the bone structure, as shown by the ratio between the bone tissue volume and the total volume (BV/TV) of each specimen (Fig. 3A).

The bones of very young individuals are very porous,

but then the bone volume fraction rapidly increases reaching its highest value at 6 to 8 months.
In mature individuals the bone volume fraction decreases due to the adaptations of the tissue to external loads or due to other physiological factors related to age and sex. Similar results were also observed and discussed by other researchers (Tanck *et al.* 2001; Ruimerman *et al.* 2005). The analysis of the two subvolumes of cancellous bone has not yielded clear data (Tab. 2). No clear age-related trends were noticed for all the single considered parameters (BV/TV, degree of anisotropy, mean trabecular spacing and connectivity), with the exception of the trabecular thickness of the subvolume 1: it increases from 2 months up to 6 to 8 months aged individuals (but with one exception of a 6 to 8 months aged individual) and then decreases in the mature ones. The absence of clear trends contrasts with results obtained by other authors (e.g. Tanck *et al.* 2001). This can be related to the dimension of the subsamples (perhaps too small), which could not be representative of the general state and could be influenced by very local characteristics of the trabecular bone. Differences between the two subvolumes are also observed; they could be explained by different external mechanical loads influencing the different regions of the talus during life. Similar results were also observed by DeSilva and Devlin (2012). The analysis of the virtual cross-sections has shown an age-related trend in the ratio BV/TV which is similar to the pattern observed when considering the whole bones. The correlation between the two data sets is very strong (r = 9.3, p<0.001). Observing the cross sections it is also possible to record an increase of the cortical bone fraction and a reduction of its porosity after the first months of life (Figg. 3B and 3C). The amount of the cortical portion is very low till the age of about 6 months and then substantially increases, reaching its maximum value between 8 and 12 months.

Subvolume 1					
	Deg.Anis	**Connectivity**	**Tb.Th. mean**	**Tb.Sp. mean**	**BV/TV**
253 vol 3	0.33	247.62	8.2	18.35	0.33
254 vol 3	0.66	147.7	5.98	50.08	0.12
TS_juv vol 3	0.68	88.62	10.52	36.22	0.27
329 vol 3	0.55	97	10.54	26.72	0.32
458 vol 3	0.41	43.1	20.5	32.94	0.53
313 vol 3	0.712	177.1	9.05	36.08	0.24
479 vol 3	0.62	63.2	11.73	31.9	0.32
160vol 3	0.61	43.37	10.39	33.64	0.25
49 vol 3	0.56	140.2	9.16	24	0.31
299 vol 3	0.62	496.7	8	20.96	0.34
47 vol 3	0.81	36.5	10.86	40.16	0.22
101 vol 3	0.768	86	10	36.01	0.25
178 vol 3	0.68	63.5	11.8	27.8	0.36
338 vol 3	0.86	83.6	6.9	34.2	0.16
Subvolume 2					
	Deg.Anis	**Connectivity**	**Tb.Th. mean**	**Tb.Sp. mean**	**BV/TV**
253 vol 4	0.82	343	8.78	14.09	0.42
254 vol 4	0.83	214	7.3	16.39	0.31
TS_juv vol 4	0.82	102.8	13.76	13.29	0.61
329 vol 4	0.67	51.7	17.88	12.91	0.69
458 vol 4	0.81	48.5	20.7	13.79	0.72
313 vol 4	0.58	974.3	11.45	15.81	0.49
479 vol 4	0.69	527.7	26.96	12.51	0.78
160vol 4	0.8	123.7	13.57	18.66	0.51
49 vol 4	0.75	129.6	14.97	14.85	0.59
299 vol 4	0.78	148.3	14.29	17.63	0.55
47 vol 4	0.81	46.5	15.31	15.7	0.58
101 vol 4	0.73	189.3	16.07	16.1	0.55
178 vol 4	0.75	234.1	13.05	16.96	0.51
338 vol 4	0.79	101.7	10.61	18.04	0.43

Tab. 2. Data collected on the two subvolumes. Abbreviations: Deg.Anis.: Degree of anisotropy (0 = isotropic, 1 = anisotropic); Tb.Th.: trabecular thickness (in µm); Tb.Sp.: trabecular spacing (in µm); BV/TV: Bone volume/Total volume; Connectivity grossly corresponds to the number of trabeculae.

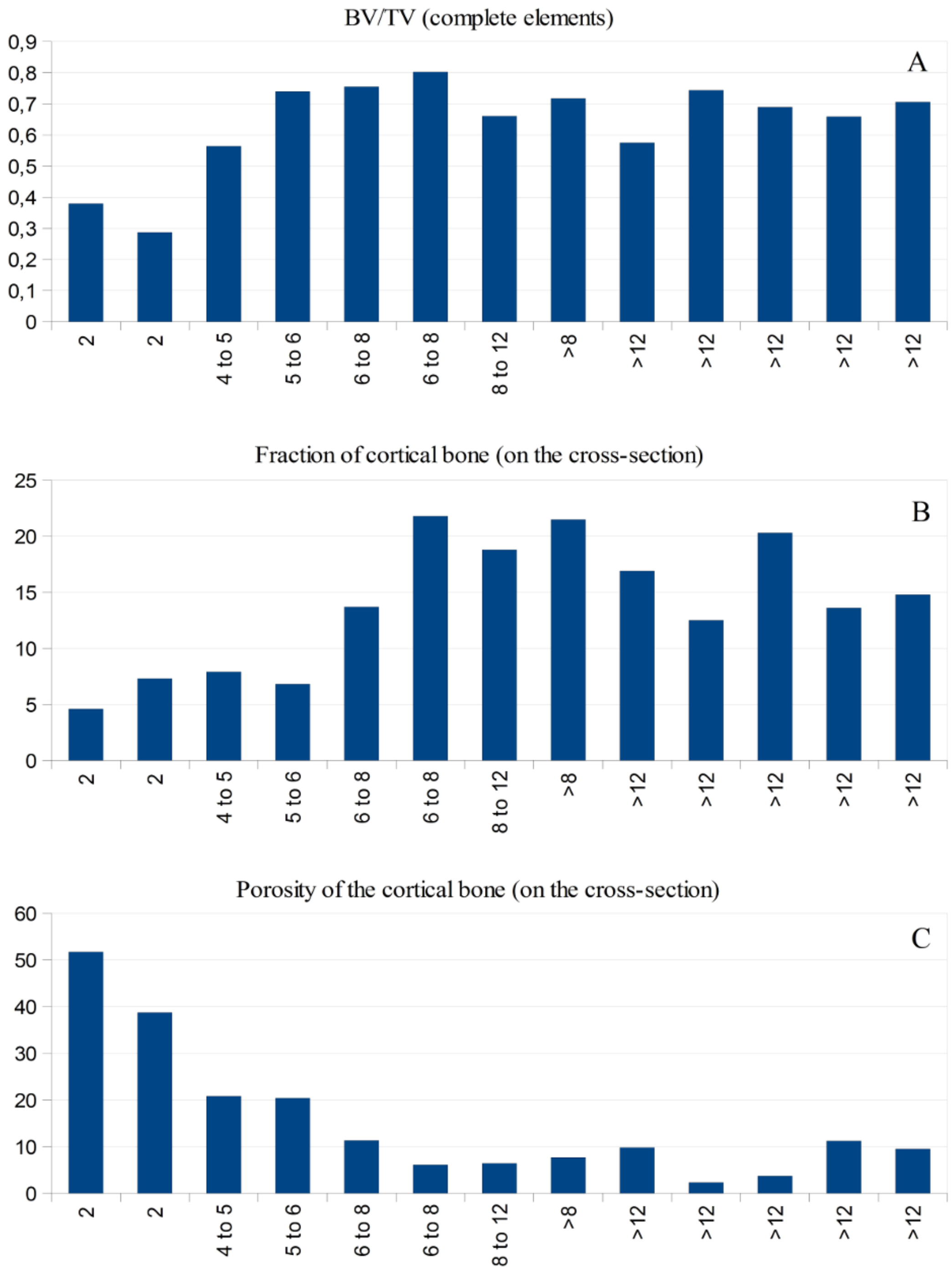

Fig. 3. Age-related diagnostic parameters measured on the specimens.

Conclusions

Microtomography is a powerful tool for understanding bone tissue development throughout vertebrates life. Results presented here show that it is possible to separate between some age-classes.

The porous bone structure of the youngest individuals (about 2 months) is significantly different from that of the older specimens.

Individuals between 4 to 6 months old can also be separated from the older ones due to their inferior amount of cortical bone and higher cortical bone porosity.

From 6 months up to adult stages, it is more difficult to assess the age-at-death of individuals.

A different sampling of sub volumes of cancellous bone and the analysis of other cross-sections should provide more information.

Results are of great interest for zooarchaeological research, allowing the discrimination between closely related taxa, showing similar juvenile bone morphology but a different adult size.

For instance the presence of small-sized individuals belonging to the genus *Canis* in prehistoric contexts can be related to the domestication if the bone structure of the identified specimens doesn't show juvenile characteristics (body growth in wolves considerably decreases after 6 months of life).

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