UNDERGRADUATE SUMMER VACATION SCHOLARSHIP AWARDS – FINAL SUMMARY REPORT FORM 2017/18

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Name of student:

Sophie Gray

Name of supervisor(s):

Dr Wendy Birch

Project Title: (no more than 220 characters)

Investigation of osteon variation throughout the human body and the impact of this variation on age estimation in a forensic context

Project aims: (no more than 700 words)

Overview

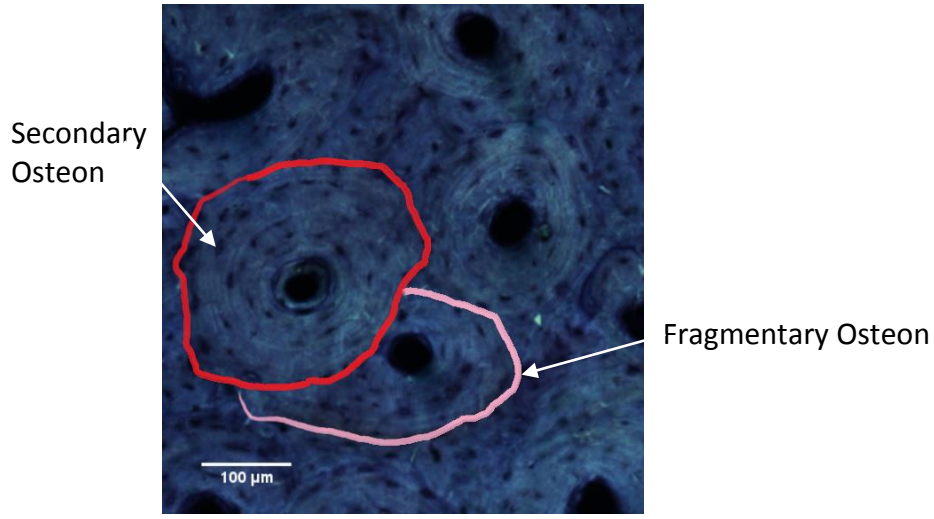
In the field of forensic age estimation there is abundant research focusing on regression equations derived from osteon counts obtained from different populations (Kersley, 1965; Ahlqvist & Damsten, 1969; Singh & Gunberg, 1970; Stout & Stanley, 1991; Yoshino et al. 1994; Maat et al. 2006; Lee et al. 2014; Pfeiffer et al. 2016). There is however, a deficit of validation studies in this area. The main aim of this project was to quantify the variation in cortical osteon density, in order to consider the impact of this variation on biological age estimation in a forensic context. This histological analysis was undertaken through a blind study of ground bone sections from two cadavers of the same chronological age but different biological sex.

In addition, the Suchey-Brooks (1990), Lovejoy et al. (1985), Iscan et al. (1984 & 1985) qualitative aging techniques were also utilised to enable a direct comparison between aging methods.

Background

In 1965 Kerley, pioneered a methodology that utilised quantitative histomorphometrics of cortical bone remodelling as a biological aging technique. In 1992, Stout and Paine modified this method and introduced a standardised variable - 'osteon density' (calculated by dividing the osteon count by the area of the region of interest (ROI)), therefore facilitating cross-study comparisons between different ROIs. The variables used to investigate osteon density of ground bone sections are the fragmentary, secondary and total osteon population density (TOPD) (Figure 1), these variables have been used to develop regression equations for aging individuals (Kersley, 1965; Ahlqvist & Damsten 1969; Singh & Gunberg 1970; Stout & Stanley 1991; Yoshino et al. 1994; Maat et al. 2006; Lee et al. 2014; Pfeiffer et al. 2016). It is the TOPD and the previously referenced regression equations that are investigated further in this project.

Figure 1: Image showing outlined secondary and fragmentary osteons from the proximal lateral femur, taken using a Leica confocal light microscope with imaging facilities (x10 mag)



Aim: To investigate osteon variation throughout the body

Different sampling sites were selected in order to investigate osteon variation throughout the body in tandem with the evaluation of the derived regression equations. Ground sections were made of the mid-shaft diaphysis and proximal/distal metaphysis of the long bones (humerus, ulna, radius, femur, tibia and fibula), the mandibular ramus at the level of the mandibular foramen, and also the mid-thoracic 6th rib (n=69). For each ground section four ROIs were identified for further imaging using light microscopy. The centre of each of these ROIs were located at 90 degrees to each other in the transverse axis of the cortical section.

Particular note should be made of the selection of the 6th rib, in lieu of the 4th rib which is commonly used for aging in a forensic context (Iscan et al. 1986). Ribs are frequently used for histomorphometric analysis and are considered to provide the most accurate results (Cho et al. 2002; Franklin, 2010). However, in this case the 6th rib was sectioned as this had been used by Stout and Paine (1992) to develop their regression equation. In addition, only one mid-thoracic sampling location was used as ribs are not load-bearing and so not greatly affected by biomechanically induced TOPD variation (Raab et al. 1991; Tommerup et al. 1993).

Aim: Evaluation of ROIs

TOPD data obtained from images of the ROIs was applied to aging regression equations (Kersley, 1965; Singh et al. 1970; Stout & Stanley, 1991; Stout & Paine, 1992; Yoshino et al. 1994; Lee et al. 2014). The variation in the resultant age at each sampling site was then analysed. The total intra- and intersectional variation in TOPD was assessed using randomised block design two-way analysis of variance (ANOVA). Therefore, this study expands on the work Chan et al. (2007), and investigates variation throughout the entire skeleton.

Aim: Investigation of bilateral symmetry

Ground bone sections were produced for the left and right sides of one cadaver to enable a comparison between each side. Analysis was performed using a paired T-test to identify any significant bilateral asymmetry, which may influence the use of osteon counts and regression equations for aging in a forensic context.

Aim: Investigation of biological sex

Both cadavers used in this project were of the same chronological age but different biological sex. Therefore, comparison of the TOPD of each respective sampling site was also carried out to identify any significant differences between the biological sexes.

Project Outcomes and Experience Gained by the Student (no more than 700 words)

Investigation of aging regression equations derived from osteon counts

The TOPD obtained from each ROI within each bone were applied to their specific regression equations. The resultant ages are displayed below.

Figure 2: Boxplots of biological age estimations derived from TOPD of each ROI within a bone when applied to aging regression equations for the left side of a female individual. The vertical whiskers show the total range of age estimations for each bone. The box shows the range of data between the quartiles, with a line within this interquartile range illustrating the median. The mean age estimation is represented by the cross. Small circles within the box plots represent data points. Small circles outside of the whiskers represent outliers. The horizontal coloured lines represent the results of the alternative aging techniques

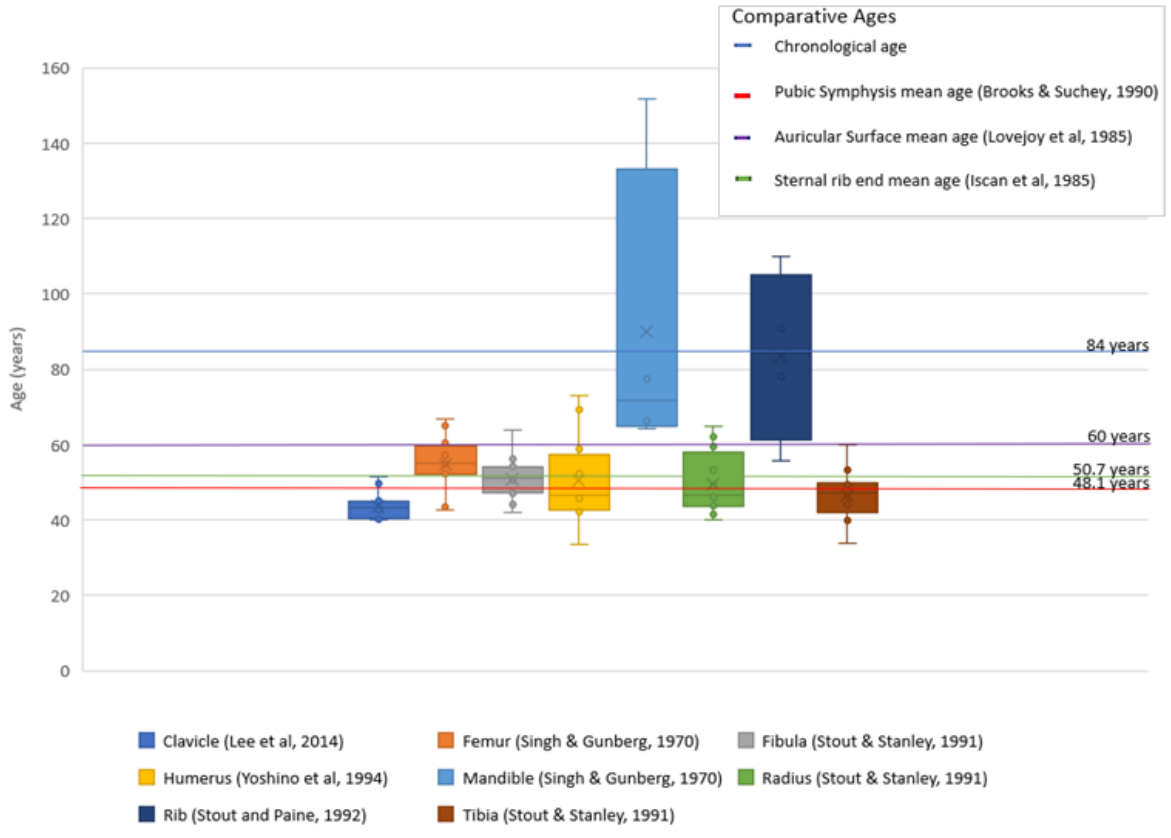
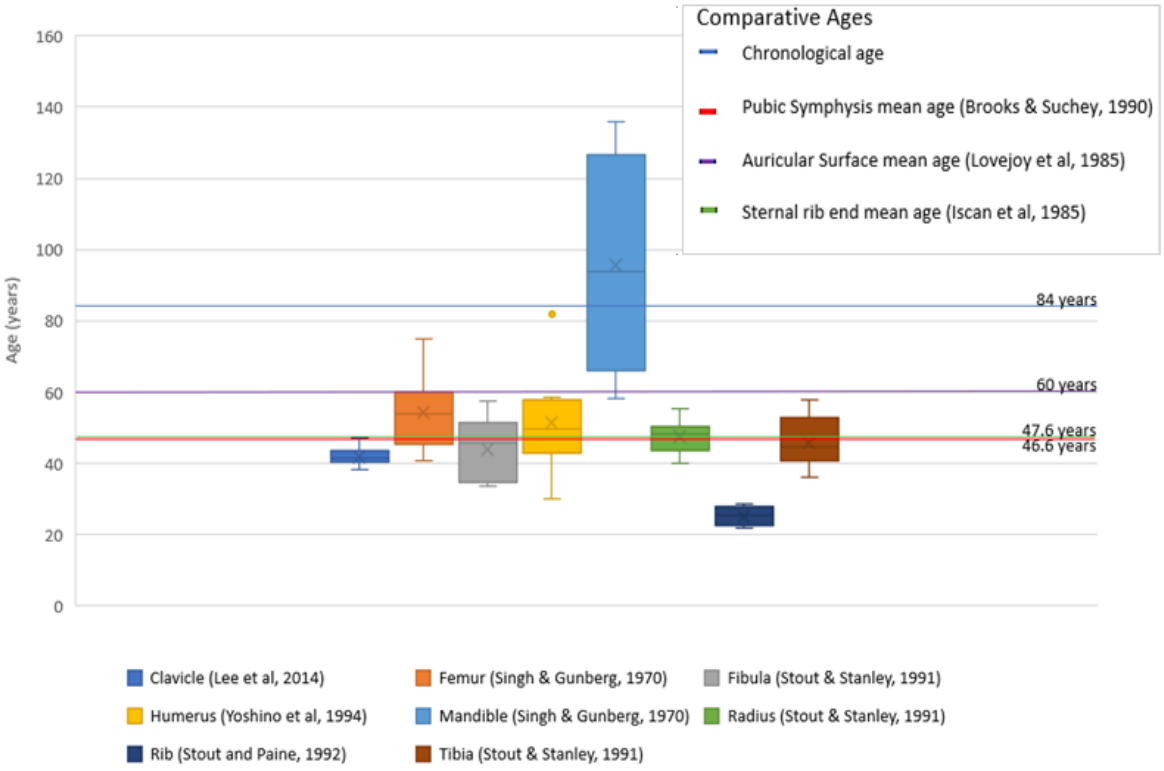


Figure 3: Boxplots of biological age estimations derived from TOPD of each ROI within a bone when applied to aging regression equations for the left side of a male individual



The chronological age of both individuals (84 years) was revealed after the data had been analysed and the alternative qualitative techniques applied, enabling a completely blind study.

Alternative qualitative aging techniques

To compare techniques, the currently used Suchey-Brooks (1990) and Lovejoy et al. (1985) pelvic aging methods and the Iscan et al. (1984 & 1985) 4th rib aging methods were investigated.

Table 1: Results obtained from the alternative qualitative aging techniques

Suchey-Brooks (1990) pubic symphysis aging technique

Biological sex	Side	Mean Age	Age Range
Female	Left	48.1	25-83
	Right	48.1	25-83
Male	Left	46.6	27-66

Lovejoy et al. (1985) auricular surface aging technique

Biological sex	Side	Mean Age
Female	Left	60 plus
	Right	60 plus
Male	Left	60 plus

Iscan et al. (1984 & 1985) 4th rib aging technique

Biological sex	Side	Mean Age	Age Range
Female	Left	50.7	32-79
Male	Left	47.6	32-67

Conclusions

For the female, the Stout and Paine (1992) estimation using the rib provided a mean of 84 years – the same as the chronological age. This was not the case for the male; Erikson, (1991) and Kim et al, (2007) suggest sexual dimorphism of bone histology be considered when deriving and using aging regression equations.

The mean age estimation for long bones (excluding the ribs) resided between 40-60 years for both individuals. The mean age estimations of the alternative qualitative aging techniques ranged from 46.6-60+. This tendency towards systematic negative bias of estimations for older individuals may be explained by a plateau in both microscopic and macroscopic changes that occurs between the 5th and 6th decade of life (Frost, 1987; Buckberry & Chamberlain, 2002; Martrille et al, 2007; Crowder, 2013; Miranker, 2016). The lateral ROI of the mandible had the lowest mean error of estimated biological to chronological age. Additionally the mandible is the only bone with a mean age estimation higher than the chronological age for both sexes, suggesting it may not be subject to the systematic underestimation of biological age of older individuals. Studies in non-humans also show bone remodelling plateauing in long bones, while remaining elevated in the mandible (Huja & Beck, 2008). The mandibular remodelling rate in a forensic context however, has not been quantified and is worthy of futher investigation.

Additional Outcomes

Accuracy of ROIs

Forensic skeletal evidence is often fragmentary, so quantification of TOPD variation allows investigation of the impact of sampling locations on histological aging techniques. Quantification of remodelling variance was completed using two-way ANOVA randomised block design (Chan et al. 2007).

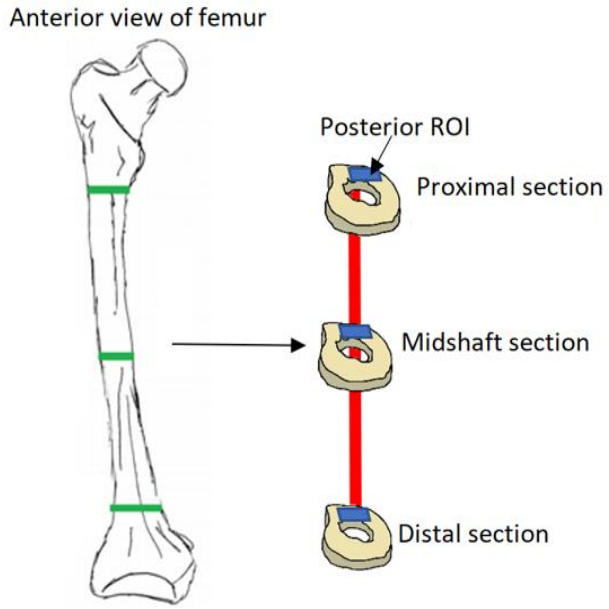
Intrasectional variation within a bone

Significant variation (p-value=0.0415) in the clavicular lateral section was identified and may possibly be explained by tension at the acromioclavicular joint (Sobol et al, 2015). Statistically significant intrasectional variation was also identified in the distal ulna (p-value=0.0187). Significant variation occurs in regions correlated to high biomechanical loads associated with distinctly human behaviour, namely clavicular asymmetry caused by handedness and intrasectional variation in the distal ulna attributed to triangular fibrocartilage induced tension with high wrist motility (Palmer & Werner, 1984).

Intersectional variation within a bone

The femoral posterior longitudinal axis (Figure 4) was the only longitudinal axis within the femur with statistically significant intersectional variation (p-value=0.0320) which is in agreement with Chan et al. (2007). Therefore, femoral posterior cortical sections should not be used for histological aging in regression equations derived from anterior, medial or lateral cortical sections. There were no other statistically significant axial variations.

Figure 4: Significant intersectional variation in TOPD was found along the posterior longitudinal axis



Bilateral symmetry

Left and right TOPD per ROI were compared using a paired T-test. The clavicle of the female (p-value = <0.05), indicates a significant and consistent difference in TOPD between the left and right at each respective ROI. The female right clavicle displayed higher TOPD, possibly due to higher biomechanical strain on the right clavicle due to right handedness (Sobol et al, 2015). No significant bilateral asymmetry was discovered for the other bones.

Biological sex

The fibula (p-value=0.025012) and 6th rib (p-value=0.002774) presented statistically significant differences in TOPD between the sexes. It should be noted that the rate of remodelling in compact bone may be influenced by multiple confounding factors other than age e.g. pathologies, diet and nutrition, genetics and biomechanical stress (Frost, 1987; Cho et al. 2002). Therefore, differences cannot be attributed to differences between biological sex alone, for conclusive results a larger sample size would be required.

Experience Gained

This project has taught me several new skills including: production and imaging of ground sections, carrying out a literature review, creating a database and using statistical analysis. Additionally, the ability to use Fiji was also developed; including use of the grid/collection stitching plugin (used by Dillon et al. (2016)), to reconstruct 276 montaged ROI grids, and the 'cell counter' tool to tabulate osteon counts (Preibisch et al, 2009). This studentship has enabled me to gain a greater understanding of what a career in anatomical research involves, I have learned that papers are not always correct and that research doesn't always go according to plan.

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Please state which Society Winter or Summer Meeting the student is intending to present his/her poster at:

The 19th congress of the International Federation of Association of Anatomists (9th-11th August 2019, London).

Proposed Poster Submission Details (within 12 months of the completion of the project) for an AS Winter/ Summer Meeting – (no more than 300 words)

Poster Title: Investigation of osteon variation throughout the human body and the impact of this variation on age estimation in a forensic context

Poster Abstract: The accumulation of osteonal remodelling events in the skeleton is correlated with increasing age. Whilst there is abundant research focusing on deriving regression equations using histological remodelling variables such as total osteonal population density (TOPD), there is a deficit of validation studies. The main aim of this project was to quantify the variation in cortical osteon density and consider its impact on biological age estimation in a forensic context. This histological analysis was undertaken through a blind study of ground bone sections from two cadavers of the same chronological age but different biological sex.

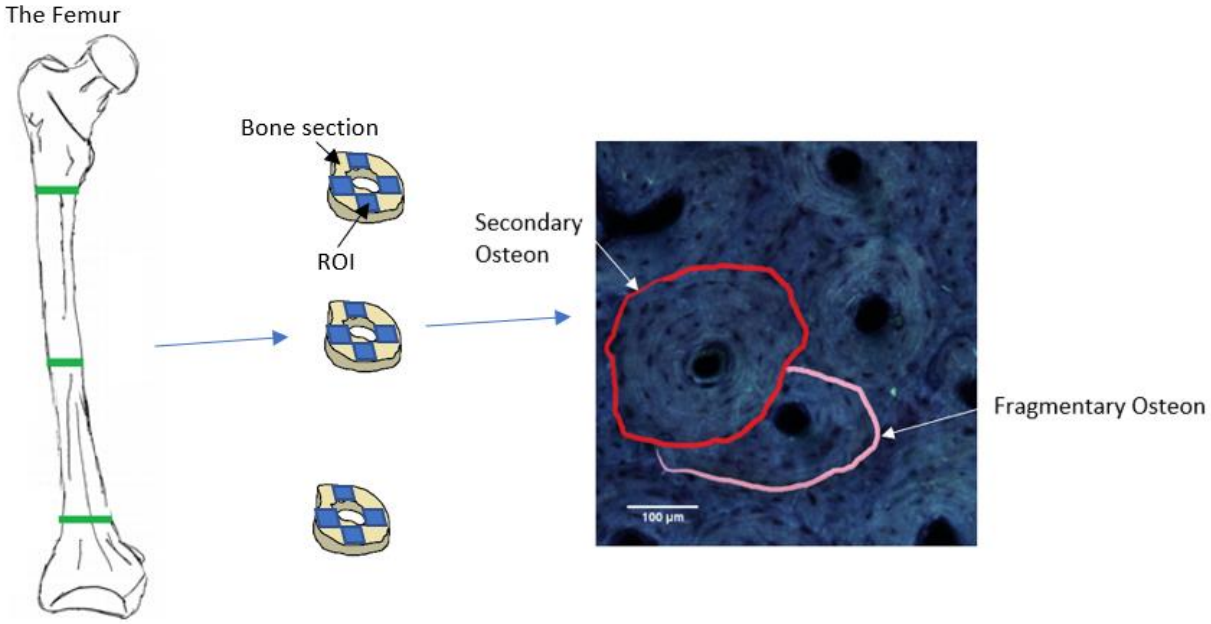
Statistically significant TOPD variation was identified in the lateral clavicle, distal ulna and in the posterior longitudinal axis of the femur. Clavicular bilateral asymmetry and sexual dimorphism of the TOPD of the fibula and 6th rib were also significant. Biological age estimations were derived from the TOPD using regression equations. The Suchey-Brooks (1990), Lovejoy et al. (1985), Iscan et al. (1984 & 1985) qualitative aging techniques were also utilised to enable a direct comparison. The mean age estimation for long bones (excluding ribs) resided between 40-60 years for both individuals. The mean age estimations of the alternative qualitative aging techniques ranged from 46.6-60+. Chronological age of the two cadavers was 84. These results illustrate that currently used aging techniques are constrained in their ability to accurately age older individuals due to the plateauing of micro and macroscopic changes in long bones which occurs between the 5th and 6th decade of life. It is suggested that further research be carried out on sampling sites not displaying negative bias, such as the mandible which, despite its large range, has the potential to enable the more accurate aging of older individuals in a forensic context due to the closeness of its resultant mean to the chronological age.

Brief Resume of your Project's outcomes: **(no more than 200-250 words)**.
The title of your project and a brief 200-250 word description of the proposed/completed project. The description should include sufficient detail to be of general interest to a broad readership including scientists and non-specialists. Please also try to include 1-2 graphical images (minimum 75dpi). NB: Authors should NOT include sensitive material or data that they do not want disclosed at this time.

Project Title: Investigation of osteon variation throughout the human body and the impact of this variation on age estimation in a forensic context

Project Resume: Osteon counts taken from ground sections are currently used in forensic osteology to estimate the biological age of unknown human remains and enable the identification of an individual in a forensic context. The most commonly used locations to obtain these counts are the mid-shaft femur or rib section. However, if osteon counts vary throughout the skeleton, or indeed within a single bone then this variation will impact on the derived age. The aim of this project was to investigate this potential variation by carrying out a blind study of ground sections taken throughout the skeletons of two cadavers of the same chronological age but different biological sex. The Suchey-Brooks (1990), Lovejoy et al. (1985), Iscan et al. (1984 & 1985) qualitative aging techniques were also utilised to enable a direct comparison.

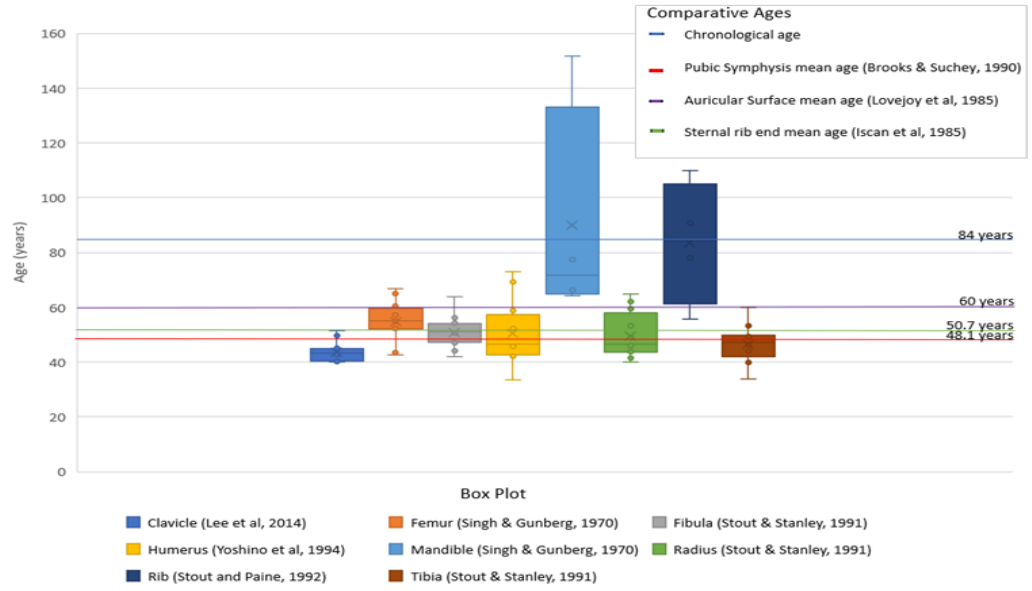
Four regions of interest (ROI) were selected to be imaged and analysed within each section, the total number of secondary and fragmentary osteons within each ROI were counted to calculate total osteonal population density (TOPD)



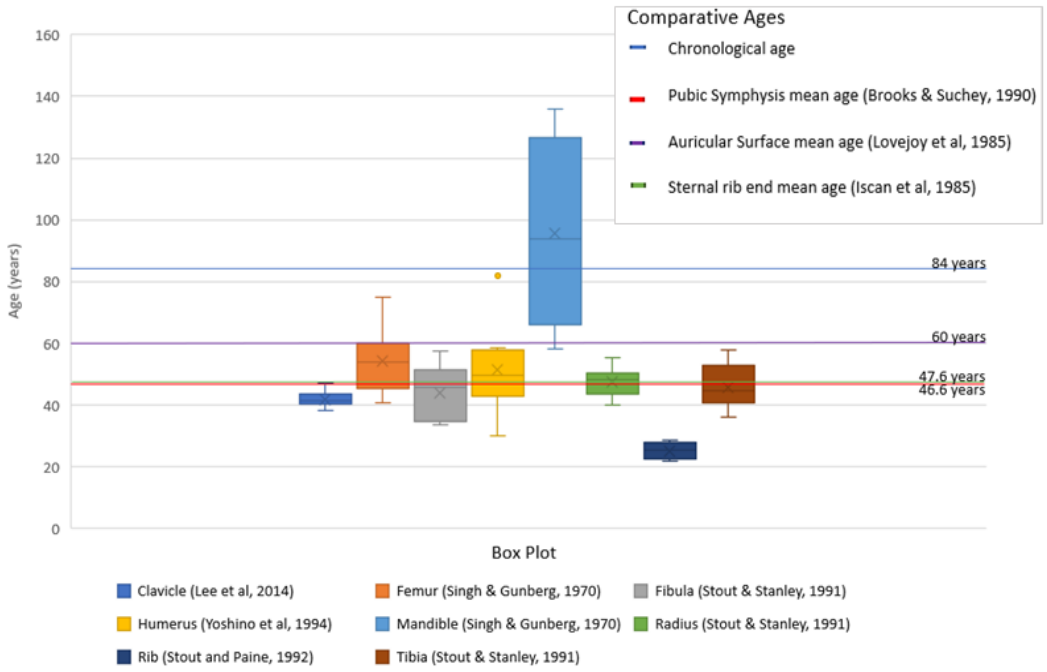
Statistically significant variation in the TOPD was identified in the lateral clavicle, distal ulna and in the posterior longitudinal axis of the femur. Clavicular bilateral asymmetry and sexual dimorphism of the TOPD of the fibula and 6th rib were also significant. This discovery of significant TOPD variation within a bone means that the selection of the sampling site for obtaining cortical bone sections for histological analysis in a forensic context needs to be carefully considered. This research indicates that the lateral ROI of the mandible had the lowest mean error of estimated biological to chronological age, this resultant osteon derived age was also closer to the chronological age than the qualitative aging techniques.

Boxplots of biological age estimations derived from TOPD of each region of interest within a bone when applied to aging regression equations for the left side of a female individual.

The vertical whiskers show the total range of age estimations for each bone. The box shows the range of data between the quartiles, with a line within this interquartile range illustrating the median. The mean age estimation is represented by the cross. Small circles within the box plots represent data points. Small circles outside of the whiskers represent outliers. The horizontal coloured lines represent the results of the alternative aging techniques



Boxplots of biological age estimations derived from TOPD of each region of interest within a bone when applied to aging regression equations for the left side of a male individual



Other comments: (no more than 300 words)

Signature of student. Ms Sophie Gray *Date 8/10/18*

Signature of supervisor Dr Wendy Birch *Date 8/10/18*

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