Faculty of Science and Technology

An experimental investigation, into the effects of thermal alteration on microstructures in mammalian long bones.

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Copyright Statement

Abstract

Thermally altered skeletal remains are among the most common categories of material recovered both from archaeological excavations and within forensic contexts. When burnt remains are encountered during a forensic investigation estimating age at death on the remains and creating a biological profile is one of the focal points of analysis. When macroscopic techniques are not viable, investigators may be obliged to turn to microscopic methods, of which the most commonly used method involves counting intact osteons and measuring their diameter. The current project presents the results of experiments in which animal proxies for human remains were subject to varying conditions of burning; the intention of which was to establish the extent of changes apparent in bone microstructure through the varying temperatures and types of burn. The results demonstrated that bones burnt in controlled circumstances displayed a varied degree of change in osteon diameter from the unburnt transverse sections and the samples burnt at 800^{o}C, along with various other environments of thermal alteration. Other variables, including the exposure of the cortical bone during the defleshing process were found to give results that hindered the distinction of the osteon structures and showed the diameter of the osteons changed before thermal alteration. These findings can assist in reconsidering the application of methods of estimating age at death specific to thermally altered remains from both forensic and archaeological contexts.

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List of Contents

Copyright Statement ii
Abstract iii
Acknowledgements iv
List of Figures ix
List of Tables xii
Chapter 1.0: Introduction 1
Chapter 2.0: Background 3
2.1: Bone structure 3
2.1.1: Bone microstructure 3
2.2: How bone microstructure can reveal age at death 4
2.2.1: Human vs non-human bone microstructure 6
2.3: Uses of animal bone as a proxy 8
2.4: Maceration and bone microstructure 8
2.5: Investigation of thermally altered remains 9
2.5.1: Forensic 9
2.5.2: Archaeological 10
2.6: Macroscopic effects of burning on bone 11
2.7: Microscopic effects of burning on bone 12
2.8: Effects of burning on micro-age assessment 13
Chapter 3.0: Aims and objectives 14
Chapter 4.0: Methods 16
4.1: Overview 16
4.1.1: Samples 16
4.2: Maceration/removing the adhering tissue 17
4.2.1: Chemical maceration 17
4.2.2: *Dermestes* maceration 18
4.3: Muffle furnace vs pyre 18
4.4: Thermal alteration of bone 19
4.4.1: Muffle furnace 19
4.4.2: Experimental burning 20
4.4.2.1: Temperature 20
4.4.2.1.1: Controlling thermal alteration 21
4.4.2.2: Duration of burning 21
4.5: Bone preparation 22
4.5.1: Cutting the bone 22

4.5.2: Thin sectioning 22
4.6: Pre- and post-burning analysis 24
4.6.1: Macroscopic analysis 24
4.6.2: Microscopic analysis 24
4.6.3: Statistical analysis 26
Chapter 5.0: Results 27
5.1: Pre-burning analysis of microstructure 27
5.2: Thermal alteration of bones 28
5.2.1: Colour change 28
5.2.2: Morphological changes 32
5.3: Post-burning analysis of microstructure 33
5.3.1: Diameter of osteon correlation 33
5.3.2: Muffle furnace burn 34
5.3.2.1: Density vs diameter size 34
5.3.2.2: Osteon diameter after varying temperatures of burning - ANOVA test 44
5.3.2.3: Femur vs humerus – Mann-Whitney U test 45
5.3.2.4: Maceration comparison – Mann-Whitney U test 45
5.3.3: Comparison animal species and burn conditions 45
5.3.3.1: Comparison of muffle furnace burn to pyre burning 47
5.3.3.2: Comparison of pig bones to deer bones 47
5.3.3.3: Comparison of pig bones to deer bones 48
5.3.3.4: Comparison of pig bones to cow bones 48
5.3.4: Experimental burns 48
5.3.4.1: ANOVA test analysis 49
5.3.4.2: T-test comparing deer to pig pyre burns 49
5.4: Comparison to human thermally altered remains 49
5.5: Summary of results 50
Chapter 6: Discussion 51
6.1: Pre-burning analysis 51
6.2: Thermal alterations to the bone 52
6.2.1: Colour change 52
6.2.2: Morphological change 52
6.3: Post-burning analysis 53
6.3.1: Muffle furnace 53
6.3.1.1: Maceration comparison 55
6.3.2: Cross species comparisons 55
6.3.3: Survival of osteons 56

6.4: Comparison of non-human proxies to human thermally altered remains 56
6.5: Redesigned experiment 56
Chapter 7: Conclusion 57
Appendices 67
Appendix 1: Complete Sample Raw Data 67
A1.1: Unburnt Samples 67
A1.1.1: Sample 18a 67
A1.1.2: Sample 18b 67
A1.1.3: Sample 19 67
A1.2: 200^{o}C Samples 68
A1.2.1: Sample 31a 68
A1.2.2: Sample 31b 68
A1.2.3: Sample 40a 68
A1.2.4: Sample 40b 69
A1.3: 600^{o}C Samples 69
A1.3.1: Sample 4a 69
A1.4: 800^{o}C Samples 69
A1.4.1: Sample 17a 69
A1.4.2: Sample 17b 70
A1.4.3: Sample 30a 70
A1.4.4: Sample 30b 70
A1.5: Cow Oil Drum Samples 71
A1.5.1: Sample 1 71
A1.5.2: Sample 2 71
A1.5.3: Sample 3 72
A1.5.4: Sample 4 72
A1.6: Deer Pyre Samples 72
A1.6.1: Sample 1 72
A1.6.2: Sample 2 73
A1.6.3: Sample 3 73
A1.6.4: Sample 4 74
A1.7: Pig Pyre Samples 75
A1.7.1: Sample 1 75
A1.7.2: Sample 2 75
A1.7.3: Sample 3 76
A1.7.4: Sample 4 76
A1.8: SHF Samples 77

A1.8.1: Sample 2 77
Appendix 2: SPSS Test Results 77
Appendix 3: Previous Experimental Methods 77
Appendix 4: Digital Gallery 77
Appendix 5: Miscellaneous 78
A5.1: Ethics Checklist 78
A5.2: Risk Assessments 80
A5.3: COSHH Forms 89

List of Figures

Figure 1: (A) Shows the plexiform bone within pig cortical bone and (B) Shows the osteons and Haversian systems within pig cortical bone (Horocholyn, 2013). (C) shows an intact osteon inside human cortical bone and (D) depicts the different structures of cortical bone (Dn) showing the non-Haversian, (Dl) lamella bone and (Do) shows the osteons (Kerley, 1965). 7

Figure 2 Two photographs showing (A) A scanning electron microscope image of hexagonal morphology at 800°C and (B) shows the fusion of the hexagonal crystals at 1200°C recrystalising (Holden et al., 1995). 13

Figure 3: Photographs of a) the bones being macerated by Dermestes maculatus and b) the bones being macerated in distilled water using Tergazyme..... 18

Figure 4: The muffle furnace used for the thermal alteration of the samples. 20

Figure 5: The chemical composition that makes up a fire. Showing how all three of these aspects are crucial in the chemical chain reaction (Schottke 2014, p. 144). 21

Figure 6: Shows photographs of a) the vacuum desiccator containing 3 bone samples and b) the Buehler Isomet 5000 Linear Precision Saw. 23

Figure 7: Schematic of the A) right femur and B) left humerus showing the cut lines. These were cut using a handheld hacksaw, as these did not need to be precise. These cuts made it easier for the samples to be embedded and later thin sectioned..... 24

Figure 8: Transverse section 1 from Sample 18a showing the plexiform bone structure present in the unburnt skeletal remains, with osteons present. Image: author. 27

Figure 9: A bar chart to show the distribution of samples according to their colour after burning, as scored according to the Munsell Soil Chart. 29

Figure 10: photographs of long bones burnt at 200°C samples 35 (A) and 3 (B). Showing the thermal alteration of the bones..... 30

Figure 11: photographs of long bones burnt at 400°C samples 2 (A) and 14 (B). Showing the thermal alteration of the bones and with how the bones begin to crack. Compared to samples 28 (C) and 36 (D), which were burnt at the same temperature and for the same duration. 31

Figure 12: photographs of long bones burnt at 600°C, showing the thermal alteration of the bones and the fissuring present on the bone. 31

Figure 13: Photographs of long bones that failed to complete their burns due to technical difficulties, but the colour changes from A and B compared to C is vastly different, but these were burnt at the same temperature. 31

Figure 14: Photographs of the burnt bones varying temperatures (A) showing the reconstructed bone at 400°C, (B) showing the fissuring from the burns and the sections of bone breaking off at 600°C, (C) a photograph of one of the bones that was burnt at 800°C showing the fracturing of the bones. 32

Figure 15: Distribution of samples according to their level of destruction post burning, including the unburnt samples, as scored according to the OHI. a score of 5 represents well preserved bone, while on the other hand a score of 0 represents very poorly preserved bone that has been destroyed by varying factors. 33

Figure 16: A bar chart to show the comparison of the average diameter of osteon, within the samples used for analysis compared to the OHI scores applied to each of the samples. 34

Figure 17: Diameter vs density of osteon, in unburnt sample 18a. 35

Figure 18: Diameter vs density of osteon, in unburnt sample 18b. 35

Figure 19: Diameter vs density of osteon, in unburnt sample 19. 35

Figure 20: Diameter vs density of osteon, in the 200°C sample 31a. 36

Figure 21: Diameter vs density of osteon, in the 200°C sample 31b. 36

Figure 22: Diameter vs density of osteon, in the 200°C sample 40a. 37

Figure 23: Diameter vs density of osteon, in the 200°C sample 40b. 37

Figure 24: Diameter vs density of osteon, in the 600°C sample 4a. 37

Figure 25: Diameter vs density of osteon, in the 800°C sample 17a. 38

Figure 26: Diameter vs density of osteon, in the 800°C sample 17b. 38

Figure 27: Diameter vs density of osteon, in the 800°C sample 30a. 38

Figure 28: Diameter vs density of osteon, in the 800°C sample 30b. 39

Figure 29: Diameter vs density of osteon, in the oil-drum burn, sample 1. 39

Figure 30: Diameter vs density of osteon, in the oil-drum burn, sample 2. 39

Figure 31: Diameter vs density of osteon, in the oil-drum burn, sample 3. 40

Figure 32: Diameter vs density of osteon, in the oil-drum burn, sample 4. 40

Figure 33: Diameter vs density of osteon, in the deer pyre burn, sample 1. 40

Figure 34: Diameter vs density of osteon, in the deer pyre burn, sample 2. 41

Figure 35: Diameter vs density of osteon, in the deer pyre burn, sample 3. 41

Figure 36: Diameter vs density of osteon, in the deer pyre burn, sample 4. 41

Figure 37: Diameter vs density of osteon, in the pig pyre burn, sample 1. 42

Figure 38: Diameter vs density of osteon, in the pig pyre burn, sample 2. 42

Figure 39: Diameter vs density of osteon, in the pig pyre burn, sample 3. 42

Figure 40: Diameter vs density of osteon, in the pig pyre burn, sample 4. 43

Figure 41: Diameter vs density of osteon, in the SHF burn, sample 2. 43

Figure 42: The diameter of osteons measured from the transverse sections analysed, showing the minimum, maximum and mean diameter in micrometers (μm) values shown in table 4. .. 44

Figure 43: The minimum osteon diameter between the varying species of animal remains used and the burn conditions the samples were burnt with. 46

Figure 44: The maximum osteon diameter between the varying species of animal remains used and the burn conditions the samples were burnt with. 46

Figure 45: The total number of osteons counted from the samples analysed used for the burns and from varying animal species. 47

Figure 46: showing the mean diameter of osteons of all species of animal used in this experiment. 49

List of Tables

Table 1: Sample analysed during the current research. 17

Table 2: Histological index values assigned to summarise the degree of diagenetic change (Hedges and Millard 1995, p203)..... 25

Table 3: Observed colour changes from transverse sections of all burn conditions. 28

Table 4: Observed colour changes from all samples thermally altered in the muffle furnace. . 29

Table 5: The total of osteons counted at each of the burn conditions, with the minimum, maximum and mean diameter of the osteons observed. 44

Table 6: The total number, minimum, maximum and mean diameter lengths of osteons for each of the species of animals used from previous experiments..... 48

Chapter 1.0: Introduction

Understanding the effects of thermal alteration to the skeleton has been a key focus of research throughout the development of forensic anthropology. In addition to application in situations where bodies have been deliberately burnt to hinder identification or to facilitate disposal, other situations where such knowledge is relevant include mass fatality incidents such as plane crashes, natural disasters or accidental fires on both small and large scales (Bennett 1999; Thompson 2005; DeHaan 2008; Ellingham *et al.* 2016). When hard tissues are exposed to extremes of heat and the chemical processes of burning, they are subject to a range of alterations that are generally well-documented at a macroscopic level including shrinkage, warping, fissuring and expansion of the bone (Hummel *et al.* 1988; Hummel and Schutkowski 1993; Herrmann and Bennett 1999; Kalsbeek and Richter 2006; Arora *et al.* 2010). The thermally induced shrinkage and expansion of bone has received growing attention in recent years. However, the survival of the microstructure after thermal alteration is a topic area that has not been systematically studied in a forensic context. This latter may have particular significance in regard to estimation of age at death, which is a particularly challenging aspect of analysis in burnt remains at a macroscopic level. Gaining knowledge in this area at the microscopic level has potential to allow for age at death estimations to become more reliable and for biological profiles to be more precise in burnt remains. Instead of applying a method used for unburnt remains to thermally altered ones. In archaeological contexts the study of thermally altered bones and microstructures were widely seen as a poor source of osteological data, however, in recent years it has become a vital utility in the reconstruction of the past (Shipman *et al.* 1984; Stiner *et al.* 1995; Lebon *et al.* 2008; Rillardon and Bracco 2008; Piga *et al.* 2016; Mamede *et al.* 2018). Forensic anthropologists may apply a method of analysis to thermally altered remains that was initially developed for unburnt bone, which when used to create a biological profile (age, sex, stature, ancestry) can alter the results (Thompson 2002; Thompson 2005; Arrowsmith, 2018). Such instances can be problematic as the biological profiles cannot be fully completed, for example, stature estimations are frequently not carried out for burnt remains, due to the thermally induced structural changes (Thompson 2002; Thompson 2005).

The estimation of age at death is one of the most difficult tasks to accomplish in forensic anthropological cases. The prevalence of thermally altered remains has increased in forensic investigations, such as mass disasters, homicides, and suicides (Imaizumi, 2015). This increase is due to the use of fire being one of the most common means to conceal or destroy evidence (Chrysostomou, 2015). The use of histological age at death estimation methods may be applied in such a case where macroscopic estimations fail to obtain a reliable determination of age. However, in order for such methods to be accepted, especially in forensic contexts, experimental data from bone samples exposed to heat at known temperature and durations are required. Various practical and ethical concerns may prevent the use of human bone in such experiments, necessitating the use of animal proxies instead. Pigs (*Sus scrofa domesticus)* are frequently used as a substitute in such experiments (Pakosh and Rogers, 2009) due to many similarities including

structure and internal anatomy (Connor *et al.*, 2017), although bone from other large mammals may also be sufficiently similar.

The most frequently used method of histological age at death estimation involves counting the number of intact osteons present in the microstructure of the bone, and the use of the Haversian canal area of these osteons (Yoshino, *et al.*, 1994; Absolonova, *et al.*, 2013). The first iteration of this histological method was created by Kerley (1965). Old osteons may surround the edge of the osteoclasts channels through Haversian bone and remain there after resorption ceases and replacement of the bone begins (Kerley 1965). The fragments of osteons increase in number with age, this is down to more and more osteons being partially destroyed, then at old age practically all complete osteons are surrounded by the fragments of older osteons (Kerley 1965). This method has had various modifications, one of which was developed by Ahlqvist and Damsten (1969) that uses percent osteonal bone, rather than the osteon count. This modification of the Kerley's (1965) method was put forward with the purpose to diminish the difficulty of separating the intact osteons from the fragmentary osteons (Stout and Stanley, 1991).

In order to address the lack of a substantial method of estimating age at death in thermally altered bone, the microstructural alteration needed to be investigated. Additionally, it was identified that different human proxies are used throughout scientific experiments. The current study presents a suit of experimental data aimed at exploring the extent to which the relevant changes are quantifiable using various animal proxies; how different burn conditions effect microstructures, with consideration also given to the effects of the defleshing process on the microstructures.

Chapter 2.0: Background

2.1: Bone structure

The bone structure of a mammal is composed of two different types: cortical and cancellous bone (Hillier & Bell, 2007). The cortical bone is the hard outer region of the bone, this is comprised of three separate sections, the periosteal zone; the mesosteal zone; and the endosteal zone (Klevezal, 1996). In comparison the cancellous bone, which is also referred to as trabecular bone, is comprised of interlinked spicular structures known as trabeculae (Hillier & Bell, 2007). Cancellous bone is located in the interior of the bone, including the epiphyses of long bones. On a microscopic level mammalian cortical bone can form as two types of bone tissue, these are woven and lamellar. Woven bone is typically temporary, with it mainly being produced during tissue repair and in response to pathogens (Hillier & Bell, 2007). Woven bone is poorly organised compared to that of lamellar bone, which has a highly organised structure (Enlow, 1963; Hillier & Bell, 2007). Plexiform bone is the primary structure within cortical bone of the long bones of large fast growing animals like cows and pigs (Hillier & Bell, 2007), with its structure similar to laminar bone, but with a more dense system of organisation. This plexiform bone is usually replaced with Haversian bone in many primates (White and Folkens, 2005, 43) but for many other mammalian groups their long bones keep their primary plexiform structure, with only sections of the bones where strong muscles attach, becoming haversian bone (Currey, 2002).

2.1.1: Bone microstructure

Recent years have seen progressive improvements in understanding the effects of burning/thermal alteration to bone at a macroscopic level (Thurman and Willmore, 1982; Buikstra and Swegle, 1989; Harbeck et al., 2011; Krap *et al.,* 2017). However, there is a significant lack of experimental research published on the associated changes in microstructure, with the most relevant article by Wolf *et al.* (2017). The latter outlined the use of microstructure in determining human age at death with cremated bone in an archaeological context. However, this issue has equal relevance to modern forensic contexts, as burning of bones is one of the most frequently used methods of concealing evidence, this increase in burning remains is due to the uncontrollable effects fire has on the bone morphology (Correia, 1997; Dirkmaat & Adovasio, 1997; Fanton *et al.,* 2006; Porta *et al.,* 2013). Further studies have focused on other aspects of microstructural changes such as, the recovery process of fracture toughness and collagen alignment within the microstructure (Martiniaková *et al.,* 2005; Ishimoto *et al.,* 2009). There is also an abundant literature on the effect of thermal alteration on skeletal remains at a macroscopic level. However, there remain gaps in current knowledge regarding aspects of the effects of thermal alteration to bone at the microstructural level. For example, the methods of age estimation of burnt bones at a microstructural level are based upon the methods applied to bones that have not been thermally altered in anyway (Thompson, 2004). Furthermore, there is a lack of understanding of the effects of burnt remains from a forensic standpoint, in comparison to an archaeological context, with a large amount of the research being conducted in this subject area

(Taylor *et al.,* 1995; Mays *et al.,* 2017). In summary therefore a significant gap persists in that no systematic study has been undertaken to investigate the effects of thermal alteration on bone microstructure in controlled experimental conditions.

Bone microstructure can be used to identify post-mortem bone alterations, pathology, age estimations and other factors that affect bone (Brits *et al.* 2014). The microstructure itself is built up of canals named haversian canals, located in the cortical bone, which allows blood vessels and nerves to travel through them. The cortical bone is built up of the basic structure known as the osteon system, the haversian canals are contained within this system along with layers of lamella surrounding these canals (Abdullah *et al.* 2018). The human skeleton undergoes constant remodelling by specialised cells with old bone being resorbed by osteoclasts, and then rebuilt by osteoblasts. This process of remodelling changes the microstructure in the cortical bone, especially with age. These structural changes are key aspects of age and sex estimations for human remains (Hummel and Schutkowski 1966; Abdullah *et al.* 2018). This histological analysis is not bound to the main skeletal structure, unlike the morphological analysis that primarily involves the study of the skull, long bones and pelvis (Thompson 2005; Brits *et al.* 2018).

The microstructure of bone is highly complex and remains incompletely understood. However, gaps in understanding about microstructural changes in response to heat have been helpfully summarised by Wolf *et al.* (2017). The microstructures in burnt human ribs can be correctly identified (Absolonova *et al.,* 2013), however, it was noted that they had a similar change in structure to the changes associated with age. The osteons present in microstructure are easily recognisable in cross-sections as a canal surrounded by concentric lamellar bone, which contain evenly spaced osteocytes (Kerley, 1965). Around the osteon there is also a reversal line that marks where osteoclastic resorption stopped, and new bone started to form (Kerley, 1965). The understanding of the microstructures after being thermally altered has been outlined in previous research (Kerley and Ubelaker, 1978). This study provided the basis for the foundations of age at death estimations using the microstructure after being burnt. This knowledge was later expanded on by counting the number of intact osteons and by using regression equations created by Kerley (1965) and modified by Kerley and Ubelaker (1978) with the work of Bradtmiller and Buikstra (1984). Albeit, whilst these studies demonstrated a general principle of how thermally altering remains affects the histomorphometric age estimations, they did not quantify its effects in detail within known parameters/circumstances of heating, due to the fact that they applied these methods to archaeological material.

2.2: How bone microstructure can reveal age at death

Age at death can be established by many methods, but in recent times the most reliable is the use of histomorphometric methods of age estimation. As one method of age estimation using the cranial sutures developed by Lisowski (1968) is seen as one of the most consistent methods (Correia, 1997). However, due to thermally altered remains having all these changes occurring during the burning this method was deemed unreliable (Lisowski, 1968). Thus, the methods used for the age estimation can limited for burnt remains, creating a less accurate estimation. From

previous studies it has also been shown that the age ranges that have been produced from thermally altered remains, have been seen to be misleading in some forensic investigations, as the range given could possibly exclude the 'right' age and thus exclude the 'right' missing person (Cunha *et al.,* 2009).

The principal accepted method of age estimation from observations of bone microstructure involves the counting of intact osteons followed by use of regression equations (Kerley, 1965; Kerley and Ubelaker, 1978). Kerley's (1965) method is based on the principle that within the cortical bone, the number of osteons and osteon fragments increase with age, while the percent of circumferential lamellar bone and the number of non-Haversian canals decrease. With two primary factors that add to estimating age at death, the first is the use of linear models to estimate, with the main assumption that the primary bone is replaced by secondary bone in a continuous process at a predictable rate, and the second is the number of intact and fragmentary secondary osteons is considered the best indicator of age at death in skeletal remains (Andronowski, *et al*. 2018). Further developments to Kerley's (1965) method are presented by Wolf *et al.* (2017) , who compared the histomorphometric age estimation and macroscopic age estimations, showing a weak correlation that the estimations corresponded until the remains were from a mature person (Wolf *et al.,* 2017). Kerley's (1965) method was also modified by Ahlqvist and Damsten (1969), which introduced the idea of using the percent of osteonal bone rather than the osteon counts. This was done to counter the difficulty of distinguishing intact osteons and fragmented osteons (Ahlqvist and Damsten 1969). However, in Ahlqvist and Damsten's (1969) method it produced age estimations with standard error of ± 6.71 years from the femoral diaphysis, compared to Kerley's determinations, which produced standard errors of ± 5.27 years of the fibular fragments and from the tibial osteons, which standard errors of ± 6.69 years. This shows that Kerley's method gave a more accurate age estimation compared to Ahlqvist and Damsten's method. Despite this, Ahlqvist and Damsten found that Kerley's method produced a correlation between the fibular fragments and age that was not found on any other bone. Thus, showing that the methods used can sometimes only provide good correlations on certain bones, meaning that the counting of the osteons may not be applicable to non-human animal remains. Along with this Ahlqvist and Damsten's highlighted the major problems that they encountered using Kerley's method, these included the as mentioned issue of the difficulty of differentiating the osteons from the osteon fragments, the rough estimations of the percentage of the lamellar bone in a circle visual field. This has proven that the histological methods for age at death estimations can be reasonably accurate (within six years of the true age) (Singh and Gunberg, 1970) and that these were used with multiple regression analyses (Singh and Gunberg, 1970). This method of age estimation however is built upon using unburnt remains and then applied if needed to burnt remains, meaning that the results could vary once applied to burnt bone.

Nonetheless, there are areas that are less well understood where a greater breadth of knowledge of bone biology remains desirable. There will need to be further research into the factors that can also have an effect on bone microstructure such as; diet and nutrition, taphonomy, disease and trauma (Andronowski *et al*. 2019). Improved understanding of such issues would help to avoid erroneous conclusions when assessing the effects of burning.

2.2.1: Human vs non-human bone microstructure

As previously mentioned, the microstructures of human vs non-human bone exhibit key differences. When differentiating human from non-human bone the analysis relies primarily on the identification of key bone tissue, the pattern of the tissue and its organisation (Andronowski, *et al.* 2018). The microstructure present in non-human mammalian bones is primarily made up of structures known as primary vascular plexiform bone (figure 1a and 1b) which differs from human Haversian systems. Such differences include the location, size and density of these systems (Lu *et al.,* 2006). This structure of plexiform bone is defined by its horizontal and rectangular shape, in a regular distribution (Mulhern and Ubelaker 2001). This primary bone structure is what makes identifying human from non-human easier due to the fact that the plexiform bone structure is rarely found in human bone, more specifically only in young humans (Jowsey, 1966; Mulhern and Ubelaker, 2001; Andronowski *et al.,* 2018; Burr, 2019). However, Haversian bone tissue can also be found within non-human compact bone (Martiniaková *et al.,* 2006), thus meaning that the presence of Haversian bone is not diagnostic of human bone and this leads to a more difficult time to distinguish between non-human and human bone (Andronowski *et al*., 2018). Methods of differentiating human from non-human bone have been proposed that rely on differences in the size of Haversian canals and the size/shape of osteons. However, these are employed with varying degrees of success and the use of these methods should be undertaken with caution (Crowder *et al*., 2018, 205). This is due to the known variables that affect the Haversian system formation, such as age and loading environment (Crowder, *et al*., 2018, 205). It is also of note that the histological appearance of bone can differ significantly in different parts of the same bone and even different areas of the same section (Enlow, 1966). This is principally related to non-human mammals having more rapid rates of growth as they mature compared to that of humans (Enlow, 1966).

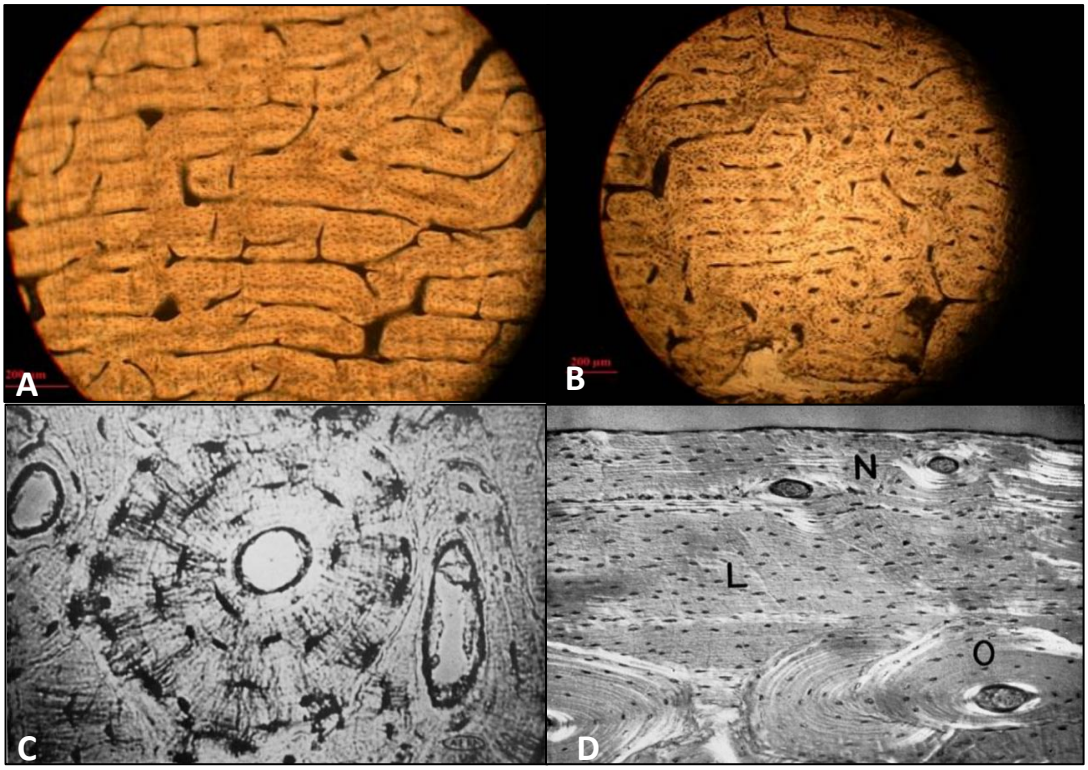

Figure 1: (A) Shows the plexiform bone within pig cortical bone and (B) Shows the osteons and Haversian systems within pig cortical bone (Horocholyn, 2013). (C) shows an intact osteon inside human cortical bone and (D) depicts the different structures of cortical bone (Dn) showing the non-Haversian, (Dl) lamella bone and (Do) shows the osteons (Kerley, 1965).

Figure 1A is a microscopic image of the plexiform bone structure found in a pig long bone (*Sus scrofa*), this structure is characterised by rounded 'brick' like structures, that swerve between each other and more divided compared to other animals for example deer plexiform bone is tightly packed, narrow and there is little space between the structures (Horocholyn, 2013). Plexiform bone is a primary bone structure within the cortical region of a long bone, and is mostly only present in fast growing animals, with it being less frequent in humans (Hiller *et al.,* 2007). Pig bones also have secondary bone structures, which include the Haversian bone and thus containing the Haversian system (Figure 1B). These Haversian systems are also known by the name secondary osteons, which as shown in figure 1B are structured alongside plexiform bone in pig long bones (Horocholyn, 2013). These osteons however, in animal bones are harder to differentiate between them (Horocholyn, 2013). Figure 1C shows a secondary osteon, which are the osteons created within the secondary bone tissue. As discussed later these osteons are used to estimate age at death in human remains (Kerley and Ubelaker, 1978; Wolf *et al.,* 2017). Along with these osteons, human cortical bone also contains other forms of bone, including the non-haversian systems and lamellar bone (figure 1dn-o). The secondary structures including the Haversian systems are surrounded by several layers of lamella bone (Figure 1d) (Hillier and Bell, 2007). These lamellar bone structures are the most abundant type within many mammalian non-human and human bones and is made up of five sublayers (Weiner *et al.,* 1999). These sublayers build up a strong 'concrete' style level of structure within the cortical bone (Weiner *et al.,* 1999).

2.3: Uses of animal bone as a proxy

The use of a proxy for human remains is widely accepted in research due to the limitations surrounding the use of human bone, these limitations are the results of both ethical and practical issues. In order to circumvent these issues, the use of porcine (*Sus scrofa domesticus*) bones as a proxy for human remains still remains one of the main proxies used in experimental research (Martiniaková *et al.,* 2006; Waterhouse, 2013; Efford, 2016). For macroscopic analysis of bone there are various animals that can be used, and these have been tested. Hillier and Bell (2007) found that pig remains were seen to be the most reliable proxy due to the morphology of the bones being similar to human bone (Dautartas *et al.,* 2018). From a histological view the use of proxies can vary due to differences in bone microstructure, but they do share similar properties as they contain plexiform bone, which is made up of lamellar bone structure and contains Haversian canal systems (Martiniaková *et al.,* 2006; Hillier and Bell, 2007). However, previous research suggests that long bone cortical thickness varies considerably between human and certain non-human species, which is clear in adult animals of a comparable size to humans having thicker and more compact cortical bone (Brothwell, 1981; Wolf, 1986; Ubelaker, 1989). So, whilst there are differences in the arrangements of human and non-human bone microstructures, there is a large degree of overlap (Brits *et al.,* 2014). However, this study was conducted with only the anterior surface of the femora being used, thus meaning that the results produced might not be able to be applied to other smaller bones in the skeleton. These pieces of research show that there is a clear method of distinguishing between non-human and human bone at a microscopic level (figure 1c and 1d) (Mulhern and Ubelaker 2001). Thus, leading to the point that proxies in scientific experiments for microstructural analysis is acceptable. With the microstructural bone tissue still being able to be compared to one another. Taking all this into account, the study is based on the understanding of unburnt bone, and how it shows that there are identifiers of the microstructures in both human and non-human of bone. Therefore, it can be put forward that these identifiers will most likely be more difficult to identify and compare within burnt human and non-human bone, but still present under microscopic analysis.

2.4: Maceration and bone microstructure

The methodologies used within the current investigation required the bones to be de-fleshed due to the equipment and potential of fire hazards. In regard to this, the method of maceration needs to be considered as using fleshed bones adds a factor that cannot easily be controlled due to the amount of meat taken off the bones by butchers. The effects of maceration on the microstructure also need consideration as this process may have an effect at this microscopic level. This issue is discussed in experimental literature concerning the comparison of enzymatic maceration compared to water maceration (Yin *et al.,* 2010). Further research papers also compare maceration techniques and the effects they have on the bone and its microstructure (Uhre *et al.,* 2014; King and Birch, 2015). The use of the beetle species; *Dermestes maculatus* and *Dermestes ater* provide a very clean form of maceration, but only if the colony of beetles are thriving and the conditions in the tanks are correct for them (Ajayi *et al.,* 2016). However, these beetles can also

be proven not as helpful in terms of microscopic analysis of the bones after maceration as comparisons from an archaeological site left indicators of microscopic edge gnawing, pits and etching on the bone, which in turn can cause destruction within the microstructure itself (Ajayi *et al.,* 2016). There have been comparisons between the different forms of maceration to see the affects these methods have on the bone and the microstructure itself (Couse and Connor, 2015; Ajayi *et al.,* 2016).

2.5: Investigation of thermally altered remains

2.5.1: Forensic

Bones that have been thermally altered in some form, both human and non-human, are frequently recovered from various contexts. These can be forensic or archaeological (Hummel and Schutkowski, 1993; Sillen and Hoering, 1993; Thompson, 2002; Thompson, 2004; Wolf *et al.,* 2017). With cremated bone being one of the most common forms of skeletal remains found in the UK (Thompson, 2004) this is solely down to that burnt skeletal remains preserve better in acidic soil compared to unburnt remains (Thompson, 2004). The forensic analysis of thermally altered remains has been an area of study since the 1940s, which has focused on the recovery/collection, the analysis and preservation of these remains (Bohnert *et al.,* 1997; Cattaneo *et al.,* 1999; Büyük and Koçak, 2009). One of the first publications, published by Krogman (1943), provided an adaptation of the heat induced macroscopic modifications in thermally altered remains (Symes *et al.,* 2015). This publication not only was one of the first studies to correspond the changes on the bone and burning, it also looked into the heat induced changes on wet and dry bone. This branch of forensic investigation was first implemented through archaeological examination of burnt remains, however, the more recent legal medical context has produced a somewhat capacious method of analysis of thermally altered remains.

These archaeological studies first came about in order to elucidate how the bones were exposed to the heat and under what conditions this occurred. For example, what were the burning methods used, the maximum temperature reached, and whether the bones were fleshed or de-fleshed, dry or fresh (Brickley, 2007; Büyük and Koçak, 2009; Symes *et al.,* 2015). These conditions were based on the examination of certain factors, these include, the colour changes of bone, the fractures present, how these fracture patterns relate to the temperature and the duration of heat exposure (Krogman, 1943 and Symes *et al.,* 2015). For instance, fatal fires such as aeroplane crashes, homicides and car fires have been examined using thermally altered remains show how varying types of burning can affect bones. Thus, leading to a need for an accurate process of creating biological profiles on thermally altered remains.

The influence of temperature on human remains, is of principal importance, however, the constant or nonconstant infliction of heat stress can have a significant increase or decrease in damage present in the microstructures of the remains. However, human interference with the burning process can alter the levels of damage on the bone and cause extra damage, this can be seen in

the murder case of a 47 year old man[1]. According to Doctor M. Smith, part of the pubic symphysis was found with a puncture within it (personal communication, Martin Smith, 15 February 2018), this indicated that there had to be some sort of interference while the bones were being burnt with a tool of some sort.

Despite this, the research published here is predominantly focused on an individual forensic case study and these cases rely on the preservation of the soft tissue due to the interpretations produced (Pope and Smith, 2004; Dirkmaat *et al.,* 2012). Consequently, these findings are difficult to apply to any other contexts, including that of an archaeological record (Dupras *et al.,* 2006). However, there are methods used to look at archaeological burnt remains.

2.5.2: Archaeological

In an archaeological context investigating burnt remains can be difficult to identify due to the fact that they are not fresh samples and the soil that they are buried in affects the colour of the bone (Symes *et al*., 2015; Irish *et al.,* 2015). A lot of the time the bones will also be very fragmented due to the environment that they are left in. So, identification can be quite difficult in regards to which bone the fragment has come from. Nevertheless, as mentioned in the above section the methods used were initially developed for archaeological contexts and applied to forensic cases thus meaning that the two at their bare minimum are similar in how the bones change via thermal alteration (Brickley, 2007; Symes *et al.,* 2015). In terms of the colour changes that bones go through in archaeological contexts these follow the same temperature levels as freshly burnt bone, but however, due to the remains being buried the colour may change due to the soil that they are buried in (Irish *et al*., 2015).

There are limitations to the biological profiles that are created for thermally altered remains found in archaeological contexts, due to the high fragmentation of the bones and also the heat-induced changes the bones go through (size, weight etc.) (Minozzi, 2015). An example of these limitations can be seen in the determination of the sex and age at death of Iron Age cremations in the North of Italy, as the bones had a large amount of heat-induced changes including shrinkage and distortion, with this the osteological standards applied to unburnt remains could only be applied to a few cases (Minozzi, 2015). Furthermore, there is the idea that cremated remains come from a section of anthropology and archaeology that is technical process that requires a specialist to analyse the remains (McKinley and Roberts, 1993), which in turn is by no means incorrect but cremations are assumed to be poor archaeological data (Williams, 2015). This is supported by White (1992) who identified the problem of distinguishing burnt bone from weathered bone in an archaeological context. White (1992) highlights the fact that there is no standardised method for determining thermally altered bone, from bones that had been fractured due to weathering.

[1] This case has been anonymised due to sensitive material which could have an effect on the family of the victim.

In comparison the conditions of the burns are becoming easier to identify with an increasing number of techniques (FTIR-ATR, Raman and Inelastic Neutron Scattering (INS)), which allows information to be extracted from archaeological remains that have been the subject of thermal alteration (Festa *et al*. 2019). Festa *et al*. (2019) found that one of the skeletons found, displayed different INS profiles, which is consistent with varying heating conditions from below 400^{o}C up to 500^{o}C or 800^{o}C to a maximum of 900^{o}C. With the increased temperatures for the ulna, femur, humerus and fibula, it suggests that the body could have been folded into a certain position (Festa *et al*. 2019). This research is beneficial to the current research as supports the common theme that the major factors that are prone to influence the thermal alteration of skeletal remains are temperature, the duration of burn and the environment in which the bone are burnt, for example the oxygen levels.

2.6: Macroscopic effects of burning on bone

The changes that occur on bone during thermal alteration can vary due to multiple factors. These are, 1. Temperature to which the bone is exposed; 2. The time of exposure; 3. The position of the bone in relation to the seat of the fire; 4. The composition of the bone; and 5. The size of the bone (Shipman *et al.,* 1984; Von Endt and Ortner, 1984; McCutcheon, 1992; Brain, 1993; Nicholson, 1993; Sillen and Hoering, 1993; Stiner *et al.,* 1995). Throughout earlier investigations of burnt remains, the roles of intensity, duration and proximity to the fire were examined, but no investigation was specifically focused on the specific temperature related changes (Webb and Snow, 1945; Buikstra and Goldstein, 1973). With more recent but still dated papers focusing on the colour change and how the structure of the bone changes post burning (Shipman, 1984). Although the sequence of colour change put forward by Shipman is widely accepted the temperatures at which these colours appear was challenged by the more recent work of Mays (2010). This demonstrates that the experiments surrounding thermal alteration on bones need to take into account all factors of thermal alteration as Mays (2010) makes sure to account for the efficacy of burning and the oxygenation of the burn in conjunction with the temperature. It is worth noting that if the bone is not heated to a high enough temperature, it will not have a drastic effect on the bone, but similar to the effects weathering has, which will need microscopic analysis to distinguish the two (Taylor *et al.,* 1995). Along with this some thermally altered remains can be misinterpreted as stained bone; particularly manganese staining - if the bones are deposited in a certain context (Shahack-Gross *et al*. 1997).

There has been an increase in methodologies being created to help determine the changes that bones are subject to as the structural integrity drastically decrease after undergoing thermal alternation, resulting in the bone exhibiting particular changes including; shrinkage, weight loss and warping (Herrmann and Bennett, 1999; Hiller *et al*., 2003). Ellingham and Sandholzer (2020) used X-ray microtomography (micro-CT) to document the volumetric and trabecular shrinkage of ribs, at 100^{o}C increments from 400^{o}C to 1000^{o}C to assist in creating biological profiles. The highest rate of shrinkage for these bones was at temperatures 900^{o}C and 1000^{o}C ($p<0.05$) (Ellingham and Sandholzer, 2020). Although the use of a micro-CT means a permeant sample is recorded without further handling of the original sample, preventing further destruction, and

allowing clear measurements to be taken (Ellingham and Sandholzer, 2020). Despite this, it is limited by the sample size as only smaller bones or fragments can be recorded and that during this experiment only bone sections were analysed so the application to practical means need to be kept in mind (Ellingham and Sandholzer, 2020). However, some have made the argument that these structural changes are caused by the presence or absence of soft tissue on the bone (Eckert *et al.,* 1988; Gonçalves *et al*., 2015). Despite this, it is important to note that the experiments reviewing this concept are limited by the lack of consistency in the results they produced and does not add to the structure of the bone via experimental research, which would be beneficial to examine. With the physical changes that bone undergoes becoming a more important field to understand, one area that has received relatively little attention is the temperature at which skeletal remains have been exposed (Krap *et al.* 2019). Krap *et al*. (2019) demonstrated the possibility of differentiating the temperature at which skeletal remains are exposed with precision and accuracy, with clear relevance for both forensic investigations and archaeological contexts. This is of interest to the current research as a general proof of concept that levels of temperature can be successfully inferred after the event.

Interpreting patterns of burning can differentiate highly across different body parts this is can even be seen on adjacent body areas (Pope and Smith, 2004). However, this research was conducted on cranial structures, which is degraded by heat and obscures the characteristic signatures of trauma in bone (Pope and Smith, 2004). Thus, making the differentiation from thermal trauma and other trauma difficult.

2.7: Microscopic effects of burning on bone

Thermal alteration of bone also takes effect on a microscopic level. The bone mineral density (hydroxyapatite) undergoes considerable recrystallisation when subject to heat, which in turn produces larger crystallites as shown in Fig. 2 (a, b) (Holden *et al.,* 1995). These changes are present after varying temperatures of burning and along with this the hexagonal type morphology of the bone was found to improve (Holden *et al.,* 1995). This is shows that the microscopic recrystallisation can be affected by varying environmental factors and also the age of the person from where the samples were obtained. Therefore, it is important to note what the burial environment was and about the sample itself, especially if the sample is from an archaeological context. However, it needs to be taken into account that this study is limited by the narrow experimental work put forward.

In Bradtmiller and Buikstra's (1984) research they put forward the idea that although microscopic age at death estimations in human bone is a widely used technique, there was little attention given to the reliability of the estimations, when the bones were thermally altered. Their research suggested shrinkage, is widely reported to not have a significant effect on the age estimation (Bradtmiller and Buikstra, 1984). In their research they also suggested in their conclusions that bone burnt at >600^{o}C retains all the necessary structures for microscopic aging techniques (Bradtmiller and Buikstra, 1984). This knowledge is useful for this current research as it demonstrates that bones burnt below 600^{o}C are usable for age estimation, and thus meaning that there should not be a drastic change on the microstructure and be able to be applied to animal

remains. Along with the recrystallisation of the microstructure the bones can undergo heat induced collagen denaturation, which can suggest a brittle fracture behaviour in bone, and this is brought on by the bones being burnt at over 150^{o}C (Todoh *et al.,* 2009). Thus, leading to that the burning of bones has a great influence on the toughness of bone.

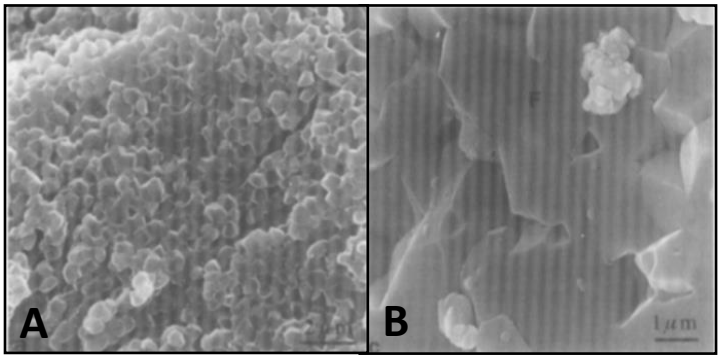

Figure 2 Two photographs showing (A) A scanning electron microscope image of hexagonal morphology at 800^{o}C and (B) shows the fusion of the hexagonal crystals at 1200^{o}C recrystalising (Holden et al., 1995).

2.8: Effects of burning on micro-age assessment

In comparison, with bones that have undergone thermal alteration, the age at death assessments can become skewed in the results as there are no methods that have been officially applied to only use on burnt bone. This is due to the fact that the method of estimating age at death has predominantly been conducted on unburnt bone as burnt remains lose the details required for these techniques to be successful, reliable and accurate. However, the use of intact osteons has been applied to burnt remains, and this can be seen in Hummel and Schutkowski (1993), where sections of bone were burnt at 1000^{o}C for an hour. This method is useful for this experimental work, as it shows that the counting of osteons can be beneficial for age estimations, however, one of their samples in their experiment came from a 76 year old female, who suffered from severe osteoporosis with corresponding limitations introduced to the reliability of the results. Wolf *et al.* (2017) give the method of determining age presented by Hummel and Schutkowski (1993), by counting the intact osteons, and using the regression equations of Kerley and Ubelaker (1978). This shows that there is only one method currently regarded as useful for application to thermally altered remains. However, these regression equations can also pose a problem due to limited correlation in the histological variables (Absolonova *et al.,* 2013). As stated in the aforementioned section bones that are thermally altered up to approximately 600^{o}C show no real effect on the factors that are required for age assessments on human remains (Bradtmiller and Buikstra, 1984).

Nevertheless, there is still a large amount of knowledge that is still in infancy, identification methods for burnt bone is one of these techniques for example (Imaizumi, 2015). Along with this there are advancements in chemical and physical analyses and DNA analysis (Imaizumi, 2015). However, once these techniques are expanded on the identification of burnt remains will become simpler for anthropologists to analyse and to estimate age with this information.

Chapter 3.0: Aims and objectives

The principle aim of this investigation is to investigate the survival of the microstructure within mammalian (pig [*Sus scrofa]*, roe deer [*Capreolus capreolus]* and cow [*Bos Taurus]*) long bones after exposure to heat at varying known temperatures and durations of burning. The respective experiments test several null hypotheses against alternative hypotheses:

1. H_0 = Bones subject to experimental heat exposure will show no observable differences in the survival of the microstructure compared to unburned bones.
    - H_a = Bones subject to experimental heat exposure will exhibit observable differences in the survival of the microstructure either as a result of increasing temperature or duration of exposure -or a combination of both.

Research objectives:

a) To design a suite of experiments whereby non-human bone proxies can be exposed to heat in controlled circumstances regarding temperature and duration.
b) To assess the effect on the survival of osteons of differing heat intensity and length of exposure.
c) To consider the above in regard to published literature concerning what is known regarding thermal alteration and the survivability of bone microstructure in humans.

2. H_0 = Osteon count and diameter size of the osteons will have no change after varying temperatures and duration of burn.
    - H_a = There will be a quantifiable difference in osteon count and diameter of osteons after varying temperatures and duration of burning.

Research objectives:

a) Investigate the effects that the burning has on the osteons of the cortical bone and how they have changed compared to the unburnt samples.
b) Undertake microscopic analysis of the osteons to count the osteons and measure their diameters pre- and post-burning.

3. H_0 = There will be no difference between using chemical maceration and maceration using *Dermestid* beetles and their effect on the bone microstructure post-burning.
    - H_a = Using two different methods of maceration will have an effect on the bone microstructure post-burning.

Research objectives:

a) Incorporate samples that have been defleshed using different methods in order to assess their effect on the microstructure of bones post-burning.

4. H_0 = Bone samples from different mammal species will not exhibit observable differences in microstructural change after thermal alteration.
    - H_a = There will be clear differences between the proxy samples from different species at a microscopic level following heat exposure.

Research objectives:

a) Investigate and compare non-human proxies to each other to explore the effect of selecting different species for thermal alteration experiments.
b) Compare the osteon structures per proxy and the extent of change following thermal alteration.

5. H_0 = There is no difference in the osteon diameter between unburnt or burnt humeri and femora.
    - H_a = There will be a clear difference between the two bone types from the burn conditions.

Research objectives:

a) Statistically compare the two bone types in two burn conditions and compare the osteon diameters of them.

Chapter 4.0: Methods

4.1: Overview

Despite the current experimental investigation being conducted to examine the heat-induced alterations in human bone microstructure, ethical and government restrictions (Human Tissue Act 2004; Cross et al. 2010) precluded the use of human remains. 20 pig (*Sus scrofa domesticus*) long bones comprised of a mix of humeri and femora were collected on October 8th, 2018 and were stored at 4°C prior to maceration. These bones were utilised in this experiment as human proxies for thermal alteration using an electric muffle furnace.

This experiment utilised the use of thin sections to analyse the microstructures. The analysis required a section that is thin enough for the light from the microscope to penetrate it and reveal the structures. This method of sample preparation had many steps to get to the final product (see Section 4.5).

The pigs utilised in this experiment were aged just under 1 year old, with a weight of around 70kg. *Sus scrofa* were chosen as the initial proxy in this experiment, due to the similarities in their bone structure to humans, hence pigs are a common human analogue in taphonomic studies (Connor et al. 2017). The bones were disarticulated and de-fleshed, except some small quantities of soft tissue remaining around the epiphyses. This amount of pre-burning alteration on the outside of the bone did not affect this experiment, as such alterations do not change the microstructure of the bone.

4.1.1: Samples

Along with 20 pig long bones mentioned previously there were other animal species used within the current study. These samples were collected from previous experiments to demonstrate the difference between species. The varying samples contained 25 pig bones thermally altered on an experimental pyre, 8 cow bones burnt in an oil drum fire, 8 deer bones burnt on another experimental pyre and finally 15 deer bones burnt in a simulated house fire (SHF), further details of these previous experiments and the methods used can be found in Appendix 3. All these samples were sections of long bone post-burning, this was to make sure that comparison across animals could be as accurate as possible. Making use of these samples allowed for more comparison on the survivability of bone microstructure in differing species of animals and also different conditions of burning. The cow bones being burnt in an oil drum allowed for an investigation on how bone microstructure is affected when exposed a less oxygenated burn, the pig bones burnt on the pyre allowed for a comparison between how a pyre effects the survivability of the bone microstructure compared to that of the samples burnt in the muffle furnace. The deer samples were used for a similar comparison, showing the difference between the bone microstructure of a deer and a pig and also how a pyre effects the microstructure compared to a simulated house fire, with varying levels of burning across the bones. Of the samples that were thermally altered the information of the burn conditions, the temperatures, the element of the bone

and the sampling area on said bone can be found below in table 1. The element for the bones that were thermally altered in previous research were difficult to identify however, it was clear that they were from long bones.

Table 1: Sample analysed during the current research.

Sample Number	Animal Species	Burn Condition	Temperature (°C)	Element	Sampling Area
19	Pig	Muffle Furnace	Unburnt	Femur	Diaphysis
18	Pig	Muffle Furnace	Unburnt	Humerus	Diaphysis
40	Pig	Muffle Furnace	200	Femur	Diaphysis
31	Pig	Muffle Furnace	200	Humerus	Diaphysis
14	Pig	Muffle Furnace	400	Femur	Diaphysis
2	Pig	Muffle Furnace	400	Humerus	Diaphysis
1	Pig	Muffle Furnace	600	Femur	Diaphysis
4	Pig	Muffle Furnace	600	Femur	Diaphysis
17	Pig	Muffle Furnace	800	Humerus	Diaphysis
30	Pig	Muffle Furnace	800	Humerus	Diaphysis
1	Pig	Pyre	700-900	Femur	Diaphysis
2	Pig	Pyre	700-900	Femur	Diaphysis
3	Pig	Pyre	700-900	Humerus	Diaphysis
4	Pig	Pyre	700-900	Humerus	Diaphysis
1	Deer	SHF	1000 Max	Long Bone	Diaphysis
2	Deer	SHF	1000 Max	Long Bone	Diaphysis
3	Deer	SHF	1000 Max	Long Bone	Diaphysis
4	Deer	SHF	1000 Max	Long Bone	Diaphysis
1	Deer	Pyre	553 Max	Long Bone	Diaphysis
2	Deer	Pyre	553 Max	Long Bone	Diaphysis
3	Deer	Pyre	553 Max	Long Bone	Diaphysis
4	Deer	Pyre	553 Max	Long Bone	Diaphysis
1	Cow	Oil Drum	300-400	Long Bone	Diaphysis
2	Cow	Oil Drum	300-400	Long Bone	Diaphysis
3	Cow	Oil Drum	300-400	Long Bone	Diaphysis
4	Cow	Oil Drum	300-400	Long Bone	Diaphysis

4.2: Maceration/removing the adhering tissue

The bones were then macerated using two different methods; one using two species of beetles (*Dermestes ater* and *Dermestes maculatus*), and the other using the enzyme activated detergent Tergazyme™ (Alconox 2017). The samples were split into two sets of twenty for equal maceration and comparison.

4.2.1: Chemical maceration

The Tergazyme™ was dissolved in distilled water in a slow cooker (Laptronix HTR-86 8.0L) with a dilution of 1:100 of Tergazyme™ to distilled water as per the directions of use displayed on the packaging of the detergent and also based on the work conducted by Selvey et al. (2018). The Tergazyme™ was dissolved in distilled water in two different ratios; 40g:4L and 50g:5L (figure 3b). The increase in the amount of distilled water was to prevent the water from evaporating below the bones in the slow cooker. The temperature was then raised to between 60 and 67°C to allow the enzyme to activate and the bones were de-fleshed partially of loose tissue and cartilage

around the distal epiphysis. Previous studies comparing different methods of factors applied to bones (Lander et al. 2013) have shown this temperature has no effect on the histology of the bone. The enzymatic process begins breaking down the flesh, allowing the remaining flesh, not already been manually de-fleshed before enzymatic maceration (Selvey et al. 2018), to be removed easily using forceps and a scalpel. These two methods were conducted to determine if there was a significant difference between the survival of the microstructure compared to one other. These bones were then stored in sample bags in a fridge.

4.2.2: *Dermestes* maceration

The first two samples that utilised the beetles were each wrapped up in tissue to prevent any odour emanating and to keep the beetles and larvae near the bone; essential as it is the larvae that do the majority of the maceration (Oliveira, 2018). The first bone was placed in whole and the second sample was placed in with the *Dermestes maculatus* and was cut into equal sections of the femoral head, diaphysis and distal epiphysis, to see how the split bone affected the maceration process by the beetles when compared to whole bone (figure 3a). It was shown that the larvae and the beetles de-fleshed the diaphysis first and then macerated the other two sections of the bone more equally as they had a similar amount of flesh present compared to the minimal amount of flesh present on the diaphysis. However, on the diaphysis the bone marrow was clear, and the beetles started eating this, thus showing that if given enough time the beetles would in fact start to damage the microstructure. Compared to the whole bone which was de-fleshed equally across all sections of the bone after 11 days the samples had been fully macerated, and both were then washed using distilled water to clean off the remaining sawdust and paper.

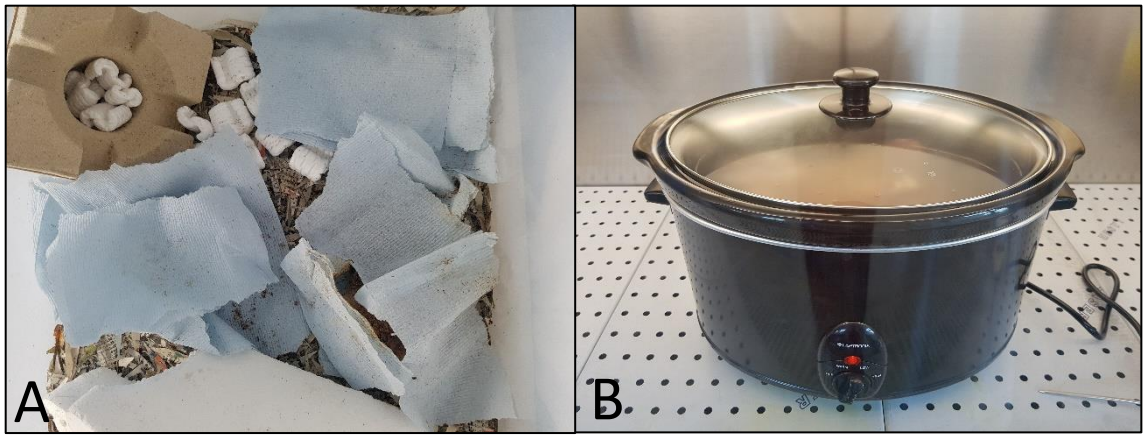

Figure 3: Photographs of a) the bones being macerated by Dermestes maculatus and b) the bones being macerated in distilled water using Tergazyme.

4.3: Muffle furnace vs pyre

Some research may support an objection that a pyre is more forensically accurate compared to the muffle furnace (Pope and Smith, 2004), however, in terms of the current project the muffle furnace provides a more controllable environment for heat exposure. Supporting the previous statement, the use of the muffle furnace over a more ‘realistic’ pyre burning has no significant effect on the thermally induced dimensional changes on the bone during or after the burning.

(Blanks, 2016). This was shown through the lack of statistically significant evidence presented in Blanks (2016) experiment between those sampled burnt in the muffle furnace and the experimental pyre (whole vs. sections and fleshed vs de-fleshed). In the experiment conducted by Blanks (2016) to compare the structural changes of bone in two different burns, one was conducted using the pyre and then the other burn was conducted using the muffle. Therefore, experiment puts forward the premise that there is no difference between the effects on the bone (Appendix 3 for full method). This research presents the argument that even though the muffle furnace is not as forensically accurate compared to a pyre it does still create the same results on the bone after burning. For the purposes of the current research the muffle furnace was more beneficial due to the controllability of the temperature and the exact time of exposure being measured exactly, along with the ease of removing the samples once the burn was completed. This also means preservation is more likely due to not having to let the fire to die down before removal. A funeral pyre style fire also has limitations if the wood is too fresh and therefore the moisture content can be too high, thus meaning the fire may not reach the desired temperature or potentially not even light in the first place (Carroll and Smith 2018). From the outset of this investigation the changes in the conditions of burn were apparent when using the muffle furnace instead of a pyre style burn. This is due to the muffle furnace burning in artificial environment compared to the chemical reactional environment in a pyre, this means there are crucial differences in how the fire burns (Blanks 2016; Carroll and Smith 2018). However, from previous experiments the effects that the two burn types have on the bones are not substantial (Blanks 2016). To support this argument of which burn type is more beneficial it was decided to look at a range of samples from different circumstances of burning to see if the burn method is important in identifying and recording the microstructure.

4.4: Thermal alteration of bone

4.4.1: Muffle furnace

After the pre-burning analysis (Section 4.6) of the thin sections, the whole bones that are not pre-cut were burnt using the muffle furnace at 200^{o}C, 400^{o}C, 600^{o}C and 800^{o}C, at 200^{o}C increments for 30 minutes for each burn. This duration of burn provided a long enough burn to show changes presented on the bone's microstructures. After maceration the bones were split into groups of two to be burnt together, creating four repeats for each temperature and time. This was done to show a clear comparison between each temperature and time of burning and to see if the changes in the microstructure were similar across the four repeats. The grouping of the bones resulted in eight remaining bones, which could be used either for further analysis of the pre-burning state or if some temperatures required another repeat. Along with this the remaining bones were used to observe the effect longer exposure to burning would have on the bones. The furnace was set to the desired temperature with time allowed to reach the specified level of heat. The furnace maintained this temperature for the time set before cooling back down (figure 4). This method was chosen as when a person uses fire to destroy skeletal remains, the fire will not instantly heat to the temperature required to destroy evidence but needs to warm up with the remains located in the fire itself. For the burning process the bones were placed on to a metal slide to allow for

two bones to be burnt at the same time, this is due to the bones being too large to fit into the ceramic crucibles that are more usually used in an electric furnace of this type. The bones were not cut up to fit into the crucibles on the grounds that the experiment needed to be as forensically accurate as possible and this method of burning allowed the bones to be burnt whole, which in most cases of concealing evidence via burning is the most commonly used method. However, due to unforeseen circumstances during the process of burning the bones the muffle furnace suffered from sealant racking around the pipe, this led to the furnace not being able to reach the desired temperature. This halted the process of burning and meant two sets of samples did not reach the temperature required.

Figure 4: The muffle furnace used for the thermal alteration of the samples.

4.4.2: Experimental burning

The additional samples included to take account of varying circumstances of burning were two deer carcasses, one burnt on an experimental pyre and the other being burnt in a domestic fire; cow remains burnt inside an oil drum to see how a poorly oxygenated fire affected the remains. These cow remains were burnt at less than 300^{o}C, shown by the colouring of the bones and how they compare to the other bones in the collection and pig remains burnt on another experimental pyre. This change in method had no effect on the comparison of the bone microstructure, this is supported by the aforementioned section on deciding whether to use the muffle furnace or a pyre. The duration of the burnings, the approximate temperature of burning, and the methods of recording are discussed below and in Appendix 3, which is the methods collected from the previous experimental research.

4.4.2.1: Temperature

The temperatures of each of the burns vary, with the domestic fire peaking at 1000^{o}C within the first 20 minutes of the burn, after which it started to decrease rapidly until reaching 80^{o}C and then continued to fluctuate (Carroll and Smith 2018). In comparison the experimental pyre peaked at 553^{o}C after 70 minutes (Carroll and Smith 2018). However, during the experimental pyre that contained the pig remains the equipment that was used to determine the temperature of burn was faulty and readings were unable to be made (Hoff-Olsen 2016). Nevertheless, from previous

studies a temperature range was able to be produced from the colour change that the bones went through during the bone, this produced temperatures somewhere between 700°C and 900°C (Hoff-Olsen 2016).

4.4.2.1.1: Controlling thermal alteration

Despite the greater degree of control in these artificial conditions, the temperature of the burns is still subject to some variation. Along with this, as mentioned above the temperatures are not kept at a consistent level throughout the burn. The bones exposure to the heat would consequently vary and thus the destruction of the microstructure could be less extensive in areas of the samples, so comparisons were made with caution.

4.4.2.2: Duration of burning

The duration of the burns varied, dependent on numerous factors, including how much oxygen the fire received and the amount of fuel for the fire to burn (figure 5) (Schottke 2014, p. 144). In the simulated domestic house fire, the burn lasted a total of 2 hours, with the carcass self-igniting after 20 minutes and the hind legs beginning to contract after 24 minutes (Carroll and Smith 2018). In comparison, the experimental pyre that was conducted on the other deer carcass lasted for a total time of 3 hours 30 minutes, with the fur becoming charred after 20 minutes and it was not until after 50 minutes that the muscles in the hind legs started to contract. The pyre started to collapse after 60 minutes, with the rest of the pyre breaking down and fully collapsing after 140 minutes. However, in comparison to the experimental pyre that contained the pig remains, the pyre lasted for 10 minutes longer at approximately 150 minutes.

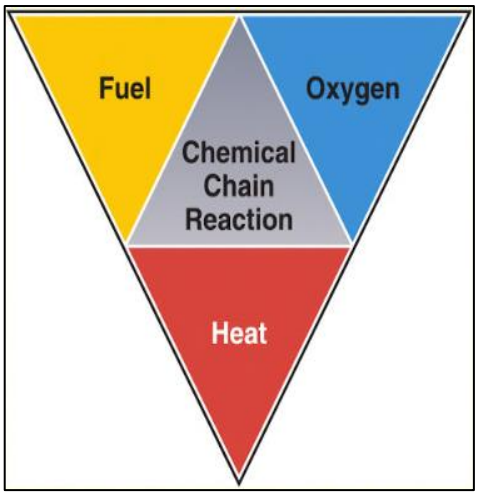

Figure 5: The chemical composition that makes up a fire. Showing how all three of these aspects are crucial in the chemical chain reaction (Schottke 2014, p. 144).

4.5: Bone preparation

4.5.1: Cutting the bone

After the process of maceration was complete, the diaphyses of the bones were cut using multiple methods of thin sectioning, some of which obtained unsatisfactory results. The unburnt bones and bones burnt at 200^{o}C had the diaphyses cut into equal sections (figure 7a and 7b), these cuts meant that there was enough to repeat if the sections were unsatisfactory for the histological analysis.

4.5.2: Thin sectioning

Thin-section (or histomorphological) analysis was employed in this study to assess the effects of burning on bone microstructure. The first thin sectioning method made use of the Nakanishi ev410-230 micro-grinder, however, the sections produced via this method, were rough and the scratch marks from the blade were visible under microscope, which distorted the microstructure, resulting in inaccurate results. This in turn led for this method to be not used for the other sections. The second method of thin sectioning made use of a hacksaw and hand cutting the sections. However, the sections produced via this method were too thick to analyse microscopically and the surface was too rough, so no focal point could be reached. As with the micro-grinder sections the scratch marks from the blade obscured the microstructure. Another method used to thin section was the use of epoxy resin and hardener. 2.5g of Buehler Epo Thin Epoxy Resin was mixed with 0.9g of Buehler Epo Thin Epoxy Hardener, this was then heated using a hotplate set to approximately 20^{o}C (between 2 and 3), with it being continuously mixed. Once fully mixed the resin was then dripped over the samples and let to stand for a minute, these samples were placed inside a vacuum desiccator and left for 10 minutes to allow the vacuum to pull the resin through the bone (figure 6a). Once the 10 minutes had elapsed the samples were left for 24 hours to set and dry in a fume cupboard. After the samples were dry, they were cut using the Buehler Isomet 5000 Linear Precision Saw (figure 6b), after a thin section was taken off, they were sanded down using a band sander on multiple different grits of paper, with water running down them. Thereafter these samples were further sanded down using carborundum powder with 600 grit mixed with distilled water using a figure of eight motion for 5 minutes.

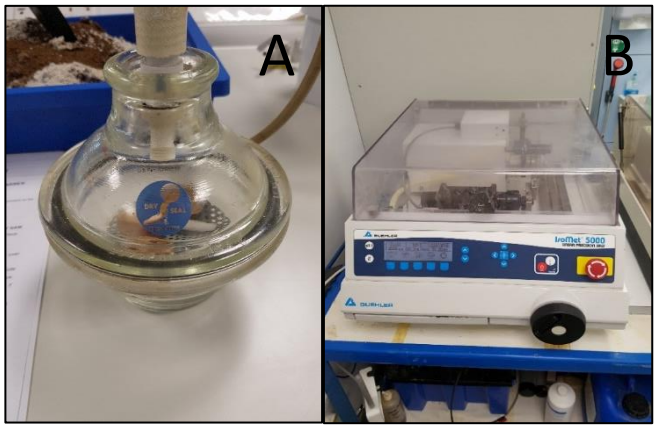

Figure 6: Shows photographs of a) the vacuum desiccator containing 3 bone samples and b) the Buehler Isomet 5000 Linear Precision Saw.

These methods all produced samples that were unsatisfactory when analysing them microscopically due to the marks left on the bone microstructure after sanding the samples were not useful for analysis. The following method began by using the Lieca SP1600 saw microtome at a speed of 600RPM to create the thin sections. The containers that were used to contain the samples were placed into were sprayed with Buehler mould release spray. The samples were then placed into the containers and embedded. The EpoThin Epoxy Resin and Hardener was used again as the embedding agent, the method used however, was different to the one that produced the samples for the Buehler Isomet. This method called for the samples to the embedded using moulds. The blocks were created by pouring the resin/hardener mixture over the samples in their moulds and left to harden for 24 hours. The quantities used ensured the samples were completely covered to be block embedded for the thin sections. The amounts used was in a ratio of 5:1.95, resin to hardener. Once the samples were set, each specimen was going to be cut using the Lieca SP1600. However, the samples were too large to fit into the mount for the Lieca SP1600, so the Buehler Isomat 5000 was used instead to create the thin sections. The samples were mounted, and these were cut to 1mm thickness at a speed of 1200RPM as there were size restrictions on the blade on the precision saw. These transverse sections were then ground down to a thickness that allowed for the light from the light microscope to pass through ~250 µm in thickness due to how fragile the bones were. The thermally altered remains that were particularly brittle, which were burnt at high temperatures were ground to a thickness that prevented the bone from being damaged or completely destroyed due to the grinding and polishing from the equipment. Once at the desired thickness the samples were polished using a much finer grit of paper at 1-6 µm to prevent more of the section being ground, but enough to remove the scratches produced due to the higher grit paper. The mounting of these thin sections on to glass allowed for these samples to be placed into a collection and used as a tool for further analysis on the microstructure of bone, pre- and post-burning.

The sections created could be later analysed more easily under a digital microscope results in the scratches produced from thin sectioning being not visible. Using this microscope allowed for a clearer analysis of the bone microstructure using the image processing and so being able to count the number of osteons clearly and thus being able to obtain a percentage of the bone microstructure, that has survived along with the diameter of these intact osteons and plexiform bone.

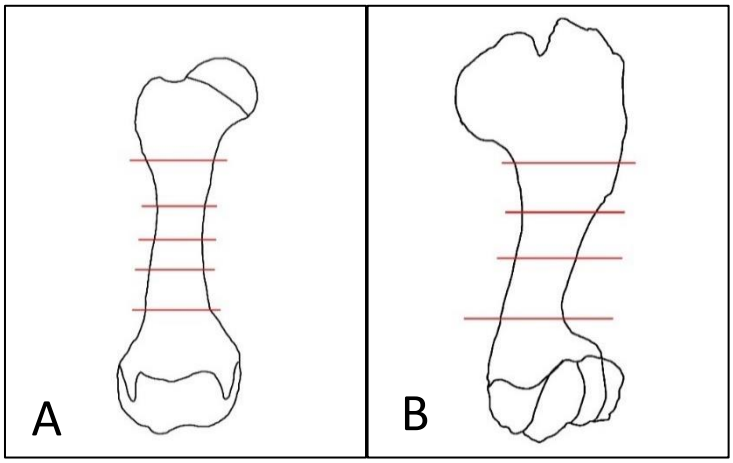

Figure 7: Schematic of the A) right femur and B) left humerus showing the cut lines. These were cut using a handheld hacksaw, as these did not need to be precise. These cuts made it easier for the samples to be embedded and later thin sectioned.

4.6: Pre- and post-burning analysis

4.6.1: Macroscopic analysis

The bones were first put through a basic macroscopic analysis, to assess how burning affected the bones morphologically compared to unburnt bones. This macroscopic analysis was only to observe any colour change the bones go through and to see how the fissuring on the bone's changes during the variations of temperature and time in each burn. Colour change was recorded using the Munsell soil chart (Munsell colour company Inc., 1975) along with any clear fissuring and fractures present on the bones post-burning.

4.6.2: Microscopic analysis

Making use of an optical light microscope the samples were photographed using a camera attachment. These pictures were then transferred to the Keyence digital microscope, the samples were analysed to see the changes in microstructure when compared to the pre-burning analysis between the number of intact osteons and the diameter of the osteons after burning. These measurements were taken to indicate the differences that are present through the varying levels of burning on the bone microstructure and to see how the osteons change, by either damage. The samples were measured and observed with magnifications of 25x, 50x, 100x and 200x to photograph the samples and to identify the microstructures. This allowed for the microstructure to be seen from varying points and to see how the structure changed at lesser magnification. Once the structures had been found they were then counted and measured. The osteons counted were the ones that were intact to show the differentiation from the pre-burning analysis using the

Oxford Histological Index (Hedges and Millard 1995). This index when counting the intact osteons, uses percentages with a scoring of 0-5 for the varying levels of preservation of the Haversian canals (Table 1). This method of scoring was implemented in this research due to the accuracy it produces and the detailed descriptions of each of the scores. On the other hand, the samples used in Hedges and Millard's (1995) study were human remains from archaeological excavations so the microstructure would differ from non-human bone as mentioned in section 2.1.1. However, this method of scoring remains transferrable to animal remains (Cuijpers, S. and Lauwerier 2008). A potential problem arises in that this system of scoring was created for the diagenetic changes undergone by bone microstructure in an unburnt condition, rather than for recording thermally induced changes. No comparable published method currently exists for application to the latter, but notwithstanding this the OHI method is arguably applicable as it is essentially descriptive, simply recording whether structures remain discernible, rather than commenting in its own right as to the cause of any loss of microstructural integrity. Despite this though the descriptions that are provided by Hedges and Millard's (1995) as they became challenging to distinguish between the scores of 1 & 4 as they were not well described and as mentioned above are based on unburnt bones.

Table 2: Histological index values assigned to summarise the degree of diagenetic change (Hedges and Millard 1995, p203).

Index	Approx. % of intact bone	Description
0	<5	No original features identifiable, other than Haversian canals.
1	<15	Small areas of well-preserved bone present, or some lamellar structure preserved by pattern of destructive foci.
2	<33	Clear lamellate structure preserved between destructive foci
3	>67	Clear preservation of some osteocyte lacunae.
4	>85	Only minor amounts of destructive foci, otherwise generally well preserved.
5	>95	Very well preserved, virtually indistinguishable from fresh bone.

These observational methods were chosen as these are the most common technique for osteon change in bone, and have been used in thermal alteration previously (Hummel and SchutkCarowski, 1966; Bradtmiller and Buikstra, 1984; Nelson, 1992). The measurements taken were the diameter of the osteon as a whole, which was compared to the unburnt samples to see how the osteons have changed after varying levels of thermal alteration, which is known to cause macroscopic changes to bone (Hummel *et al.* 1988; Hummel and Schutkowski 1993; Herrmann and Bennett 1999; Kalsbeek and Richter 2006; Arora *et al.* 2010; Blanks, 2016; Carroll and Smith, 2018). Thus, showing that it was likely to predict that these same changes would be exhibited on a microstructural level.

4.6.3: Statistical analysis

As an essentially quantitative study the current project presents descriptive statistics as the principle presentation of results, by analysing the diameter of the osteons pre- and post-burning, how each burn condition affected the microstructure compared to one another, and how each of the different species bone microstructure is affected by thermal alteration. Inferential tests were applied to apparent patterns within these data in order to test the strength of observed differences. As the results were comprised of discrete samples of continuous data T-tests or Mann-Whitney U tests were used when comparing the results of two of the samples, with ANOVA tests applied when more than two samples were compared.

These statistical methods were used after reviewing the raw data obtained and how it can be analysed. With the main bulk of the raw data compared one temperature of thermal alteration to the unaltered samples the use of a t-Test or a Mann-Whitney U test was the best option as it compared the osteon diameter sizes and would show if there is a significant statistical difference between the unburnt and the burnt samples. Before each of the tests conducted a normality test on the data would be conducted to see if the date for the samples are normally distributed or not by conducting a kilmonogorov smirnov or shapiro wilks test depending on sample size. These tests dictated which tests were usable further along in analysis.

Chapter 5.0: Results

5.1: Pre-burning analysis of microstructure

The pre-burnt bones that were analysed by digital microscope (DM) showed the plexiform bone structures as being organised and rectangular in shape (Figure 8). The plexiform bone structures varied in length from 407µm to 1732µm in the unburnt samples, with the width of these structures varying as well with measurements of 114µm to 154µm (full list of measurements in Appendix 1). Within these unburnt samples there were a number of secondary osteon bands and thus Haversian canals. The number of osteons were counted to have an overall count of 93 with a mean diameter of 34.68µm from a sample size of 11 transverse sections from two different bones. These figures varied between bones however, for the femur compared to the humerus with osteon diameter showing a minimum of 11.26µm and 13.35µm respectively and a maximum diameter of 61.36µm and 125.55µm respectively. These unburnt remains across all three sections and then all 11 transverse thin sections displayed an OHI score of 5 for the osteons present and visible in the bone.

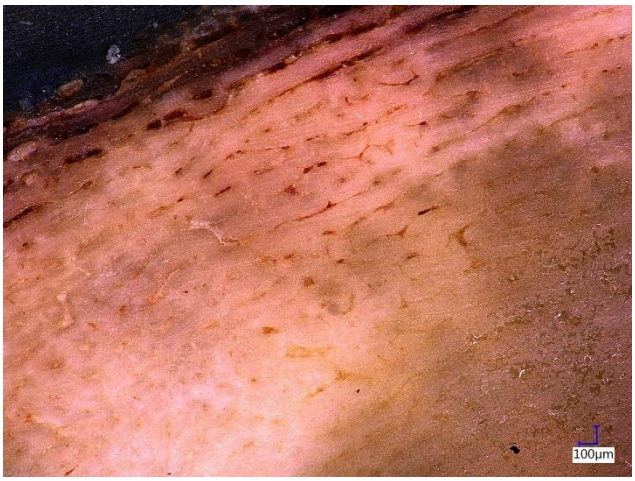

Figure 8: Transverse section 1 from Sample 18a showing the plexiform bone structure present in the unburnt skeletal remains, with osteons present. Image: author.

5.2: Thermal alteration of bones

5.2.1: Colour change

Colour change was observed on all the bone samples used in the current study. The tables below demonstrate the colour changes observed from the bones across all manners of burning within this experimental investigation. In table 2 'Y' denotes the hue colour yellow within the Munsell soil chart pre- and post-burning (Appendix 3).

Table 3: Observed colour changes from transverse sections of all burn conditions.

Sample	Burn Condition	Munsell Soil Colour Value (Hue)	Munsell Soil Colour Value (Value/Chroma)
19	Unburnt	2.5Y	8/2
18	Unburnt	2.5Y	8/2
40	200˚C	2.5Y	8/4
31	200˚C	2.5Y	8/2
14	400˚C	2.5Y	2/0
2	400˚C	2.5Y	2/0
1	600˚C	2.5Y	7/0
4	600˚C	2.5Y	7/0
17	800˚C	2.5Y	8/0
30	800˚C	2.5Y	8/0
Pig 1	Pyre	2.5Y	8/0
Pig 2	Pyre	2.5Y	5/0 (2/0 interior)
Pig 3	Pyre	2.5Y	6/0
Pig 4	Pyre	2.5Y	3/0 (8/0 interior)
Deer 1	SHF	2.5Y	2/0
Deer 2	SHF	2.5Y	8/4
Deer 3	SHF	2.5Y	3/0
Deer 4	SHF	2.5Y	4/4 and 2/0
Deer 1	Pyre	2.5Y	8/2
Deer 2	Pyre	2.5Y	8/2
Deer 3	Pyre	2.5Y	8/0
Deer 4	Pyre	2.5Y	8/0
Cow 1	Oil Drum	2.5Y	3/0
Cow 2	Oil Drum	2.5Y	2/0
Cow 3	Oil Drum	2.5Y	2/0
Cow 4	Oil Drum	2.5Y	2/0

Table 2 indicates the colour change of all the samples used for the thin sectioning from unburnt bone to 800ºC and then for the samples collected from the other experimental burns. This table also indicates the differential colour changes a bone goes through in both an artificial conditional burn and an experimental pyre, with most of the bone samples undergoing calcination in the higher temperatures of the burns.

Table 4: Observed colour changes from all samples thermally altered in the muffle furnace.

	Hue											
	2.5Y											
	Value/Chroma											
Burn Condition	2/0	4/0	5/0	5/6	6/0	6/2	7/0	7/2	8/0	8/2	8/4	8/6
Unburnt										N=2		
200˚C				N=1						N=3	N=1	N=1
400˚C	N=3						N=1	N=1				
600˚C	N=1	N=2	N=2		N=1	N=1	N=3		N=3			
800˚C					N=2		N=2		N=2			

Table 3 n=x where 'x' equals the number of bone samples that displayed that colour. Sample 28 that was burnt at 400ºC had a humeral head and half the diaphysis displaying the colour 2/0, while its distal epiphysis had an observed colour 7/2. In contrast sample 14 which was also burnt at 400ºC had an observed colour of 2/0 over the whole bone. For the majority of the samples burnt, 600ºC and above had multiple observed colours over the exterior of the bone and on the interior of the bone, for example sample 4 displayed multiple colours across the bone fragments with the humeral head having an observed colour of 8/0, but the diaphysis and interior of the bone displaying a colour of 4/0 and 2/0 respectively. However, the samples burnt at 800ºC both displayed the same colours throughout the bones as a whole with the colours 6/0, 7/0, 8/0.

The results of the Munsell soil chart are presented in Figure 9, with a variation of colour noted across the samples at each of the temperatures of the burns. In the Munsell Soil chart the score for the value at hue 2.5Y had varying colours form white (8) to black (2), with stages of grey throughout the two at 3, 4, 5, 6 and 7. Combined with the chroma it produces differing levels of these values producing more yellow and brown colours for the lower temperature.

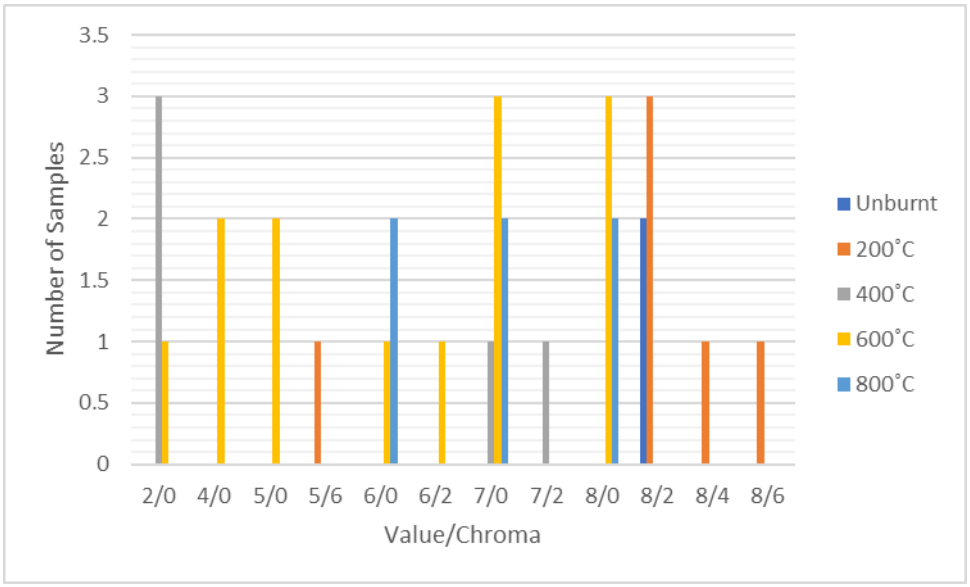

Figure 9: A bar chart to show the distribution of samples according to their colour after burning, as scored according to the Munsell Soil Chart.

The samples burnt at 200ºC (samples 35 and 31) showed a colour change from the unburnt bone colour to a more yellow colour on the diaphysis and yellow/brown on the epiphysis (Figure 10). Compared to the bones burnt at 400ºC, which displayed an increased colour change, from the white/red colour before burning to black with greyish ends. Along with this the second set of bones burnt at 400ºC (samples 2 and 14) showed areas of the bone burning to produce a blue colour present on the diaphysis and the epiphyses (Figure 11 A&B). This colour change can support the work of Mays (2010) with his descriptions of the changes in colour from varying temperature ranges, with the temperatures that the bones from this experiment were burnt at, falling into the ranges provided. However, the timings of these burns varied, which in turn demonstrates that this blue colour change starts to occur at approximately 30 minutes at 400ºC. Despite this the first set of bones burnt at 400ºC (Figure 11 C&D) (samples 28 and 36) did not have as much of this blue colour present. The blue colour of the bones after burning is much more prominent once the bones have been burnt at approximately 600ºC (Figure 12) (samples 1 & 4) at no longer than 30 minutes again. A set of bones were burnt at 800ºC and this produced a white colour across the whole bone (Figure 14C), which demonstrates that between 600ºC and 800ºC the colour change between blue and white is somewhere within half an hour.

However, with the bones that did not complete their burn, samples, 37 and 38 (Figure 13A and 12B), compared to sample 33 (Figure 13C), the colour changes were quite different even though the temperatures they reached were around the same range (400-450ºC). With 37 and 38 being completely black all over, while 33 had the white and blue colouring that is seen on sample 1 which is burnt at 600ºC (Figure 12A). In summary these variations in colour showed that the extent of which the organic material is burnt away within the different samples varies substantially.

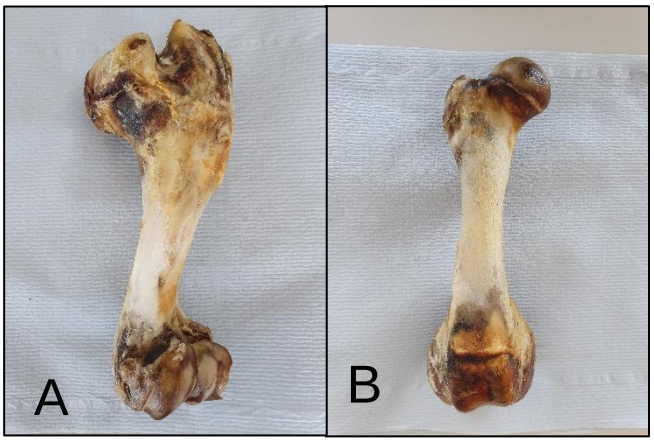

Figure 10: photographs of long bones burnt at 200ºC samples 35 (A) and 3 (B). Showing the thermal alteration of the bones.

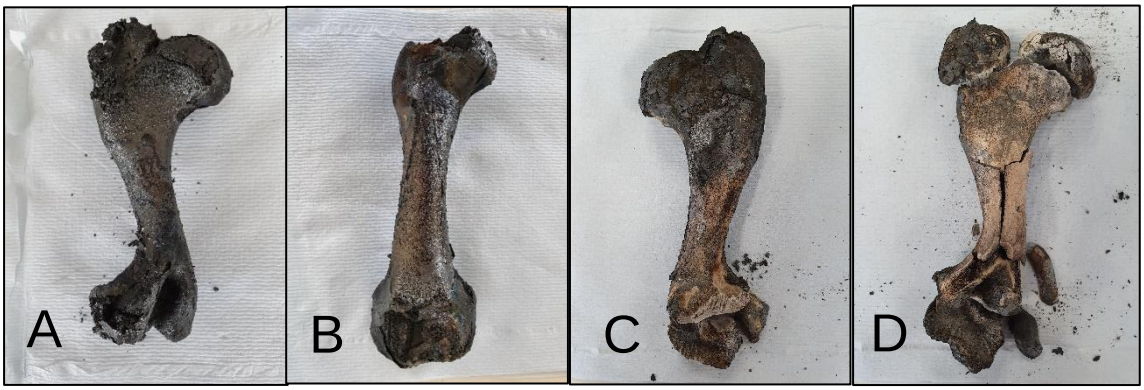

Figure 11: photographs of long bones burnt at 400°C samples 2 (A) and 14 (B). Showing the thermal alteration of the bones and with how the bones begin to crack. Compared to samples 28 (C) and 36 (D), which were burnt at the same temperature and for the same duration.

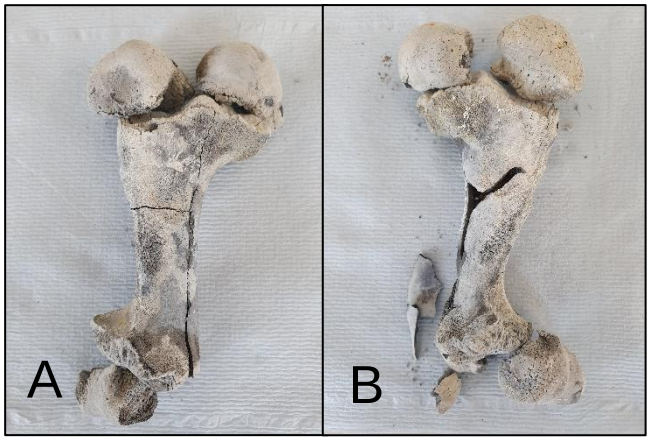

Figure 12: photographs of long bones burnt at 600°C, showing the thermal alteration of the bones and the fissuring present on the bone.

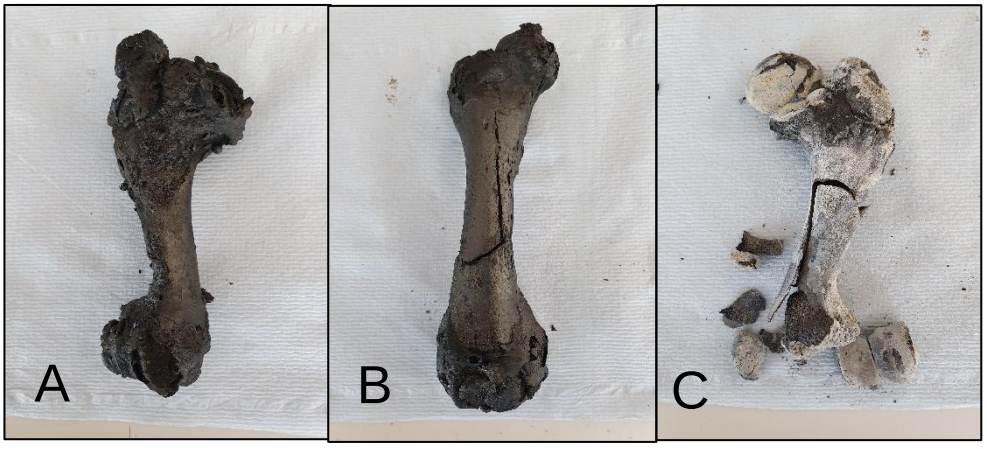

Figure 13: Photographs of long bones that failed to complete their burns due to technical difficulties, but the colour changes from A and B compared to C is vastly different, but these were burnt at the same temperature.

5.2.2: Morphological changes

The remains that were collected from the muffle furnace clearly showed a varying amount of damage that has occurred over the duration of the two different time frames at the four varying temperatures. One of the bones burnt at 400ºC cracked and split into separate sections, due to the heat and being as fragile as it is, but it was able to be reconstructed to show the original shape with the remains that were still present (Figure 14A). The bones that were burnt at over 200ºC all showed fissuring and also flaking of the cortical surface. Along with these changes most of the epiphyses on the bones burnt above 200ºC became detached from the diaphysis. At 600ºC the bones again showed fissuring, which was often so extensive that the diaphysis became fragmented, exposing the interior of the medullary cavity to further thermal alteration (Figure 14B). The bones that were burnt at 800ºC became even more severely fragmented (Figure 14C) with some parts of the cortex having effectively peeled outwards due to a combination of warping and cracking. In such cases the respective warped portions then rapidly fell apart, leaving the epiphyses as the most intact part of the bone.

As expected, there was an obvious size difference between the bones burnt at 800ºC and the unburnt samples. This size difference was also clear in the 600ºC sample but was less apparent for the bones burnt bunt at 200ºC and 400ºC, without measurement.

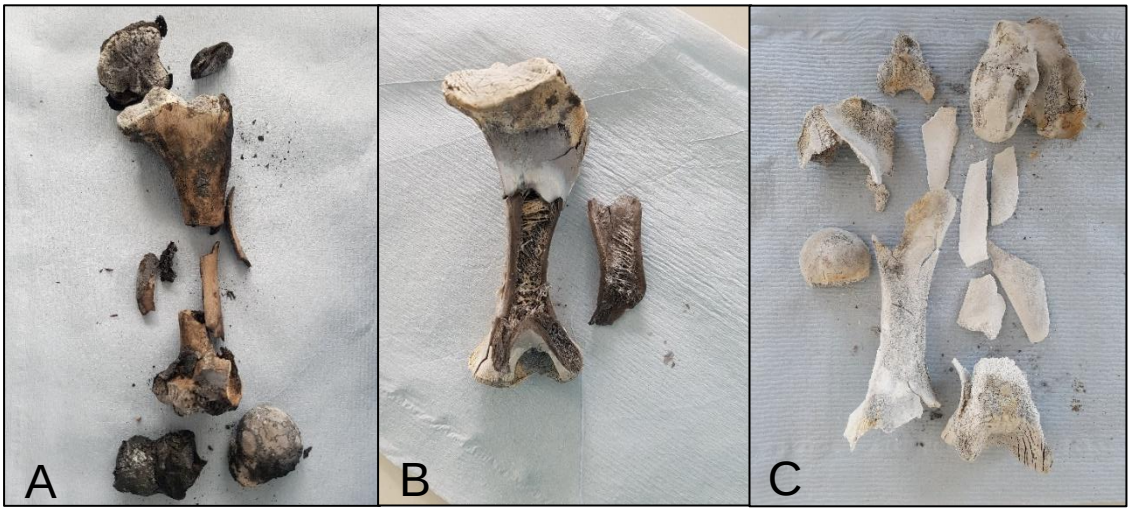

Figure 14: Photographs of the burnt bones varying temperatures (A) showing the reconstructed bone at 400ºC, (B) showing the fissuring from the burns and the sections of bone breaking off at 600ºC, (C) a photograph of one of the bones that was burnt at 800ºC showing the fracturing of the bones.

5.3: Post-burning analysis of microstructure

A total of 75 transverse sections were analysed, of which 64 were thermally altered in varying circumstances (muffle furnace, open pyre, oil drum and simulated house fire) whilst the remaining 11 were unaltered apart from the maceration process. The sample information being previously mentioned in section 4.1.1. Following microscopic analysis, an OHI score was given for each of the samples (see Figure 15 and Appendix 3). As previously mentioned, while the OHI provided a good structure for the study of thermally altered bone microstructure, it was found during this study that there was not a dedicated system of scoring burnt bone microstructure, so applying this scale became challenging. However, using the descriptions provided the scoring could be conducted by using the levels of destruction as the main focus of the scores, this was due to the bones not displaying the same structures as the bones used in the OHI, due to different factors being applied to the bone, so the levels of destruction via thermal alteration was analysed instead of the degeneration of the bone. The full collection of micro-photographs can be found in Appendix 4, and these photographs show the detail of the bone microstructure, however, due to limited equipment these were the clearest photographs that could be produced.

Figure 15: Distribution of samples according to their level of destruction post burning, including the unburnt samples, as scored according to the OHI. A score of 5 represents well preserved bone, while on the other hand a score of 0 represents very poorly preserved bone that has been destroyed by varying factors.

5.3.1: Diameter of osteon correlation

The average diameter of the osteons was compared to the temperature of burns and the OHI scores given in order to investigate the possibility of a relationship between osteon size and temperature. There was an overall marginal decrease in both average osteon diameter and OHI score with increasing temperature exposure (Figure 16).

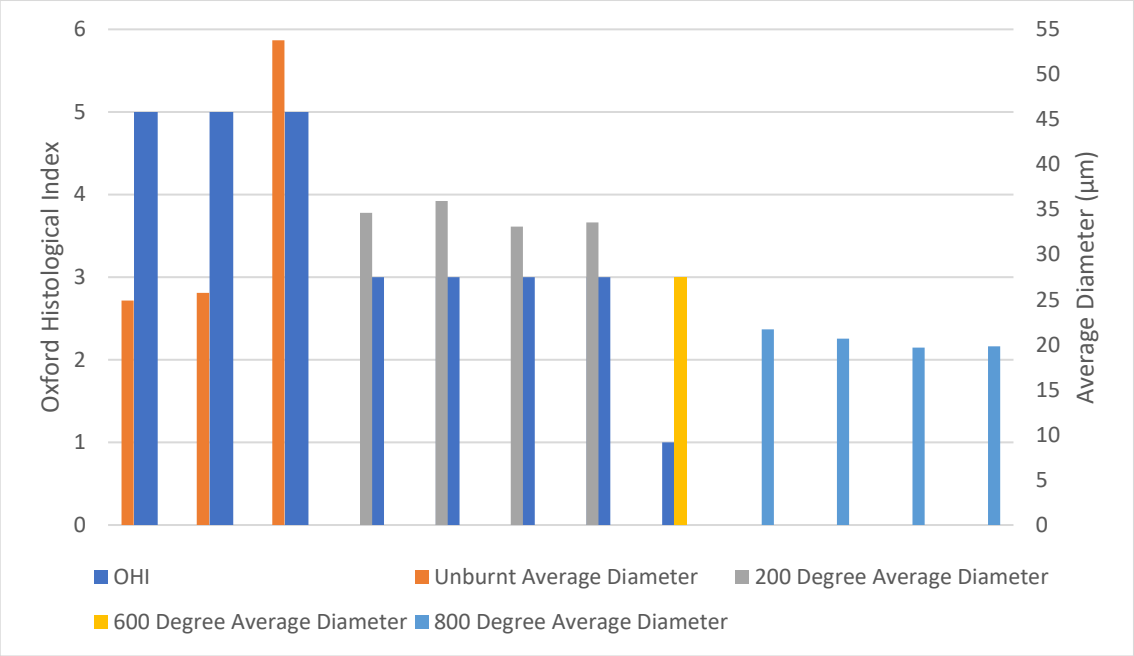

Figure 16: A bar chart to show the comparison of the average diameter of osteon, within the samples used for analysis compared to the OHI scores applied to each of the samples.

5.3.2: Muffle furnace burn

The samples burnt in the muffle furnace are the only bones that could be compared across all temperatures due to the other samples being obtained post burning, leaving no unaltered bones present. In some cases, the samples had become completely carbonised, which caused difficulties for microscopic analysis, as the light could not penetrate. The samples that were unable to be analysed were the samples burnt at 400^{o}C and also three samples burnt at 600^{o}C.

5.3.2.1: Density vs diameter size

From the samples that were analysed, multiple tests were completed., the raw data from these tests can be found in Appendix 2. The first of these tests compared the density of osteons against their diameters. This was conducted by studying the graphs created from the measurements taken. When analysing these results it was clear that there was mix across all the samples of weak positive or weak negative correlations between the variables two across all the burn types (figures 17-41), with a statistical significance at $p<0.05$.

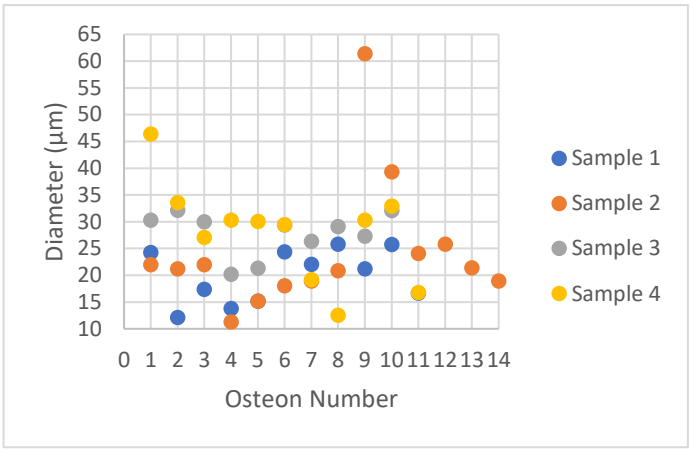

Figure 17: Diameter vs density of osteon, in unburnt sample 18a.

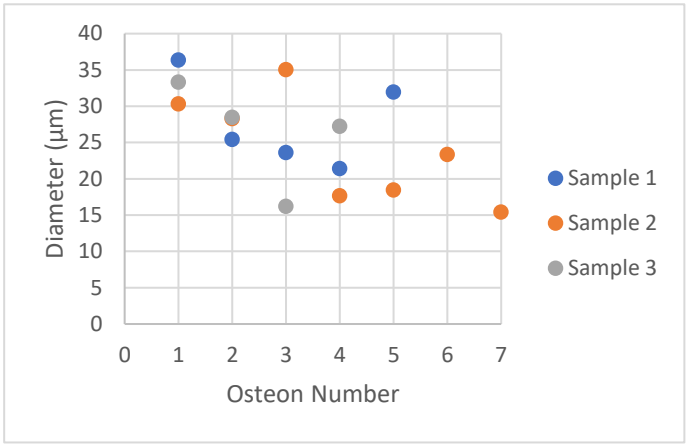

Figure 18: Diameter vs density of osteon, in unburnt sample 18b.

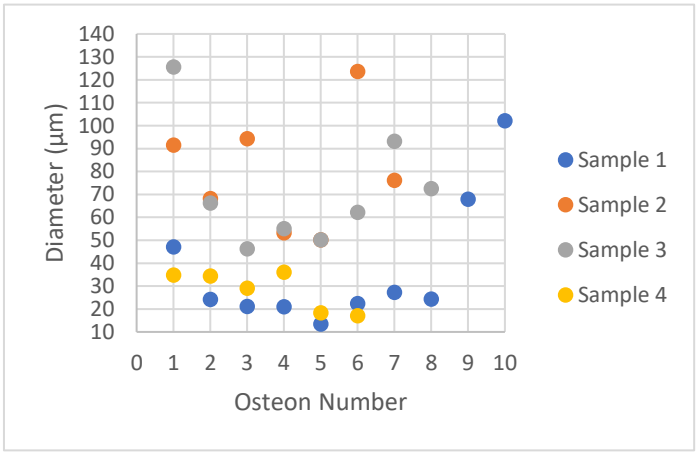

Figure 19: Diameter vs density of osteon, in unburnt sample 19.

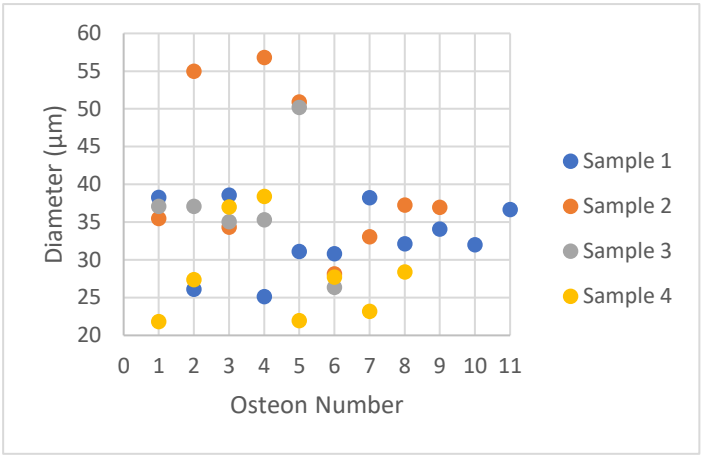

Figure 20: Diameter vs density of osteon, in the 200ºC sample 31a.

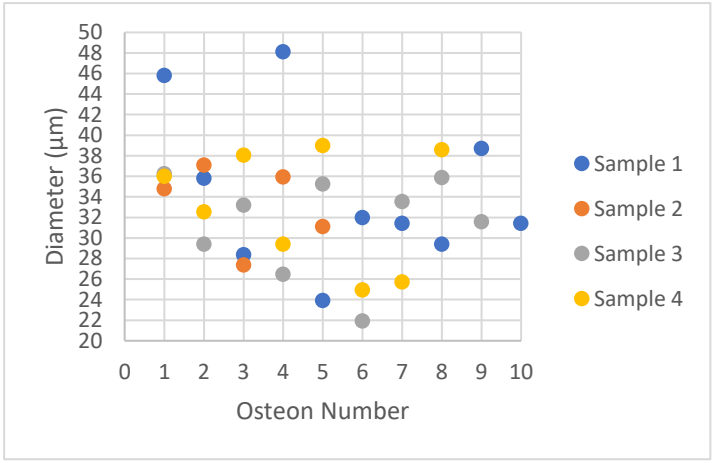

Figure 21: Diameter vs density of osteon, in the 200ºC sample 31b.

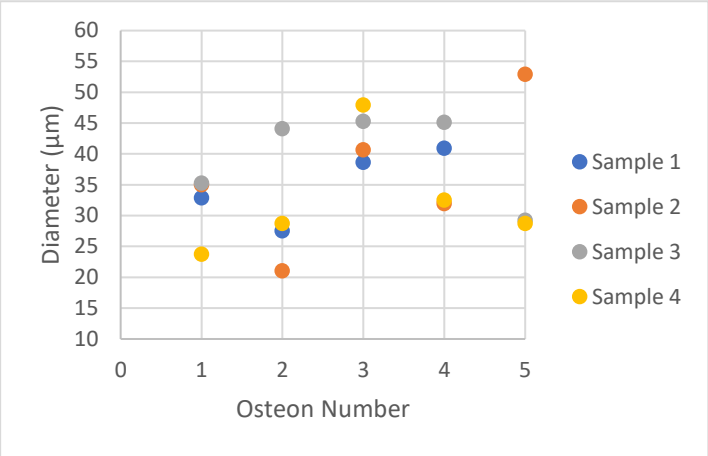

Figure 22: Diameter vs density of osteon, in the 200ºC sample 40a.

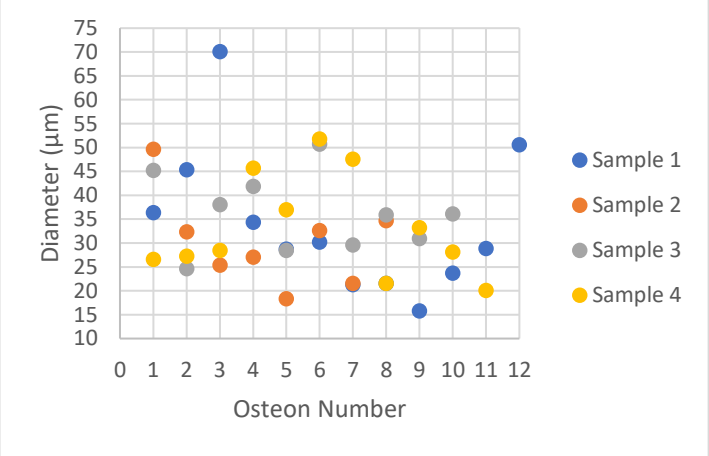

Figure 23: Diameter vs density of osteon, in the 200ºC sample 40b.

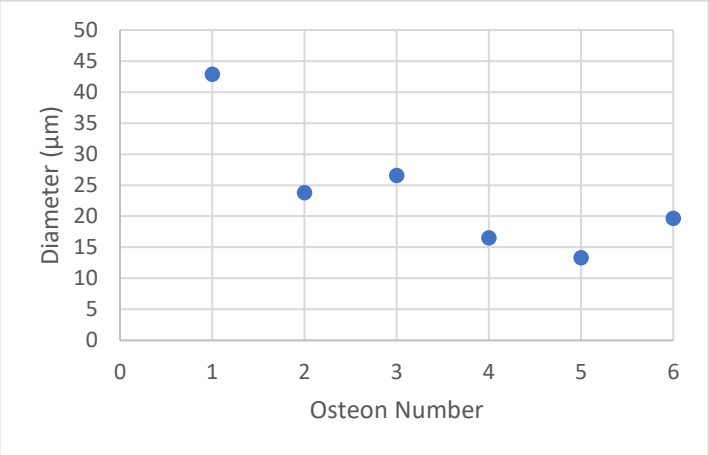

Figure 24: Diameter vs density of osteon, in the 600ºC sample 4a.

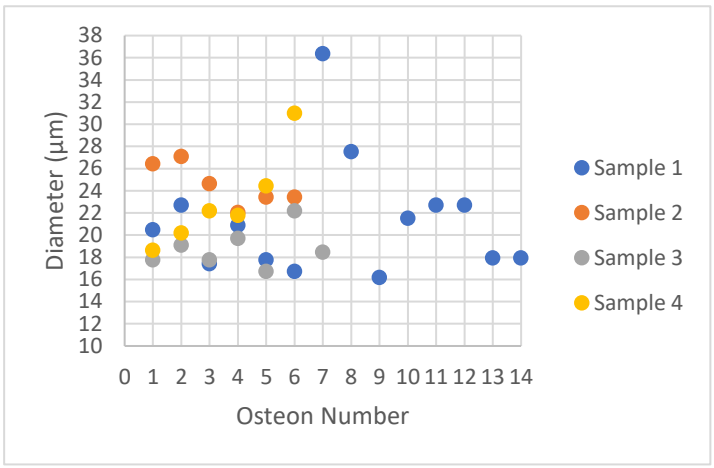

Figure 25: Diameter vs density of osteon, in the 800ºC sample 17a.

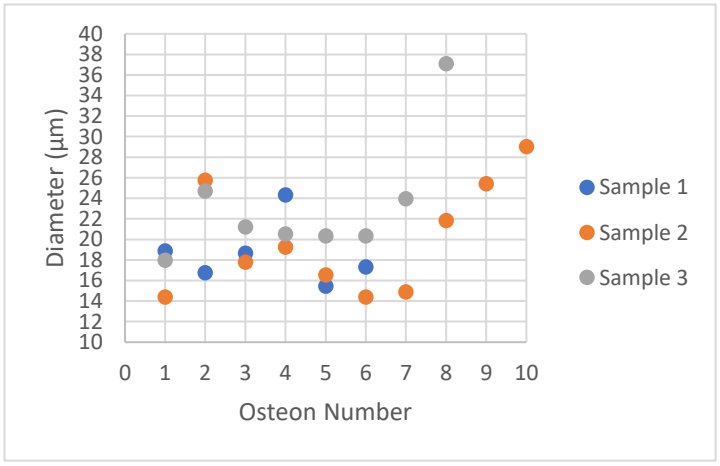

Figure 26: Diameter vs density of osteon, in the 800ºC sample 17b.

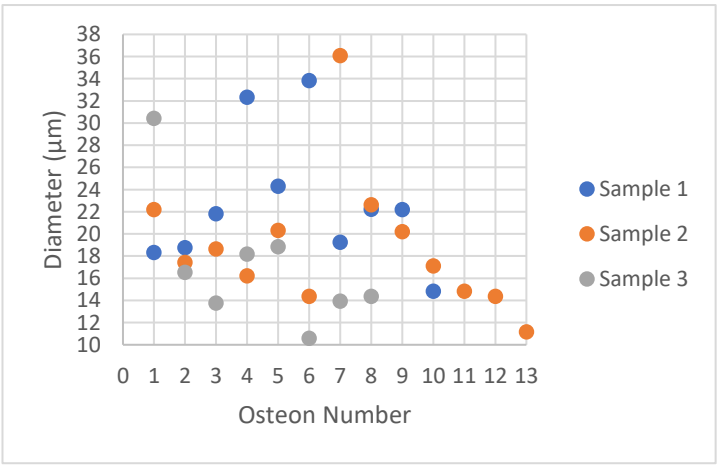

Figure 27: Diameter vs density of osteon, in the 800ºC sample 30a.

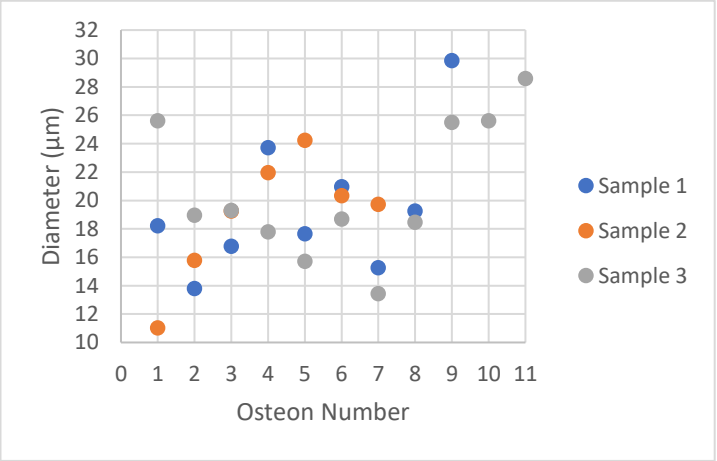

Figure 28: Diameter vs density of osteon, in the 800°C sample 30b.

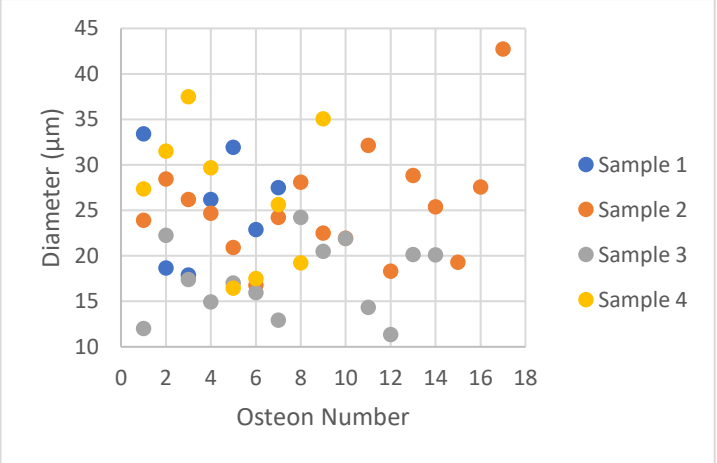

Figure 29: Diameter vs density of osteon, in the oil-drum burn, sample 1.

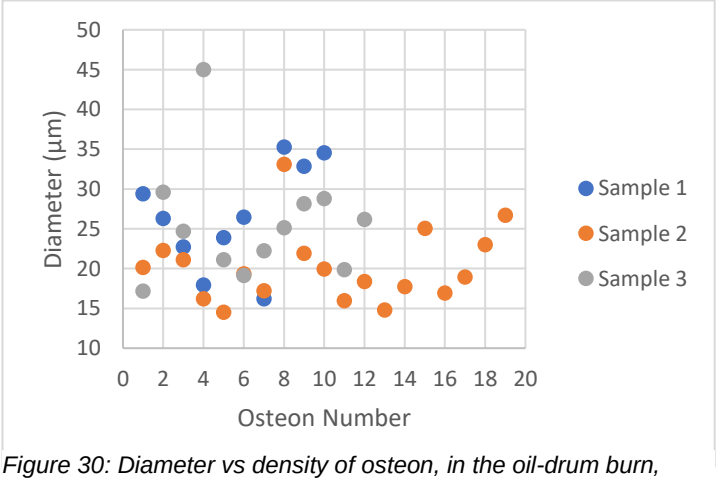

Figure 30: Diameter vs density of osteon, in the oil-drum burn, sample 2.

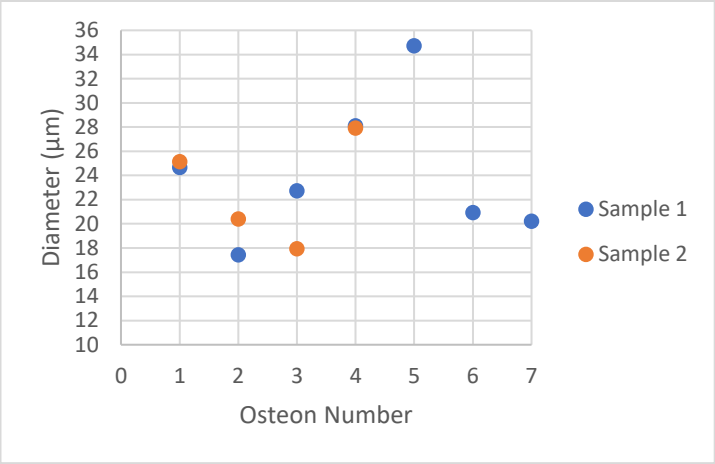

Figure 31: Diameter vs density of osteon, in the oil-drum burn, sample 3.

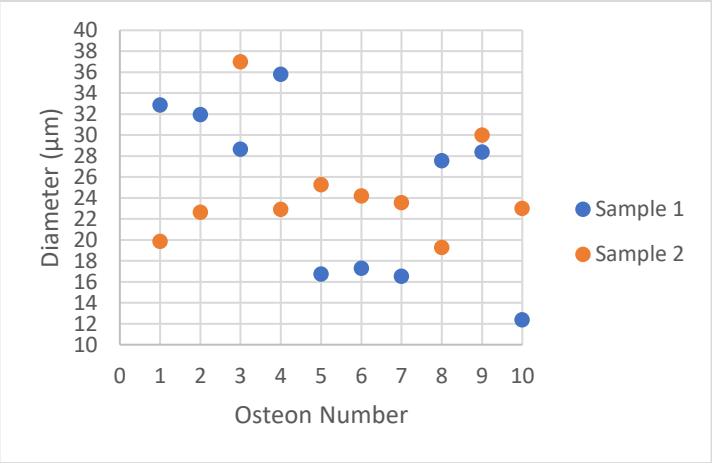

Figure 32: Diameter vs density of osteon, in the oil-drum burn, sample 4.

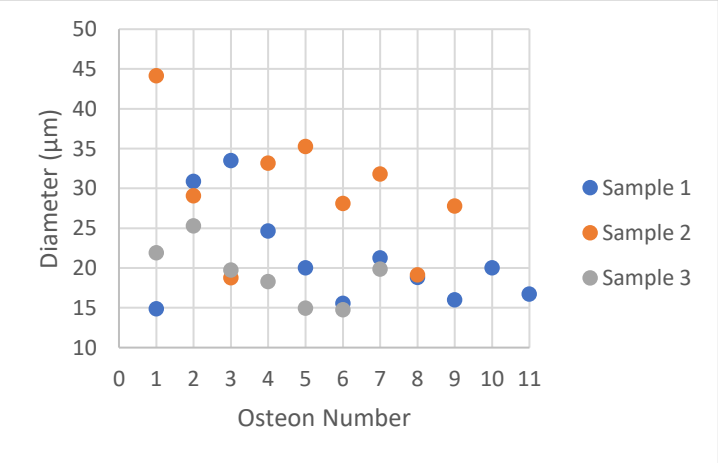

Figure 33: Diameter vs density of osteon, in the deer pyre burn, sample 1.

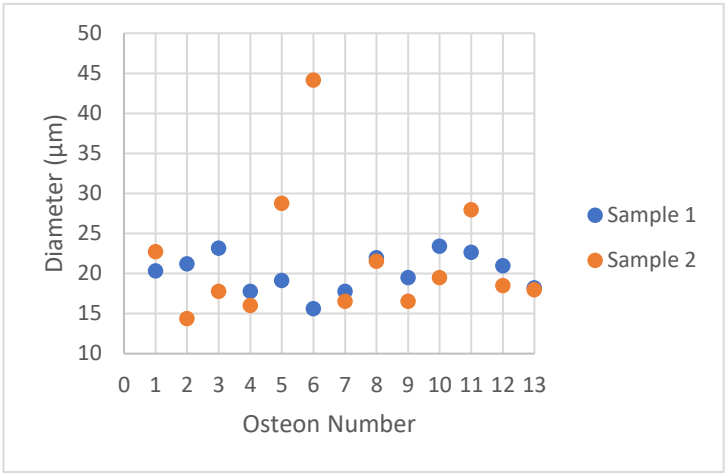

Figure 34: Diameter vs density of osteon, in the deer pyre burn, sample 2.

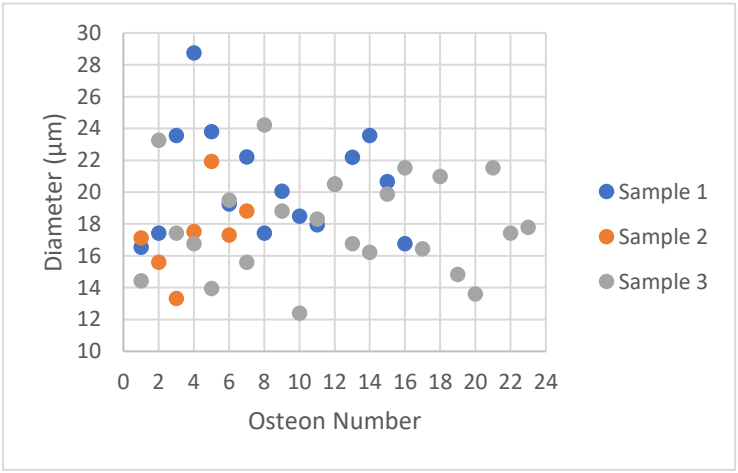

Figure 35: Diameter vs density of osteon, in the deer pyre burn, sample 3.

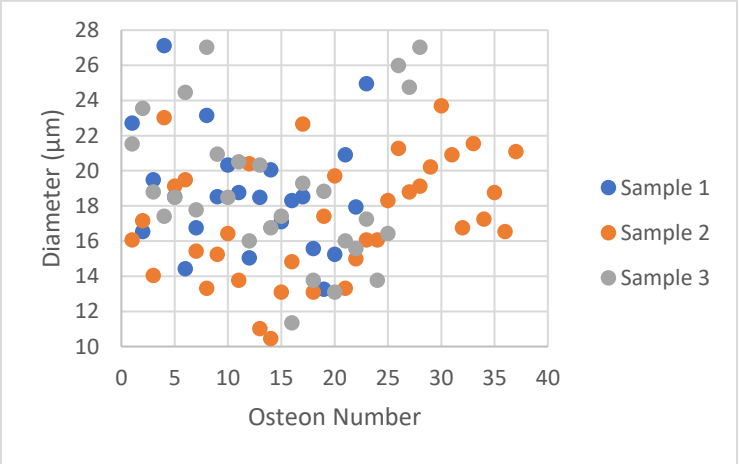

Figure 36: Diameter vs density of osteon, in the deer pyre burn, sample 4.

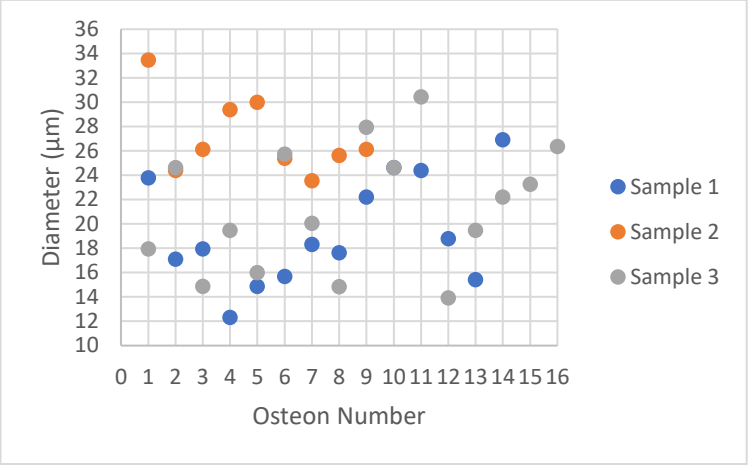

Figure 37: Diameter vs density of osteon, in the pig pyre burn, sample 1.

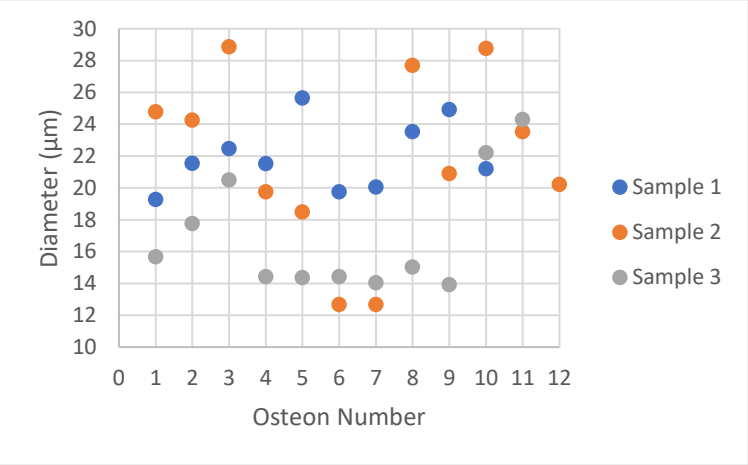

Figure 38: Diameter vs density of osteon, in the pig pyre burn, sample 2.

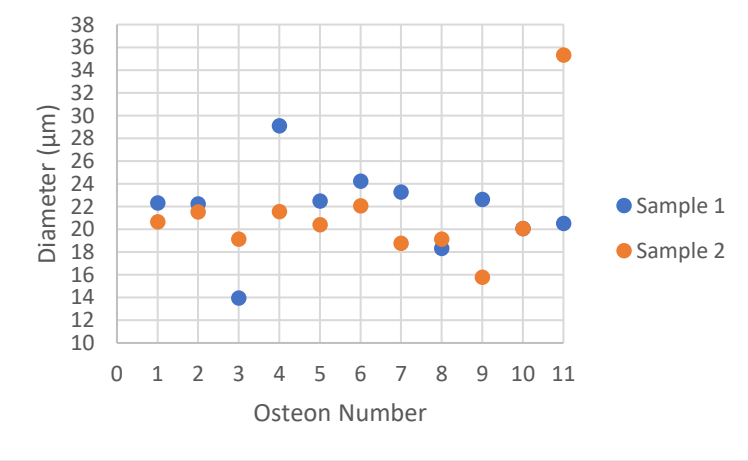

Figure 39: Diameter vs density of osteon, in the pig pyre burn, sample 3.

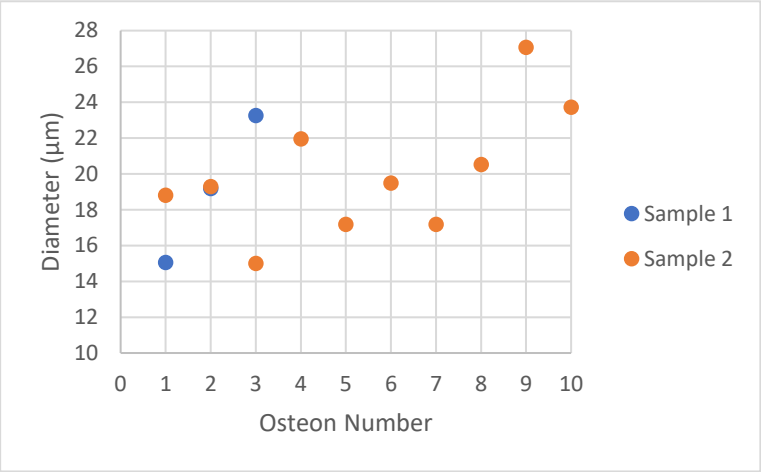

Figure 40: Diameter vs density of osteon, in the pig pyre burn, sample 4.

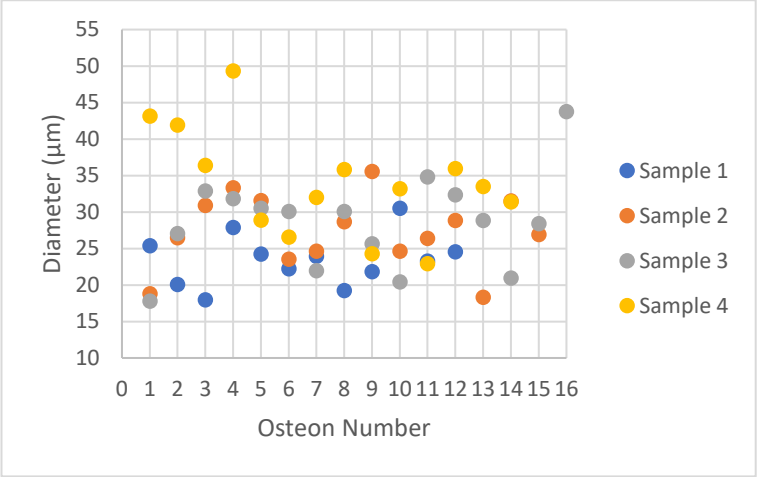

Figure 41: Diameter vs density of osteon, in the SHF burn, sample 2.

5.3.2.2: Osteon diameter after varying temperatures of burning - ANOVA test

The data used in this test showed the bones at the unburnt and 200ºC levels are not normally distributed, while the bones altered at 600ºC and 800ºC are normally distributed, this explains why there were parametric and non-parametric tests being conducted on these results. Results from comparing the samples across all temperatures saw a decrease in osteon diameter from the unburnt bone with a mean osteon diameter of 34.68µm, 200ºC with a mean diameter of 34.10µm, 600ºC with a mean diameter of 23.79µm and the samples burnt at 800ºC had a mean diameter of 20.62µm (Table 4; Figure 42). There was a statistically significant difference between the different burn conditions as determined by a one-way ANOVA (F (3,329) = 23.35, p= <0.001). A Tukey post hoc test revealed that there was no statistically significant difference between osteon diameter at 200ºC (34.10 ± 8.96µm, *p* = >0.05) and 600ºC (23.62 ± 10.49µm, *p* = >0.05) compared to the unburnt samples (34.68 ± 23.66µm). Alongside this the samples burnt at 200ºC showed no significant difference to either 600ºC (*p* = >0.05), with the 600ºC samples also showing no statistically significant difference to the 800ºC samples (*p* = >0.05). However, the osteon diameter between the unburnt samples and the samples thermally altered at 800ºC was statistically significant (*p* = <0.001) and also the samples burnt at 200ºC showed a statistically significant difference to the samples burnt at 800ºC (*p* = <0.001).

Table 5: The total of osteons counted at each of the burn conditions, with the minimum, maximum and mean diameter of the osteons observed.

Temperature (°C)	Total	Min (µm)	Max (µm)	Mean (µm)
Unburnt	93	11.262	125.553	34.68002151
200	126	15.78	70.11	34.10404762
600	6	13.34	42.87	23.79166667
800	108	10.6	37.08	20.61787037

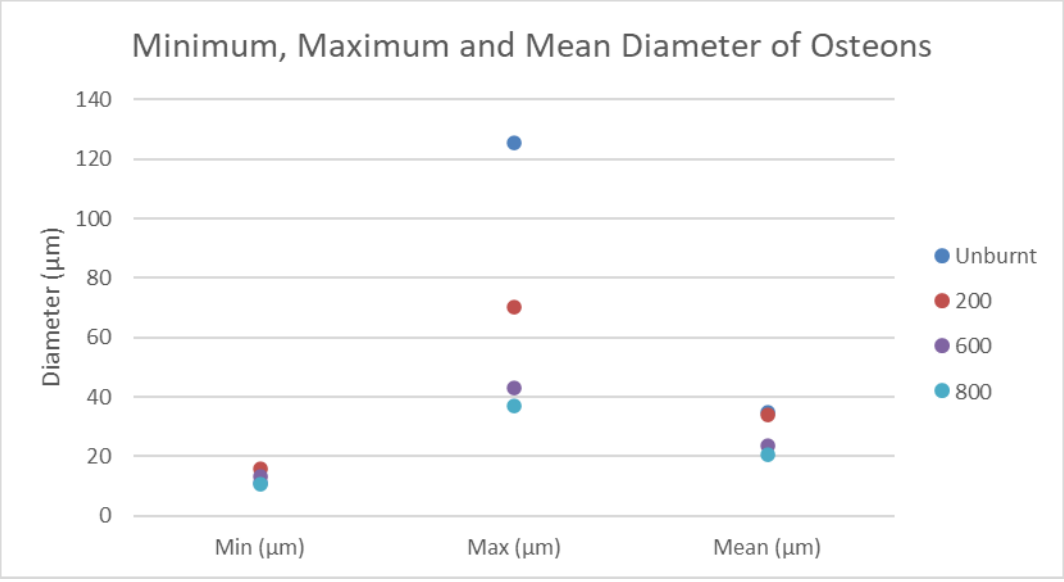

Figure 42: The diameter of osteons measured from the transverse sections analysed, showing the minimum, maximum and mean diameter in micrometers (µm) values shown in table 4.

5.3.2.3: Femur vs humerus – Mann-Whitney U test

A Mann-Whitney U test was conducted and shows that there was a statistically significant difference between the femora (median = 24.31) and humeri (median = 50.06) from the unburnt samples (U = 1513, p = <0.001) osteon diameter size. However, when comparing femora (median = 32.35) and humeri (median = 35.04) from the samples that were thermally altered at 200^{o}C showed that there was no statistically significant different between the two bone types (U = 2193.5, p = >0.05) osteon diameters.

5.3.2.4: Maceration comparison – Mann-Whitney U test

Results from the Mann-Whitney U test comparing the bones defleshed by chemical maceration (median = 34.09) to those that were delfeshed using the *Dermestes* beetles (median = 32.87), which were later thermally altered at 200^{o}C showed that there was no a statistically significant difference (U = 690.50, p = >0.05) between the maceration techniques. However, when comparing how the beetles affected the microstructure between a whole sample (sample 18, median = 24.31) and one that is cut into three sections (sample 19, median = 50.06). A Mann-Whitney U test showed that there was a statistically significant difference (U = 1513, p = <0.001) between the bone put in whole with the beetles to the one that was cut into three sections. This result was expected due to the cortical bone being exposed initially so there would be destruction on the microstructure from the beginning. This comparison was conducted to be applied to forensic cases and the decomposition of remains and if there are any effects on the microstructure if a body is cut up or not, from this test it shows that there would.

5.3.3: Comparison animal species and burn conditions

Similar to the pig bones thermally altered in the muffle furnace, the data collected on the different animal species showed varied distribution, with the oil drum samples, the pig pyre samples and the simulated house fire samples were all normally distributed, while the deer pyre samples were not normally distributed, which as previously mentioned meant both parametric and non-parametric tests were used. From the data collected there were differences apparent in the minimum, maximum and the total number of osteons present between samples from the different species, post-burning (figures 43- 45). The minimum osteon diameter varied across all animals and the burn conditions of these animals (figure 43). In comparison the maximum osteon diameter showed a negative correlation across the sample types (figure 44). Results of osteon diameter of these animals pre-burning are as followed with a deer showing a diameter of osteon pre-burning at ~100μm (Skedros *et al.* 2011). The measurements of the diameters of cow osteon were a maximum of ~149μm to a minimum of ~118μm in humerus and a maximum of ~195μm to a minimum ~128μm in femur (Zedda *et al.* 2008). This shows that as with pig remains the osteon size decreases as temperature increases. The following tests were conducted to compare the differences between all species of animal used within the current research, with the comparison

to the pig samples burnt within the muffle furnace. This was done to give a verdict on their applications as a proxy for human remains for scientific experiments.

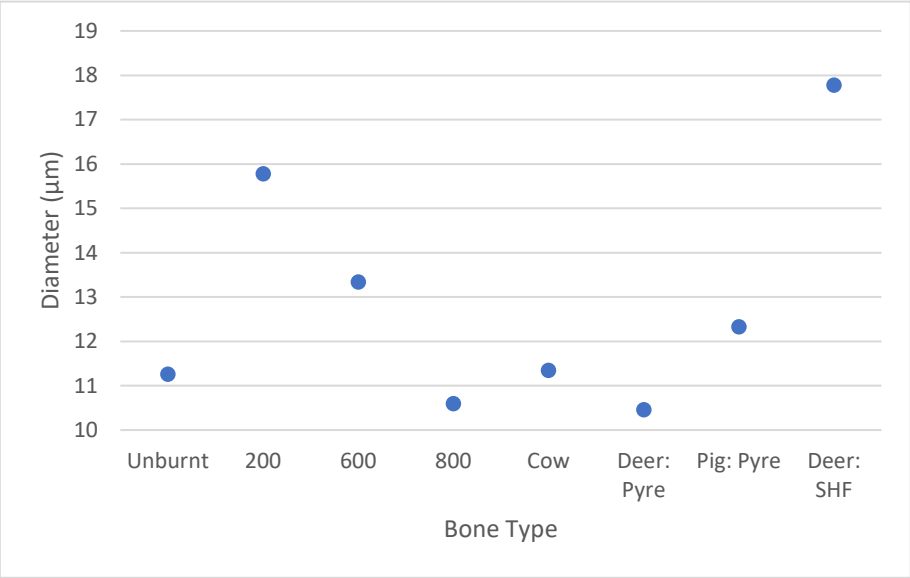

Figure 43: The minimum osteon diameter between the varying species of animal remains used and the burn conditions the samples were burnt with.

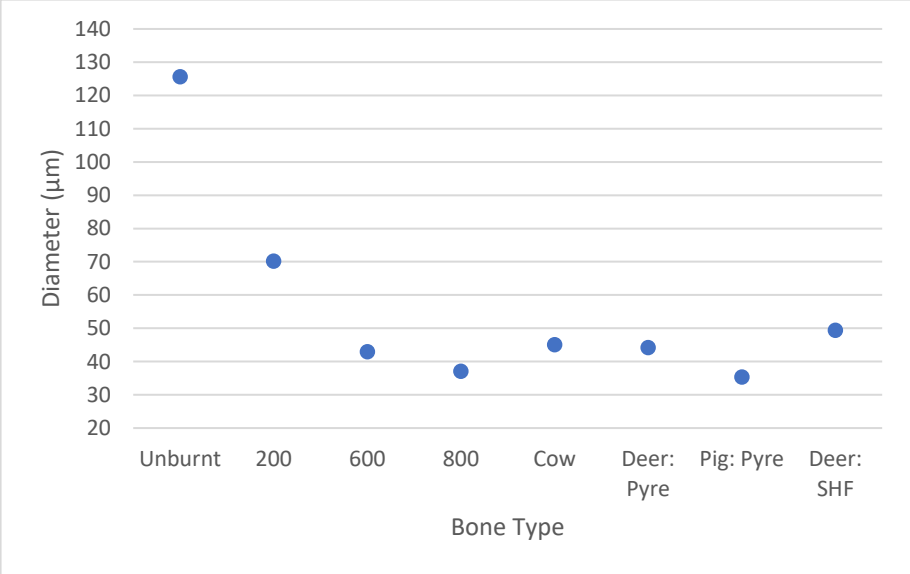

Figure 44: The maximum osteon diameter between the varying species of animal remains used and the burn conditions the samples were burnt with.

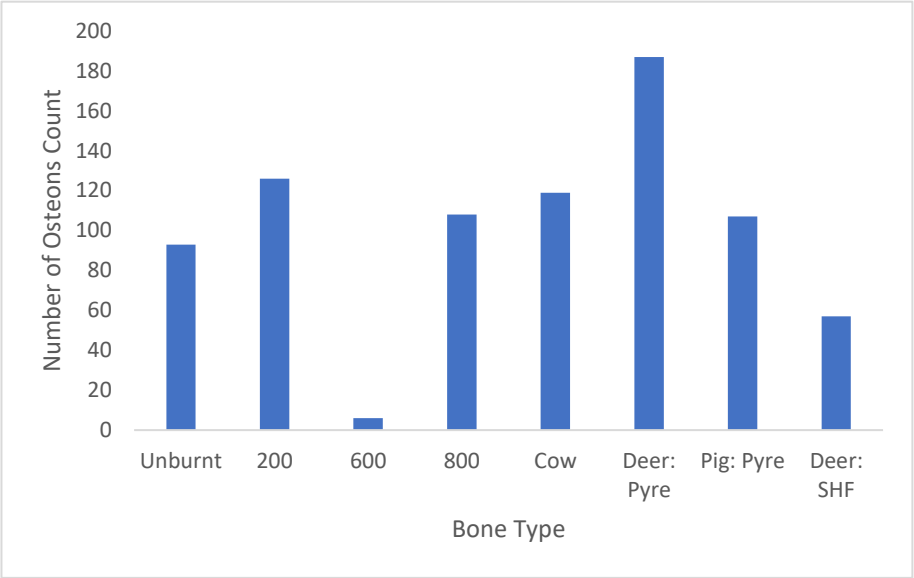

Figure 45: The total number of osteons counted from the samples analysed used for the burns and from varying animal species.

5.3.3.1: Comparison of muffle furnace burn to pyre burning

For this comparison samples had to be selected that displayed similar macroscopic changes to those of the samples burnt within the pyre (colour, facture patterning), these were the 600ºC and the 800ºC samples. This study found that thermally altering remains in a muffle furnace at 600ºC (23.79 ± 10.49µm) had no statistically significant difference to burning the samples in a pyre style burn (21.09 ± 4.72µm), $t_{(111)} = 1.255$, $p = >0.05$. Along with this by conducting a t-test on the 800ºC samples compared to the pyre burn showed also that there was no statistically significant difference between burning in a muffle furnace (20.62 ± 5.34µm) to a pyre style burn (21.09 ± 4.72µm), $t_{(213)} = -.691$, $p = >0.05$. This concurred with Blanks' (2016) study which similarly found no significant difference between the results of pyre burning and heating in a furnace.

5.3.3.2: Comparison of pig bones to deer bones

Following on from the previous test when comparing the muffle furnace samples to the deer pyre meant that again only samples that shared similar changes and conditions could be used, which meant only the 800ºC samples could be compared. This study found that thermally altered pig remains (20.62 ± 5.34µm) had a statistically significant difference to that of the deer samples burnt in the pyre (19.50 ± 5.03µm), $t_{(293)} = 1.792$, $p = <0.05$.

5.3.3.3: Comparison of pig bones to deer bones

Again, the macroscopic changes and the conditions of the burns had to be taken into account when it came to comparing the 800°C muffle furnace samples to the simulated house fire samples. From this study it was found that the muffle furnace samples (20.62 ± 5.34µm) had a statistically significant difference to that of the simulated house fire samples (28.48 ± 6.74µm), $t_{(163)}$ = -8.202, p = <0.001. However, due to the sample size of the simulated house fire results the results will not be as accurate as they should be.

5.3.3.4: Comparison of pig bones to cow bones

As with the previous tests comparing the muffle furnace samples to different environments of burning only one temperature of the samples burnt in the furnace could be used (800°C) against the cow bones thermally altered in the oil drum. From this study and a t-test it was found that the samples burnt in the furnace at 800°C (20.62 ± 5.34µm) had a statistically significant difference to the cow bone samples thermally altered in the oil drum (23.59 ± 6.54µm), $t_{(225)}$ = -3.732, p = <0.001. This helps understand how a deoxygenated fire affects the bone microstructure post burning and how a cow's microstructure changes compared to a pig.

5.3.4: Experimental burns

The samples obtained from the different experimental burns showed a varying number of osteons with the mean osteon diameter for cow at 23.59µm, the deer burnt in the pyre had an mean osteon diameter of 19.50µm, the pig burnt within the pyre as well had an mean osteon diameter of 21.09µm and the deer burnt within the simulated house fire had an mean osteon diameter of 28.48µm (Table 5; Figure 46). These tests were conducted to compare if there were differences between the samples collected of two difference species from a pyre experiment.

Table 6: The total number, minimum, maximum and mean diameter lengths of osteons for each of the species of animals used from previous experiments.

Animal: Burn Type	Total	Min (µm)	Max (µm)	Mean (µm)
Cow: Oil Drum	119	11.35	44.98	23.59168067
Deer: Pyre	187	10.46	44.18	19.5040107
Pig: Pyre	107	12.33	35.32	21.09327103
Deer: SHF	57	17.78	49.32	28.48421053

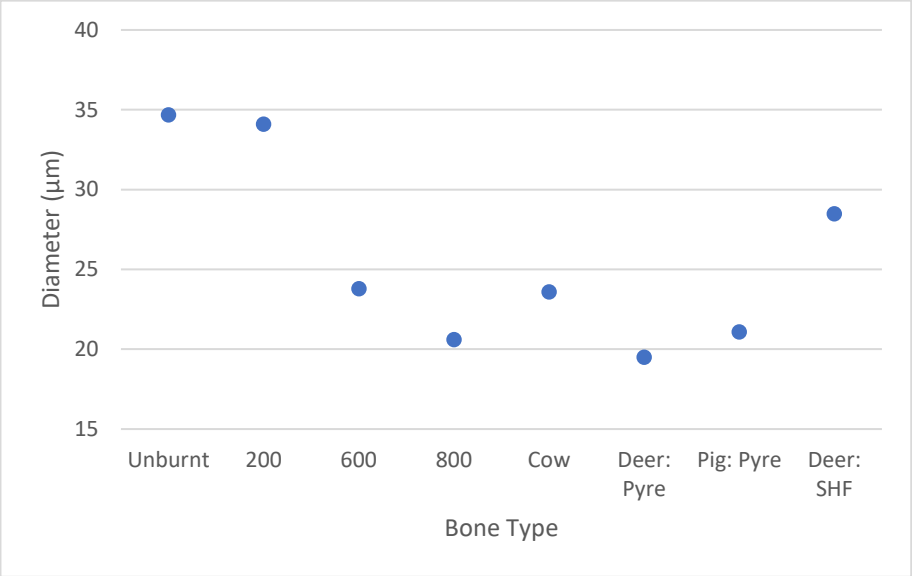

Figure 46: showing the mean diameter of osteons of all species of animal used in this experiment.

5.3.4.1: ANOVA test analysis

There was a statistically significant difference between the burn conditions as determined by a one-way ANOVA ($F(3,466) = 41.882$, $p = <0.001$). A Tukey post hoc test revealed that the pyre used to burn the pig remains (21.09 ± 4.72µm) had a statistically significant difference to the samples burnt within the oil drum (23.59 ± 6.54µm, $p = <0.01$) and the samples burnt in the SHF (28.48 ± 6.74µm, $p = <0.001$). However, there was no statistically significant difference between the pyre used to burn the pig remains and the pyre that was used to burn the deer remains (19.50 ± 5.03µm, $p = >0.05$).

5.3.4.2: T-test comparing deer to pig pyre burns

This test was conducted to compare how differing species of animal in the same burn conditions affect the bone microstructure and if there is a drastic difference. This study found that comparing the pig bones thermally altered in the pyre (21.09 ± 4.72µm) to the deer bones burnt in the pyre (19.50 ± 5.03µm) shows a statistically significant difference between the two animal species, $t_{(292)} = 2.665$, $p = <0.01$.

5.4: Comparison to human thermally altered remains

Comparing to previous studies conducted on human remains there are similarities with the results produced within this research. With the average diameter of the osteons decreasing as temperature increases, with the average diameter at the unburnt state being 193.66µm, at 700°C these bones had an average diameter of 171.67µm, and at 800°C the bones had an average diameter of 143.94µm (Absolonova *et al.*, 2013). Showing that the other structures within the bone does not interfere with the osteons and they will act in the same way when being altered thermally.

5.5: Summary of results

In summary the results that were collected during this experiment conformed to expectations derived from previous studies and generally accepted views regarding the effects of thermal alteration on microstructure and bone structure. Where there are limitations in the data this has been taken into account when considering a possible redesigned experiment, however, the data as a comparison of animal bones and thermal alterations effects on them still apply.

Firstly, the first ANOVA test comparing the burns from the muffle furnace demonstrated what was predicted in the primary analysis that there was a clear significant different between the osteon diameters between the high temperatures and the low temperatures produced by the muffle furnace.

Secondly, the comparison of the bone types (femur vs humerus) gave mixed results, with the unburnt samples not giving the expected results of there being no difference between the bones microstructures, but in fact showing a large significant difference between the two. Unlike the samples burnt at 200^{o}C.

Thirdly, the techniques used for defleshing the samples showed that when analysing the microstructures, the different processes applied have no noticeable effect on the osteons when the bone is defleshed via either chemical maceration or *Dermestes maculatus* or *ater* whole. Compared to when the microstructural bone is exposed to the beetles (after being cut into sections), the microstructure displayed a change in diameter compared to the bones that were defleshed whole.

Fourthly, the burn conditions showed that when comparing osteon diameter change in samples heated ‘artificially’ as opposed to open air burning, there is no difference in the size. This point concurs with the results obtained by Blanks (2016) on a microscopic level and shows that for control in thermal alteration experiments a muffle furnace produces a predictable alteration on the microstructures.

Finally, when comparing the non-human proxies to one another it was clear that the results showed a significant difference between the species of animal used in this experiment. However, when analysing these results, this difference can be put down to the total number of osteons counted in each of the burn conditions. The species of animals used in this study displayed the same diameter size decreasing as the human samples did (Absolonova *et al*., 2013), thus showing that the proxies used within the current experiment provide an accurate comparison for human microstructural changes.

Chapter 6: Discussion

The current research is one of few controlled studies that have been conducted using thermally altered animal remains as proxies for microstructural analysis. It aimed to develop a method of analysis for exploring the survival of the osteons and how they change throughout the burning process. Unsurprisingly, the results demonstrated that the survival of plexiform bone and osteon structures, in terms of both size and number covary with temperature and duration of heat exposure, as well as being affected by different circumstances of burning. These results are relevant with regard to differentiating human bone from animal bone, along with distinguishing the histological traits pertinent to age at death estimations for forensic cases that involve thermal alteration of a victim.

6.1: Pre-burning analysis

As expected, the unburnt remains displayed similar results to previous experimental studies (Martiniaková *et* al., 2006; Horocholyn, 2013). The OHI scores given to the unburnt bones are due to the fact that the bones have not been put through any major alteration, be it freezing or burning, whilst bacterial decomposition was prevented through defleshing. The maceration process that the bone underwent caused little to no damage to the bone microstructure as the bones were macerated whole, which meant that the tergazyme could only break down the soft tissues and not penetrate the interior of the bone and more exclusively the bone microstructure. The number of intact osteons were counted on the optical light microscope, which produced a clear image for counting while displaying the structural gaps the plexiform bones shows in each animal, which is supported by studies previously mentioned (Zedda *et al*. 2008; Felder *et al*. 2017).

The unburnt samples, however, vary in the number of the samples obtained from the original bones (sample 19), meaning that only one chunk could be taken for thin sectioning. This was as a consequence of many methods applied when the embedding began, which caused some of the samples to be damaged beyond use. This is was due to the use of a drying oven to try and allow the resin and hardener to pass through the cortical bone more easily.

6.2: Thermal alterations to the bone

6.2.1: Colour change

Throughout all the burns that were conducted the differential colour change present on the bones from both the muffle furnace and the experimental burns conformed to the sequence of colour progression put forward by Shipman *et al.* (1984). The current study produced results at variance from Shipman *et al.* (1984) in regard to the temperatures at which these colours occur but instead agreed with the more recent work of Mays (2010) and Devlin and Herrmann (2015). This was because Shipman ***et al.'s*** (1984) research only considered the temperatures, and they did not incorporate any other factor such as oxygenation. This is the most likely explanation as to why researchers were unable to reproduce the sample colours within repeat experiments, as was also the case in the current investigation (Table 2, p.29).

Table 3 (p.30) detailed that the samples burnt in the muffle furnace exhibited variations in colour across the bone, with certain bones having a different colour on the epiphysis to the diaphysis. This differential colour change can be attributed to the amount of the periosteum left on the bone. In contrast to the experimental samples, there were some samples from deer pyre which had a drastic colour difference across a single bone, with one end of the diaphysis being 8/0 but the other displaying a colour of 2/0. This differential colour change can be attributed to the shielding of part of the bone that did not fully come in contact with the flame from the burn. The use of the Munsell colour soil chart though has its limitations as the result is dependent upon the establishment of a match to a colour contained within the charts. As this technique is susceptible to variation in lighting that can affect the interpretation of the colour and produce errors in colour choice.

6.2.2: Morphological change

The fractures that occurred on the bones during burning are caused by dehydration and demineralisation of the bone, which caused a rough appearance (Holden *et al*., 1995). The heat induced fracture patterns observed were present on a large amount of the fragments in one form or another. However, there were also breakages present on the bones, and this was most likely a result of the recovery process from the muffle furnace as the bones would be stuck to the metal plates. Along with this, the analysis of the bones, when moving the more fragile samples would cause the bones to break, which compromised the data obtained. The samples collected from the muffle furnace showed longitudinal and transverse fractures, with concentric cracks on the epiphyses of the bone. The samples also displayed exfoliated breakages. The samples collected from the experimental fires exhibited longitudinal, transverse, thumbnail fractures.

It has been seen in previous studies that certain fracture patterns caused by thermal alteration – specifically thumbnail fractures – can arise due to the condition of the bone pre-burning (Asmussen, 2009; Gonçalves *et al*., 2015). The main condition that affects these fractures is if the bone is burnt in a fleshed state or a dry state (DeHaan, 2002; Ubelaker, 2009). These fractures are also visible on some of the samples burnt within the muffle furnace and from the pig pyre burn. However, the reliability of these thumbnail fractures as identifiers are not reliable on their own, but will require further supporting evidence (Gonçalves *et al*., 2015). A previous study

discovered that the heat induced fractures occurred mainly when the bone was exposed to the heat, compared to while the bones are cooling down (Bohnert *et al.*, 1998). These fractures caused by the drying and cooling down of the bone would also result in fractures that would appear postmortem (Symes *et al*., 2015). Nonetheless, rapid fluctuations to the temperature exposed to the bone can cause an increase in fragmentation and fracturing (Bohnert *et al.*, 1998). Therefore, it can be seen that the fractures and cracks along the bones are very normal to be present in the distributions displayed on the bones at these temperatures. Thus, supporting the findings from previous studies (Bohnert *et al*., 1998; Asmussen *et al.*, 2009).

The remains from the simulated house fire demonstrated several stages of thermal decomposition from the preservation of charred soft tissue to fully calcinated bone, producing an inconsistency in the degree of burning. Previous studies support the fact that lower temperature and shorter burns result in the same manner of an inconsistent burnt across the samples (Bohnert *et al*., 1998; Herrmann., 1977).

6.3: Post-burning analysis

6.3.1: Muffle furnace

The samples that were burnt in the muffle furnace showed a clear decrease in the diameter of the osteons as the temperature of the burn increases. From the 75 transverse sections that were produced only 41 of these samples were able to be analysed in sufficient detail, due to the carbonisation of a large majority of the samples at 400^{o}C and 600^{o}C, and the light from the microscope used to photograph the transverse sections could not penetrate through the section. Nonetheless, from the samples obtained the correlation of a decrease in osteon diameter still stands. Despite there being faults with the burns, the second set of bones burnt at 800^{o}C however, suffered from these faults in the muffle furnace, due to sealant around the pipe cracking and preventing the furnace from reaching the desired temperature. During this burn the 30 minutes was prematurely started when the furnace reached 750^{o}C and left to run for the duration of time, with the final temperature being recorded at 790^{o}C. This set of samples is an anomaly in the results but can help depict more accurate time frame on the destruction of the microstructure between 600 and 800 and at what point the changes on the bone occur. Along with this there was another malfunction with the muffle furnace which led to one more set of bones not completing their cycle of burning, with this one getting to approximately 380^{o}C. To counter act this problem the remaining bones were burnt via a pyre, which in turn created another avenue of questions on how an unnatural controlled burn compares to a natural uncontrolled burn.

The samples that were given OHI score numbers show a typical decrease in score as the temperature of the burn increases, which is supported by the work conducted by Cuijpers and Lauwerier (2008). However, as previously mentioned, when using the OHI scores on burnt bone the scoring of 1-4 became more challenging to distinguish due to the variation of score not being as well described in Hedges and Millard's (1995) research compared to the descriptions for the scores 0 and 5. It is suggested that a more quantifiable method of scoring is added to the OHI, as this would reduce interobserver errors present when using the current OHI when analysing the thermal alteration of microstructural aspects of the cortical bone.

The use of the muffle furnace was purely due to the ease of controllability with the temperatures and the duration of the burn, so that when the burns were being conducted an exact temperature and duration could be reached compared to the pyre burn. However, the statistical analysis that was conducted on the samples collected from the pig pyre burn and samples that were burnt at a similar temperature in the muffle furnace, showed that there is no significant difference between the bones burnt in both of the types (Chapter 5.3.3.1). This finding was supported by the work completed by Blanks (2016). This is important for future studies of burning bones whether they are researching questions associated in the archaeological or forensic science field. This addresses the argument often put forward in studies surrounding thermally altered bone, which use the muffle furnace conditions as opposed to the pyre style burn or a simulated fire scene, which is often deemed the more realistic burn conditions. This suggests that neither of the two environments of thermal alteration produce more scientifically robust results that are more applicable to real world situations and scenarios at a microstructural level. Previous research provides the same conclusion on a macroscopic level, with the dimensional changes (Blanks, 2016). Other research studies support this conclusion with the summation that there is no significant influence in the thermally induced dimensional changes of bone in a fleshed or un-fleshed state (Blanks, 2016).

The first test that was conducted was to assess any correlation between the density and diameter of the osteons present. Despite there being a statistical significance between these results, the graphs produced after analysis showed a weak relationship between these medians, which was expected due to osteons varying in their density within cortical bone (Skedros *et al*., 2011) (figures 17-41). Due to the quality of image and human error in counting the osteons some could be missed, however, this would most likely not affect the result obtained as the minimum diameter of the osteons present in the unburnt bone was 11.26μm, with a total of 93 osteons, compared to the minimum diameter of the osteons present in the bones thermally altered at 200^{o}C that was 15.78μm, with a total of 126 osteons. Thus, showing that if there was going to be a correlation between these medians the minimum diameter would need to be smaller within the bones thermally altered at 200^{o}C. Nonetheless, potential of human error when counting the osteons needs to be taken in account when comparing the burn conditions.

When comparing femur to humerus to see if one of the types of long bones shows a more scientifically robust result on the number and diameter of osteons, the results for the bones thermally altered at 200^{o}C showed the expected results of there was no significant difference between the two types of long bone when measuring the diameter of osteons. However, in comparison the bones that were not altered thermally showed a significant difference between the femur and humerus results, this was most likely due to the maceration process leaving only a section of sample 19 available for analysis, instead of the normal two sections for thin sectioning. Along with the previously mentioned error of not burning two of each bone type at 600^{o}C and 800^{o}C, meaning that they could not undergo statistical analysis (Zedda *et al.,* 2008).

6.3.1.1: Maceration comparison

Analysis of the samples that were macerated using the two different methods, showed that when the bones were macerated whole, it led to no significant difference in diameter of the osteons. Thus, meaning that for future scientific research when it comes to macerating bones for microstructural analysis the process used does not affect them, if using a chemical maceration in water or if using the *Dermestes* beetles. However, when the bone has been cut into sections before the maceration process the diameter of the osteons showed a significant difference (Couse and Connor, 2015). This result was however expected due to the cortical bone being exposed from the start of defleshing process, as a consequence the microstructure can be altered as the beetles ate the sections of bone. However, in realistic forensic cases the body is most likely not going to be macerated before the burn so this factor of alteration to the cortical bone would not be present in a forensic case if unmacerated. Nonetheless the bones found in a forensic case, the cortical bone could be visible during the burn, due to them being cut for burning, thus meaning there will be direct heat being put onto the cortical bone altering it in a different way to the bones in this research (Couse and Connor, 2015).

6.3.2: Cross species comparisons

Comparisons of the different animal types to the pig remains that were thermally altered using the muffle furnace, showed they produced varying levels of significance from the statistical test completed. The deer bones that were thermally altered using the pyre showed a significant difference when compared to similar bones from the muffle furnace, but only minor changes comparison to some of the other animals and burn types were found. This is represented in the statistical test between the deer thermally altered within the simulated house fire and the muffle furnace, which produced a much larger significant difference compared to the deer in the pyre burn and the samples from the muffle furnace. There are two factors that could produce this difference between the SHF and the pyre deer burn, the first is that the burn across the deer carcass was not uniform as previously spoken about, the second factor is the number of osteons counted and measured between the two sets of data. Finally, the last animal tested against the pig in the furnace was the cow remains burnt within an oil drum to see how a low oxygenated burn affects structure, again the test conducted showed a significant difference between the pig and the cow, however, this was expected due to the size difference between the two species as a cows cortical bone is much larger compared to the pig (Martiniaková *et al*., 2006).

Comparing the structures of these different animals to the pig samples from this research, showed that they all have very similar microstructural bone despite the size if it was plexiform bone, osteon structures and Haversian canals (figure 17-41), which is supported by previous studies that compared the size of varying mammals microstructural bone (Felder *et al.,* 2017). The minimum osteon diameter of each animal type showed a closeness between the cow, the deer: pyre and the pig: pyre, however the deer thermally altered within the simulated house fire varied from this with the largest minimum diameter of osteon present throughout the samples analysed.

6.3.3: Survival of osteons

From the samples that were used within this study it showed that when thermal alteration is applied to the bones the survivability of the microstructure can be seen through the samples as the temperature increases. However, distinguishing between intact and fragmentary osteons when using the counting method that was applied in this research showed difficulty, due to the light source used on the microscope and the quality of photo, as previously mentioned some of the structures could be missed in the analysis, thus, skewing the result of the survivability. Nonetheless from the measuring of the osteons it shows that the structures can still hold their shape and are visible for analysis post burning. The structure that changed the most over the course of the burns was the plexiform structure around the cortical bone (figure 17-41) this can be caused due to the cortical bone cracking and flaking off due to the charring or calcining that occur when the bone is exposed to fire (Kerley, 1970).

6.4: Comparison of non-human proxies to human thermally altered remains

When compared to human thermally altered remains, there are clear similarities across mammalian species in the changes of the microstructures between unaltered and thermally altered remains. It is relatively easy to identify these structures when unaltered but when the same specimen is experimentally cremated the distinction of the microstructures becomes more difficult (Hummel and Schutkowski, 1993). On the contrary, the current research shows further understanding of how the structures change after thermal alteration. The knowledge and method used can be applied to human remains so the distinction of microstructures can become easier when looking at thermally altered human remains. As the microstructures from the human results showed a decrease as the temperature increases, thus showing that as even though the structures are smaller, they undergo the same destruction and shrinking as the proxies used.

6.5: Redesigned experiment

As for further work a redesigned experiment could work for a deeper comparison of the bones. As in the current research it was designed to give it forensic and archaeological accuracy, which in terms of data produced some limitations. In this redesigned experiment the bones would be macerated the same way as in the current experiment as it did the desired job. However, the bones would be then cut into sections and analysed under the microscope to obtain the diameter of the osteons in set areas of the unburnt bones and then the same samples would be burnt at varying degrees for the same duration of time (30 minutes). This means that the same sample would be analysed against itself showing a direct comparison of osteon diameter in the sample microstructure. In comparison to the experimental burns like pyres, this cannot not be conducted in the same way as if the bones were put in sections they would most likely be destroyed, so these would have to be compared in a similar way to the current research, with the bones being thermally altered then compared to unburnt samples. This would also lead to a greater sample size which in turn would lead to greater analysis and comparisons.

Chapter 7: Conclusion

The nature and extent of physical changes that occur on a microscopic level in bones subject to thermal alteration are well documented. Supported by the current experimental study that has demonstrated that physical changes can also be quantified at a microstructural level. Showing that:

- There was a clear observable difference in the survival of the microstructure when comparing unburned bones to the samples burnt at the varying temperatures.
- There was a quantifiable difference in the osteon count and diameter of these osteons post-burning when comparing all the burn conditions.
- When comparing maceration techniques on bones that had been thermally altered showed there was no statistically significant difference between these. However, when the comparison of a whole bone and one that had been cut into sections was conducted in the *Dermestids* it showed a statistically significant difference. Which in turn can help with the assumption that if remains were cut into sections before burying the microstructure would be more degraded than that of bones buried whole.
- Comparing the bones from the different mammal species exhibited showed there to be a statistically significant difference between any of the statistical analyses conducted during this research.
- From the comparisons of the femora and humeri microstructures showed differences in statistical analysis with the unburnt samples showing a statistically significant difference between the samples. However, the samples burnt at 200^{o}C showed no statistically significant difference between the two bone types.

The changes observed in osteon size and diameter were noted across the different species of animal, it is reasonable to conclude that this is a general occurrence in all mammals and therefore it can be reasonably expected to happen in human bones as well. However, the magnitude of changes in osteon size between the species of animals involved (pig, deer and cow) raised the question of which species constitutes the closest proxy to human bone, in regard to such experiments. From the current experiment it showed that pig remains will display the same destruction patterns in both a lab-based burn inside a muffle furnace and within an experimental pyre.

Assessment of the effects of the duration of heat exposure was subject to limitations imposed by technical problems experienced with the muffle furnace. Along with this further challenges became apparent when applying published methods of osteon counting to thermally altered remains, as it was not always possible to accurately count the number of osteons present in the sections due to the carbonization of the bone and identifying osteons became harder between the temperatures of 400^{o}C and 600^{o}C. However, the other experimental burn samples analysed during the project indicated that osteon size is not widely affected by lower temperatures (<400^{o}C) regardless of the duration of exposure. This means that the methods of identification and bone microstructure analysis did not need to be changed to take the effects of thermal alteration into account at the temperatures below 400^{o}C. The process to deflesh the bones was taken into

consideration as regards to the effects it has on osteon structure and the impact it would have on the outcome of results from the experiments. However, such effects can have little implication in a forensic context due to the likelihood that the remains would be burnt fleshed and then buried or deposited elsewhere. Ultimately, the findings from the current research present some useful points, which caution against the straightforward application of microstructural analysis methods developed from unburnt bone. Instead the necessity is indicated for further investigations to establish the specific extent of likely thermally induced changes to bone microstructure in humans. The current results also possess significance with regard to the distinction between thermally altered human remains and non-human remains at a histological level, provide a further contribution to both forensic investigations and archaeological contexts, with the clear indications of how the bone microstructures differentiate in diameter and also the density of these osteons and the plexiform bone compare to each other when analysed.

When using existing student datasets these can come with their own limitations, with the main one being that these experiments were conducted at least a year before the current research. Moving the boxes could cause more damage to the bones thus in turn leading to potentially more damage to the microstructure. However, this can only be tested with future work being conducted in this area of research. The other obvious limitation to using these datasets is that the temperatures are not controlled in the same manner as the bones that were thermally altered in the muffle furnace, but that is unlikely to have a major factor on the outcome of the results. Along with this the duration of burn had limitations as the samples collected from the previously experiments all had varying levels of exposure time to the fire, which compared to the muffle furnace that had a set duration could have an affect on the results. However, this would need further in-depth research to support. Finally the use of the cow remains from the barrel, had their own limitations, which were that this experiment was completed some years ago, so the exact temperatures and durations of the burn were not able to be obtained, so estimations of temperature had to be made based on the other previous experiments and from the bones thermally altered in the current research.

For further work it is necessary that human remains are used to gain a better understanding of these changes to the microstructure within human remains, in both forensic investigations and archaeological contexts. With the use of human remains as a comparison to non-human remains allows for an identifier of the differences between animal microstructure and human microstructure. Along with this from background research, a new method of estimating age at death solely set to analyse thermally altered remains needs to be created. This is as a consequence of the current methods being applicable, however, the methods can give a range that is too large to assist investigations greatly, due to the larger age groups.

From conducting this experiment, it is also suggested that a larger sample size should be used, compared to the one collected within the current research. With the number of unburnt samples being considerably larger for more comparisons, this would allow for a better comparison between the temperatures applied to the remains. More time is also recommended on the thin sectioning of the samples due to the bones that were altered at 400^{o}C and 600^{o}C having issues when it

came to analysing them, as this became a factor of the carbonation on the bones and the light not being able to be penetrated through. Thus, leading to them not being able to be used in the comparative statistical analysis against other samples.

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Appendices

Appendix 1: Complete Sample Raw Data

A1.1: Unburnt Samples

A1.1.1: Sample 18a

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	24.242	21.97	30.303	46.363
2	12.121	21.226	32.141	33.574
3	17.356	21.97	29.998	27.068
4	13.785	11.262	20.172	30.289
5	15.152	15.17	21.347	30.029
6	24.374	18.007	29.415	29.45
7	22.022	18.939	26.34	19.14
8	25.802	20.844	29.083	12.512
9	21.212	61.364	27.291	30.289
10	25.758	39.314	32.102	32.915
11	16.667	24.076		16.787
12		25.802		
13		21.387		
14		18.939		

A1.1.2: Sample 18b

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	36.369	30.32	33.302
2	25.424	28.283	28.456
3	23.623	35.02	16.212
4	21.381	17.641	27.228
5	31.927	18.433	
6		23.32	
7		15.385	

A1.1.3: Sample 19

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	47.03	91.463	125.553	34.826
2	24.087	68.091	66.149	34.31
3	21.084	94.269	46.154	29.01
4	20.99	53.216	55.067	36.055
5	13.35	50.061	50.061	18.347
6	22.36	123.642	62.205	17.003
7	27.177	76.119	93.119	
8	24.292		72.511	
9	67.925			
10	102.023			

A1.2: 200ºC Samples

A1.2.1: Sample 31a

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	38.29	35.49	37.08	21.81
2	26.12	54.97	37.08	27.37
3	38.58	34.33	35.04	37
4	25.14	56.82	35.29	38.39
5	31.11	50.91	50.17	21.94
6	30.81	28.13	26.38	27.7
7	38.23	33.07		23.16
8	32.14	37.26		28.38
9	34.09	36.94		
10	32			
11	36.65			

A1.2.2: Sample 31b

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	32.89	35.04	35.29	23.8
2	27.56	21.1	44.13	28.77
3	38.68	40.69	45.32	47.96
4	40.97	32	45.14	32.54
5		52.94	29.3	28.77

A1.2.3: Sample 40a

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	45.8	34.8	36.25	36
2	35.83	37.08	29.4	32.54
3	28.38	27.37	33.19	38.07
4	48.13	35.93	26.46	29.4
5	23.93	31.11	35.25	38.98
6	32		21.94	24.96
7	31.42		33.53	25.74
8	29.4		35.89	38.6
9	38.7		31.59	
10	31.42			

A1.2.4: Sample 40b

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	36.4	49.61	45.25	26.61
2	45.32	32.35	24.65	27.23
3	70.11	25.38	38.07	28.46
4	34.38	27.03	41.85	45.67
5	28.75	18.32	28.46	37
6	30.22	32.63	50.71	51.78
7	21.28	21.56	29.61	47.56
8	21.53	34.71	35.93	21.53
9	15.78		30.91	33.19
10	23.71		36.08	28.11
11	28.88			20.06
12	50.59			

A1.3: 600ºC Samples

A1.3.1: Sample 4a

Osteon Number	Sample 1 (µm)
1	42.87
2	23.8
3	26.55
4	16.54
5	13.34
6	19.65

A1.4: 800ºC Samples

A1.4.1: Sample 17a

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	20.51	26.46	17.78	18.65
2	22.73	27.12	19.13	20.22
3	17.43	24.65	17.78	22.22
4	20.92	22.05	19.72	21.81
5	17.78	23.45	16.76	24.47
6	16.76	23.45	22.22	31.03
7	36.4		18.49	
8	27.56			
9	16.21			
10	21.53			
11	22.73			
12	22.73			
13	17.95			
14	17.95			

A1.4.2: Sample 17b

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	18.85	14.37	17.95
2	16.76	25.74	24.68
3	18.65	17.78	21.21
4	24.31	19.25	20.51
5	15.44	16.54	20.33
6	17.3	14.37	20.33
7		14.89	23.93
8		21.81	37.08
9		25.41	
10		29.01	

A1.4.3: Sample 30a

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	18.32	22.19	30.44
2	18.77	17.43	16.54
3	21.84	18.65	13.78
4	32.35	16.21	18.2
5	24.31	20.33	18.85
6	33.84	14.37	10.6
7	19.25	36.08	13.94
8	22.22	22.63	14.37
9	22.19	20.22	
10	14.84	17.12	
11		14.84	
12		14.37	
13		11.16	

A1.4.4: Sample 30b

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	18.2	11.02	25.6
2	13.78	15.78	18.96
3	16.76	19.25	19.28
4	23.71	21.94	17.78
5	17.65	24.22	15.71
6	20.95	20.33	18.67
7	15.25	19.72	13.43
8	19.25		18.46
9	29.84		25.48
10			25.6
11			28.59

A1.5: Cow Oil Drum Samples

A1.5.1: Sample 1

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	33.41	23.93	12.02	27.37
2	18.67	28.46	22.25	31.5
3	17.91	26.2	17.41	37.49
4	26.2	24.68	14.93	29.68
5	31.93	20.92	17.03	16.44
6	22.9	16.76	15.95	17.52
7	27.49	24.22	12.94	25.65
8		28.11	24.23	19.25
9		22.49	20.51	35.06
10		21.94	21.91	
11		32.14	14.35	
12		18.32	11.35	
13		28.85	20.15	
14		25.38	20.12	
15		19.29		
16		27.56		
17		42.73		

A1.5.2: Sample 2

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	29.4	20.12	17.17
2	26.29	22.25	29.58
3	22.69	21.11	24.68
4	17.94	16.2	44.98
5	23.88	14.49	21.1
6	26.44	19.32	19.13
7	16.2	17.18	22.22
8	35.26	33.08	25.14
9	32.85	21.91	28.13
10	34.54	19.93	28.77
11		15.95	19.87
12		18.35	26.15
13		14.79	
14		17.72	
15		25.05	
16		16.92	
17		18.93	
18		22.97	
19		26.7	

A1.5.3: Sample 3

Osteon Number	Sample 1 (µm)	Sample 2 (µm)
1	32.89	19.87
2	31.98	22.66
3	28.67	37
4	35.83	22.93
5	16.76	25.29
6	17.3	24.22
7	16.54	23.55
8	27.56	19.29
9	28.38	30.01
10	12.39	23.03

A1.5.4: Sample 4

Osteon Number	Sample 1 (µm)	Sample 2 (µm)
1	24.68	25.14
2	17.43	20.4
3	22.73	17.95
4	28.11	27.94
5	34.73	
6	20.95	
7	20.22	

A1.6: Deer Pyre Samples

A1.6.1: Sample 1

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	14.89	44.18	21.94
2	30.89	29.09	25.29
3	33.53	18.81	19.76
4	24.65	33.19	18.32
5	20.06	35.29	14.99
6	15.59	28.13	14.79
7	21.28	31.81	19.87
8	18.85	19.17	
9	16.02	27.81	
10	20.06		
11	16.76		

A1.6.2: Sample 2

Osteon Number	Sample 1 (µm)	Sample 2 (µm)
1	20.33	22.73
2	21.21	14.37
3	23.16	17.78
4	17.78	16.02
5	19.13	28.75
6	15.59	44.13
7	17.78	16.54
8	21.94	21.53
9	19.49	16.54
10	23.39	19.49
11	22.63	27.94
12	20.95	18.49
13	18.2	17.95

A1.6.3: Sample 3

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	16.54	17.12	14.43
2	17.43	15.59	23.26
3	23.55	13.33	17.43
4	28.75	17.52	16.76
5	23.8	21.94	13.94
6	19.25	17.3	19.49
7	22.22	18.81	15.59
8	17.43		24.22
9	20.06		18.81
10	18.49		12.39
11	17.95		18.32
12	20.51		20.51
13	22.19		16.76
14	23.55		16.21
15	20.66		19.87
16	16.76		21.53
17			16.44
18			20.99
19			14.84
20			13.61
21			21.53
22			17.43
23			17.78

A1.6.4: Sample 4

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	22.73	16.07	21.53
2	16.54	17.17	23.55
3	19.49	14.05	18.81
4	27.12	23.03	17.43
5	18.53	19.13	18.49
6	14.43	19.49	24.47
7	16.76	15.44	17.78
8	23.16	13.33	27.03
9	18.53	15.25	20.95
10	20.33	16.44	18.49
11	18.77	13.78	20.51
12	15.05	20.4	16.02
13	18.49	11.02	20.33
14	20.06	10.46	16.76
15	17.12	13.1	17.43
16	18.32	14.84	11.36
17	18.53	22.66	19.29
18	15.59	13.1	13.78
19	13.27	17.43	18.85
20	15.25	19.72	13.1
21	20.92	13.33	16.02
22	17.95	14.99	15.59
23	24.96	16.07	17.26
24		16.07	13.78
25		18.32	16.44
26		21.28	26
27		18.81	24.77
28		19.13	27.03
29		20.22	
30		23.71	
31		20.92	
32		16.76	
33		21.56	
34		17.26	
35		18.77	
36		16.54	
37		21.1	

A1.7: Pig Pyre Samples

A1.7.1: Sample 1

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	23.8	33.48	17.95
2	17.12	24.4	24.65
3	17.95	26.15	14.89
4	12.33	29.4	19.49
5	14.89	30.01	16.02
6	15.69	25.41	25.74
7	18.32	23.55	20.06
8	17.65	25.65	14.84
9	22.22	26.15	27.94
10	24.65		24.65
11	24.4		30.44
12	18.81		13.94
13	15.44		19.49
14	26.92		22.22
15			23.26
16			26.38

A1.7.2: Sample 2

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)
1	19.29	24.8	15.69
2	21.56	24.28	17.78
3	22.49	28.88	20.51
4	21.53	19.76	14.43
5	25.65	18.49	14.37
6	19.76	12.69	14.43
7	20.06	12.69	14.05
8	23.55	27.7	15.05
9	24.93	20.92	13.94
10	21.21	28.77	22.22
11		23.55	24.31
12		20.22	

A1.7.3: Sample 3

Osteon Number	Sample 1 (µm)	Sample 2 (µm)
1	22.32	20.66
2	22.22	21.53
3	13.94	19.13
4	29.09	21.56
5	22.49	20.4
6	24.22	22.05
7	23.26	18.77
8	18.32	19.13
9	22.63	15.78
10	20.06	20.06
11	20.51	35.32

A1.7.4: Sample 4

Osteon Number	Sample 1 (µm)	Sample 2 (µm)
1	15.05	18.81
2	19.17	19.29
3	23.26	14.99
4		21.94
5		17.17
6		19.49
7		17.17
8		20.51
9		27.06
10		23.71

A1.8: SHF Samples

A1.8.1: Sample 2

Osteon Number	Sample 1 (µm)	Sample 2 (µm)	Sample 3 (µm)	Sample 4 (µm)
1	25.38	18.81	17.78	43.12
2	20.06	26.46	27.03	41.92
3	17.95	30.91	32.89	36.4
4	27.89	33.3	31.81	49.32
5	24.22	31.57	30.49	28.88
6	22.22	23.55	30.09	26.55
7	23.93	24.65	21.94	31.98
8	19.25	28.67	30.09	35.83
9	21.84	35.55	25.65	24.28
10	30.49	24.65	20.4	33.19
11	23.26	26.38	34.8	22.93
12	24.56	28.85	32.35	35.93
13		18.32	28.85	33.48
14		31.5	20.95	31.38
15		26.92	28.38	
16			43.77	

Appendix 2: SPSS Test Results

*See B.4 in B Folder for the data from the statistical tests conducting within this experiment.

Appendix 3: Previous Experimental Methods

* See B.3 in B folder for experimental methods from previous studies and spoken about in the thesis.

Appendix 4: Digital Gallery

*See B.1 in B folder for additional digital data of whole bones and see B.2 in B folder for additional digital data of thin section analysis.

Appendix 5: Miscellaneous

A5.1: Ethics Checklist

Bournemouth University

Initial Research Ethics

Note: *All researchers* must complete this brief checklist to identify any ethical issues associated with their research. Before completing, please refer to the BU *Research Ethics Code of Practice* which can be found www.bournemouth.ac.uk/researchethics. School Research Ethics Representatives (or Supervisors in the case of students) can advise on appropriate professional judgement in this review. A list of Representatives can be found at the aforementioned webpage. **Sections 1-5 must be completed by the researcher and Section 6 by School Ethics Representative/ Supervisor prior to the commencement of any research.**

1 RESEARCHER DETAILS						
Name	**Callum Arrowsmith**					
Email	**carrowsmith@bournemouth.ac.uk**					
Status	☐ **Undergraduate**		☒ **Postgraduate**		☐ **Staff**	
School	☐ **BS**	☐ **AS**	☐ **DEC**	☐ **HSC**	☐ **MS**	☐ **ST**
Degree Framework & Programme	**Masters by Research, Science and Technology**					
2 PROJECT DETAILS						
Project Title	**An experimental investigation, of the survival of the microstructures in long bones after a varying temperature and time of burning, with a link to age at death.**					
Project Summary *Sufficient detail is needed; include methodology, sample, outcomes etc*	**Using pig remains and other species of animals, to see how temperature changes microstructures of long bones on a microscopic level.**					
Proposed Start & End Dates	**17th Sept 2018 – 11th Sept 2019**					
Project Supervisor	**Martin Smith**					
Framework Project Co-ordinator						

3 ETHICS REVIEW CHECKLIST – PART A			
I	Is approval from an external Research Ethics Committee (e.g. Local Research Ethics Committee (REC), NHS REC) required/sought?	☐ **Yes**	☒ **No**
II	Is the research solely literature-based?	☐ **Yes**	☒ **No**
III	Does the research involve the use of any dangerous substances, including radioactive materials?	☐ **Yes**	☒ **No**
IV	Does the research involve the use of any potentially dangerous equipment?	☐ **Yes**	☐ **No**
V	Could conflicts of interest arise between the source of funding and the potential outcomes of the research? (see section 8 of BU Research Ethics Code of Practice).	☐ **Yes**	☒ **No**
VI	Is it likely that the research will put any of the following at risk: Living creatures?	☐ **Yes**	☒ **No**
	Stakeholders?	☐ **Yes**	☒ **No**
	Researchers?	☐ **Yes**	☒ **No**

Research Ethics Checklist (Graduate School & CRE) December 2010

	Participants? The environment? The economy?	☐ Yes ☐ Yes	☒ No ☒ No
VII	Does the research involve experimentation on any of the following: Animals? Animal tissues? Human tissues (including blood, fluid, skin, cell lines)? Genetically modified organisms?	☐ Yes ☒ Yes ☐ Yes ☐ Yes	☐ No ☐ No ☒ No ☒ No
V111	Will the research involve prolonged or repetitive testing, or the collection of audio, photographic or video materials?	☒ Yes	☐ No
IX	Could the research induce psychological stress or anxiety, cause harm or have negative consequences for the participants or researcher (beyond the risks encountered in normal life)?	☐ Yes	☒ No
X	Will the study involve discussion of sensitive topics (e.g. sexual activity, drug use, criminal activity)?	☐ Yes	☒ No
XI	Will financial inducements be offered (other than reasonable expenses/ compensation for time)?	☐ Yes	☒ No
XII	Will it be necessary for the participants to take part in the study without their knowledge / consent at the time?	☐ Yes	☒ No
XII	Are there problems with the participant's right to remain anonymous?	☐ Yes	☒ No
XIV	Does the research *specifically* involve participants who may be vulnerable?	☐ Yes	☒ No
XV	Might the research involve participants who may lack the capacity to decide or to give informed consent to their involvement?	☐ Yes	☒ No

4 ETHICS REVIEW CHECKLIST – PART B

Please give a summary of the ethical issues and any action that will be taken to address these.

Ethical Issue: Animal remains gathered from a butcher and previous experiments.	**Action: No ethical issues or actions required.**

5 RESEARCHER STATEMENT

I believe the information I have given is correct. I have read and understood the BU Research Ethics Code of Practice, discussed relevant insurance issues, performed a health & safety evaluation/ risk assessment and discussed any issues/ concerns with a School Ethics Representative/ Supervisor. I understand that if any substantial changes are made to the research (including methodology, sample etc), then I must notify my School Research Ethics Representative/ Supervisor and may need to submit a revised Initial Research Ethics Checklist. By submitting this form electronically I am confirming the information is accurate to my best knowledge.

Signed		**Date**	**21/01/2019**

6 AFFIRMATION BY SCHOOL RESEARCH ETHICS REPRESENTATIVE/ SUPERVISOR

Satisfied with the accuracy of the research project ethical statement, I believe that the appropriate action is:

The research project proceeds in its present form	☐ Yes	☐ No
The research project proposal needs further assessment under the School Ethics procedure*	☐ Yes	☐ No
The research project needs to be returned to the applicant for modification prior to further action*	☐ Yes	☐ No

** The School is reminded that it is their responsibility to ensure that no project proceeds without appropriate assessment of ethical issues. In extreme cases, this can require processing by the School or University's Research Ethics Committee or by relevant external bodies.*

Reviewer Signature		**Date**	
Additional Comments			

A5.2: Risk Assessments

About You & Your Assessment

Name	Callum Arrowsmith
Email	carrowsmith@bournemouth.ac.uk
Your Faculty/Professional Service	Faculty of Science and Technology
Is Your Risk Assessment in relation to Travel or Fieldwork?	No
Date of Assessment	30/07/2019
Date of the Activity/Event/Travel that you are Assessing	01/08/2019

What, Who & Where

Describe the activity/area/process to be assessed	Using epothin resin and hardener, to embed samples to use the Leica SP1600 saw microtome
Locations for which the assessment is applicable	Dorset House and Christchurch
Persons who may be harmed	Staff, Student

Hazard & Risk

Hazard	Inhalation
Severity of the hazard	Medium
How Likely the hazard could cause harm	Medium
Risk Rating	Medium

Control Measure(s) for Inhalation :

Work in fume cupboard

With your control measure(s) in place - if the hazard were to cause harm, how severe would it be? Medium

With your control measure(s) in place - how likely is it that the hazard could cause harm? Low

The residual risk rating is calculated as: Low

Hazard	Severing or Cutting
Severity of the hazard	Medium

How Likely the hazard could cause harm	Medium
Risk Rating	Medium
Control Measure(s) for Severing or Cutting: do not put hands inside when machine is on Keep safety cover on microtome while in use	
With your control measure(s) in place - if the hazard were to cause harm, how severe would it be? Medium	
With your control measure(s) in place - how likely is it that the hazard could cause harm? Low	
The residual risk rating is calculated as: Low	
Hazard	Burning/Burns
Severity of the hazard	Medium
How Likely the hazard could cause harm	Medium
Risk Rating	Medium
Control Measure(s) for Burning/Burns: Controlled movements Wear correct PPE Work in fume cupboard	
With your control measure(s) in place - if the hazard were to cause harm, how severe would it be? Medium	
With your control measure(s) in place - how likely is it that the hazard could cause harm? Low	
The residual risk rating is calculated as: Low	

Review & Approval

Any notes or further information you wish to add about the assessment	
Names of persons who have contributed	Callum Arrowsmith
Approver Name	Auto Approved by Callum Arrowsmith
Approver Job Title	[Not Applicable]

Approver Email	Auto Approved by carrowsmith@bournemouth.ac.uk
Review Date	

Uploaded documents

No document uploaded

Bournemouth University Faculty of Science & Technology Risk Assessment Approved

Author: Callum Arrowsmith

To submit a risk assessment, click on the 'new risk assessment tab' and complete steps 1-4.

It is important that you choose your equipment or bench space booking from the 'equipment being used' in tab 2 and that you **upload a risk assessment and any relevant COSHH (control of substances hazardous to health) forms to tab 3 'upload documents'**. Risk assessment forms can be downloaded from https://bmth.siso.co/fst/import/data/temp/scitechriskassessmen_1562748679.xls

You will be unable to collect equipment or access the laboratories without completing tab 4. Please choose **YOUR** supervisor from the 'approval lecturer box', click 'save' and then choose 'send for approval'. You only need to complete a new risk assessment if your activity changes. Repeated bookings for the same activity are covered by your original risk assessment can be uploaded for subsequent bookings.

Low risk activities such as using a camera and taking measurements in the anthropology labs etc do not need supervisor approval and do not need to be formally assessed but you are still required to describe the activity in section 1 and send the risk assessment form to Dean Burnard in tab 4.

1. Describe the Activity

Risk Assessment Title: Creating thin sections

Date: 01/08/2019

Supervisors Name: Martin Smith

Description: Using epothin resin to embed samples and using the Leica SP1600 saw microtome to create thin sections of bone samples for microscopic analysis.

Risk assessment online at: https://risk.bournemouth.ac.uk/assessment/print/120928d9-2e1e-496c-bf0f-5cf15422525b

2. People at Risk

Primary Risk Assessment holder:

3. Equipment being used

No	Name	Stored	Collection	Return	Approved
1	DG41 Microscopy Lab Station 1 (Stereo/HP)		02/08/2019 09:00	30/08/2019 15:59	✔

No	Name	Stored	Collection	Return	Approved
2	Leica SP1600 Saw Microtome (Bournemouth University)	Already stored in Dorset House Lab			

4. Locations(s)

No Locations(s) Added

5. Emergency Procedures

Sector	Name	Contact Details
First Aided		
Nearest Hospital		
Local Police Station		

6. List potential Risks / Activities

No Risk(s) Added

7. Attached Documents

coshhassessmentforme_1564436734.docx
Epo Thin Hardener COSHH Form

coshhassessmentforme_1564436757.docx
Epo Thin Resin COSHH Form

epothinhardenerdatas_1564436775.pdf
Epo Thin Hardener Datasheet

epothinresindatashee_1564436789.pdf
Epo Thin Resin Datasheet

8. Approval

Approved: 30/07/2019 09:50

Written By: Martin Smith - When: 30/07/2019 08:14
This appears to have the relevant COSHH forms uploaded but doesn't actually include a risk assessment -needs to have the risk assessment form filled in as well as the COSSH forms supplied.

Bournemouth University Faculty of Science & Technology Risk Assessment Approved

Author: Callum Arrowsmith

To submit a risk assessment, click on the 'new risk assessment tab' and complete steps 1-4.

It is important that you choose your equipment or bench space booking from the 'equipment being used' in tab 2 and that you **upload a risk assessment and any relevant COSHH (control of substances hazardous to health) forms to tab 3 'upload documents'**. Risk assessment forms can be downloaded from https://bmth.siso.co/fst/import/data/temp/scitechriskassessmen_1562748679.xls

You will be unable to collect equipment or access the laboratories without completing tab 4. Please choose **YOUR** supervisor from the 'approval lecturer box', click 'save' and then choose 'send for approval'. You only need to complete a new risk assessment if your activity changes. Repeated bookings for the same activity are covered by your original risk assessment can be uploaded for subsequent bookings.

Low risk activities such as using a camera and taking measurements in the anthropology labs etc do not need supervisor approval and do not need to be formally assessed but you are still required to describe the activity in section 1 and send the risk assessment form to Dean Burnard in tab 4.

1. Describe the Activity

Risk Assessment Title: Using Muffle Furnace

Supervisors Name: Martin Smith

Description: Use of the muffle furnace to heat pig bones to look at the micro-structures of the long bones.

2. People at Risk

Primary Risk Assessment holder: Callum Arrowsmith

Contact Information: 07889898465

Name	Group	Role	Contact Number
	Staff		
	Students		

3. Equipment being used

No	Name	Stored	Collection	Return	Approved
1	Muffle furnace		08/01/2019 15:30	22/01/2019 15:29	✓
2	Muffle furnace		11/03/2019 09:00	20/03/2019 16:59	✓
3	Muffle furnace		01/04/2019 09:00	04/04/2019 16:59	✓
4	Muffle furnace		11/04/2019 09:00	23/04/2019 16:59	✓
5	Muffle furnace		09/05/2019 15:30	14/05/2019 16:59	✓
6	Muffle furnace		28/05/2019 09:00	28/05/2019 16:59	✓
7	Muffle Furnace (Dorset House)	Already in Dorset House			
8	Muffle Furnace ()				

4. Locations(s)

Location	Date at Location
Dorset House/Fern Barrow Road, Poole/ BH12 5BB	23/10/2018

5. Emergency Procedures

Sector	Name	Contact Details
First Aided	Damian Evans	
Nearest Hospital	The Royal Bournemouth Hospital	01202 303626
Local Police Station	Dorset Police	01202 222222

6. List potential Risks / Activities

Location	Hazard	Groups at risk?	Potential Outcome and Likelihood of Incident	Control Measure	Risk Level
	use of laboratory use of any science laboratory	Staff Students	**Potential Outcome: Minimal Likelihood of Incident: Unlikely Moderate Risk Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and limited. Risks reduction measures should be implemented within a defined period. Where the moderate risk is associated with extremely harmful consequences, further assessment may be necessary to establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.**	Correct PPE to be worn at all times	
Dorset House/Fern Barrow Road, Poole/ BH12 5BB	Working with muffled furnace	Staff Students	**Potential Outcome: Serious Likelihood of Incident: Unlikely Moderate Risk Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and limited. Risks reduction measures should be implemented within a defined period. Where the moderate risk is associated with extremely harmful consequences, further assessment may be necessary to establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.**	Make sure that the furnace is cold before leaving alone. Tell people that the furnace will be in use. Have hot plates in front for items to rest on while cooling.	**Tolerable Risk No additional controls are required. Consideration may be given to a more cost-effective solution or improvement that imposes no additional cost burden. Monitoring is required to ensure that the controls are maintained**
Dorset House/Fern Barrow Road, Poole/ BH12 5BB	Burning of hands	Staff Students	**Potential Outcome: Minor Likelihood of Incident: Unlikely Moderate Risk Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and limited. Risks reduction measures should be implemented within a defined period. Where the moderate risk is associated with extremely harmful consequences, further assessment may be necessary to establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.**	Wear the right gloves to work with the samples. Put signs out to say the samples will be hot. Handle with the correct tools.	**Tolerable Risk No additional controls are required. Consideration may be given to a more cost-effective solution or improvement that imposes no additional cost burden. Monitoring is required to ensure that the controls are maintained**

7. Attached Documents
No Document(s) Added

8. Approval
Approved: 24/10/2018 09:35

Bournemouth University

Bournemouth University Faculty of Science & Technology Risk Assessment Approved

Author: Callum Arrowsmith

To submit a risk assessment, click on the 'new risk assessment tab' and complete steps 1-4.

It is important that you choose your equipment or bench space booking from the 'equipment being used' in tab 2 and that you **upload a risk assessment and any relevant COSHH (control of substances hazardous to health) forms to tab 3 'upload documents'**. Risk assessment forms can be downloaded from https://bmth.siso.co/fst/import/data/temp/scitechriskassessmen_1562748679.xls

You will be unable to collect equipment or access the laboratories without completing tab 4. Please choose **YOUR** supervisor from the 'approval lecturer box', click 'save' and then choose 'send for approval'. You only need to complete a new risk assessment if your activity changes. Repeated bookings for the same activity are covered by your original risk assessment can be uploaded for subsequent bookings.

Low risk activities such as using a camera and taking measurements in the anthropology labs etc do not need supervisor approval and do not need to be formally assessed but you are still required to describe the activity in section 1 and send the risk assessment form to Dean Burnard in tab 4.

1. Describe the Activity

Risk Assessment Title: Maceration of Bones using slow cooker

Supervisors Name: Martin Smith

Description: Using Tergazyme (enzyme) to macerate pig remains using a slow cooker with distilled water in. This will be contained in a fume cupboard in C139.

2. People at Risk

Primary Risk Assessment holder: Callum Arrowsmith

Contact Information: carrowsmith@bournemouth.ac.uk

3. Equipment being used

No	Name	Stored	Collection	Return	Approved
1	Slow cooker (Owner by you)				
2	Slow cooker (Damian Evans)	In C139			

4. Locations(s)

Location	Date at Location
Christchurch House C139, Fern Barrow, Poole, BH12 5BB	12/11/2018

5. Emergency Procedures

Sector	Name	Contact Details
First Aided		
Nearest Hospital	Royal Bournemouth Hospital	01202 303626
Local Police Station	Dorset Police	01202 222222

6. List potential Risks / Activities

Location	Hazard	Groups at risk?	Potential Outcome and Likelihood of Incident	Control Measure	Risk Level
	Chemicals / fuels / high-energy Handling Chemicals/Fuels/High-Energy Substances/Processes Generating Hazardous Materials, Dusts or Fumes.	Staff Students	**Potential Outcome: Minimal Likelihood of Incident: Unlikely Moderate Risk Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and limited. Risks reduction measures should be implemented within a defined period. Where the moderate risk is associated with extremely harmful consequences, further assessment may be necessary to establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.**	Wear correct personal protective equipment to prevent irritation.	**Tolerable Risk No additional controls are required. Consideration may be given to a more cost-effective solution or improvement that imposes no additional cost burden. Monitoring is required to ensure that the controls are maintained**
	Hot or cold materials or liquids Working near or with hot or cold surfaces, materials or liquids.	Staff Students	**Potential Outcome: Minor Likelihood of Incident: Unlikely Moderate Risk Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and limited. Risks reduction measures should be implemented within a defined period. Where the moderate risk is associated with extremely harmful consequences, further assessment may be necessary to establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.**	To put signs saying that container will be hot and that the contents is hot.	**Tolerable Risk No additional controls are required. Consideration may be given to a more cost-effective solution or improvement that imposes no additional cost burden. Monitoring is required to ensure that the controls are maintained**

7. Attached Documents

coshhassessmentform._1541598535.docx
COSHH form for the tergazyme

8. Approval

Approved: 21/11/2018 09:09

Bournemouth University

Bournemouth University Faculty of Science & Technology Risk Assessment Approved

Author: Callum Arrowsmith

To submit a risk assessment, click on the 'new risk assessment tab' and complete steps 1-4.

It is important that you choose your equipment or bench space booking from the 'equipment being used' in tab 2 and that you **upload a risk assessment and any relevant COSHH (control of substances hazardous to health) forms to tab 3 'upload documents'**. Risk assessment forms can be downloaded from https://bmth.siso.co/fst/import/data/temp/scitechriskassessmen_1562748679.xls

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Low risk activities such as using a camera and taking measurements in the anthropology labs etc do not need supervisor approval and do not need to be formally assessed but you are still required to describe the activity in section 1 and send the risk assessment form to Dean Burnard in tab 4.

1. Describe the Activity

Risk Assessment Title: Using hacksaw to cut off end of bone

Supervisors Name: Martin Smith

Description: Using a Hacksaw to remove the two epiphysis to then use microscopic analysis.

2. People at Risk

Primary Risk Assessment holder: Callum Arrowsmith

Contact Information: carrowsmith@bournemouth.ac.uk

3. Equipment being used

No Equipment Added

4. Locations(s)

Location	Date at Location
Christchurch House C139, Fern Barrow, Poole, BH12 5BB	

5. Emergency Procedures

Sector	Name	Contact Details
First Aided		
Nearest Hospital	Royal Bournemouth Hospital	01202 303626
Local Police Station	Dorset Police Station	01202 222222

6. List potential Risks / Activities

Location	Hazard	Groups at risk?	Potential Outcome and Likelihood of Incident	Control Measure	Risk Level
Christchurch House C139, Fern Barrow, Poole, BH12 5BB	Using a vice and hacksaw to saw off the two epiphysis.		**Potential Outcome: Minor Likelihood of Incident: Unlikely Moderate Risk Efforts should be made to reduce the risk, but the costs of prevention should be carefully measured and limited. Risks reduction measures should be implemented within a defined period. Where the moderate risk is associated with extremely harmful consequences, further assessment may be necessary to establish more precisely the likelihood of harm as a basis for determining the need for improved control measures.**	Wear appropriate protection equipment with control over the saw.	**Tolerable Risk No additional controls are required. Consideration may be given to a more cost-effective solution or improvement that imposes no additional cost burden. Monitoring is required to ensure that the controls are maintained**

7. Attached Documents

No Document(s) Added

8. Approval

Approved: 04/12/2018 15:11

A5.3: COSHH Forms

Bournemouth University *COSHH Assessment Form*

1. Assessor:	2. Assessment Date:	3. Assessment Review:		
C. Arrowsmith	24/10/2018	Next Review Date:	Reviewed on: Date:	Reviewed By:

4. Summary of process or method (or make specific reference to written protocol to be used) :

Macerating of flesh off pig long bones using the enzyme Tergazyme in 55ºC simmering distilled water.

5. Key Activity/Task (in relation to exposure potential e.g. mixing, filling, spraying, etc.):	6. People who could come to harm (number & roles e.g. students)
Macerating flesh	Students and staff (2-8)
7. Duration of Exposure (minutes, hours and how often):	**8. Location and Conditions of Use** (e.g. lab, room, temp etc.)
Several days, at any one time.	Christchurch House Labs

	9. Hazardous ingredients: (copy form/add more rows as req'd)	10. Quantities Used	11. Workplace Exposure Limit (WEL)	12. Hazard and Precaution statements	13. Actual Potential Route of Exposure (E.g. by inhalation)	14.Datasheet Attached? Y/N
A	Sodium tripolyphosphate	1.8Kg	N/A	**Hazard statements**: H319 Causes serious eye irritation. **Precautionary statements**: P264 Wash skin thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing. P501 Dispose of contents and container as instructed in Section 13.	Inhalation, contact with skin and eyes, swallowing	
B					•	
C					•	

15. Control Measures

1) Engineering controls – Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use or handling.

2) Personal Protective Equipment – Lab coat, safety goggles, nitrile gloves (or latex if allergic to nitrile) must be worn at all time.

3) Storage – Store in a cool, well-ventilated area.

Now mark in the letters from the list of 'Hazardous Ingredients' above to indicate potential danger:

16. Indication of Danger				17. Route of Exposure		18. Chemical State		19. Flammability		20. Volatility		21. Dust rating	
Very Toxic		Irritant	A	Inhalation	All	Solid	A	Flammable		Low		Low	
Toxic		Sensitiser		Skin Contact	All	liquid		Highly flammable		Medium		Medium	
Corrosive		Carcinogen		Eye Contact	All	Gas/vapour		Extremely flammable		High		High	
Harmful		Mutagenic		Swallowing	All			Oxidising					
Biological Agent		Toxic to reproduction		Injection				Explosive					

22. First Aid Procedures (as advised from Material Safety Data Sheet)

If inhaled	If skin contact	If eye contact	If swallowed	If injected
Move to fresh air If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation Immediate medical attention is required. Maintain an unobstructed airway. Loosen clothing as necessary and position individual in a comfortable position.	Wash off immediately with plenty of water and soap for at least 15 minutes. Obtain medical attention if irritation persists.	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.	Do not induce vomiting. Call a physician or Poison Control Centre immediately. Rinse mouth thoroughly.	Obtain medical attention immediately.

23. Spillage Procedures: →	Clean up wearing gloves and with paper towel.
24. Disposal Arrangements	

Collection	Swill down sink	Evaporation	In normal waste	Other

25. Are the risks adequately controlled? (Write 'Yes' or 'No'):	Yes

If you decide that the controls in Section 15. are sufficient, skip to section 27. If you decide that the risks are NOT adequately controlled (or you're not sure), then you will need to give special instructions to control the risk.

26. Special Instructions to control the risk:

27. Ensure those affected are informed of the Risks & Controls - Confirm how this will be done e.g. by issuing written instructions:

Summary information and verbal instruction will be given to those performing the procedure

Bournemouth University *COSHH Assessment Form*

1. Assessor:	2. Assessment Date:	3. Assessment Review:		
C. Arrowsmith	29/07/2019	Next Review Date:	Reviewed on: Date:	Reviewed By:

4. Summary of process or method (or make specific reference to written protocol to be used) :	6. People who could come to harm (number & roles e.g. students)
Using Epo Thin Resin for embedding samples.	Staff and students (2-3)
5. Key Activity/Task (in relation to exposure potential e.g. mixing, filling, spraying, etc.):	
Mixing and pouring	
7. Duration of Exposure (minutes, hours and how often):	**8. Location and Conditions of Use** (e.g. lab, room, temp etc.)
4 hours per day	C221/C139

	9. Hazardous ingredients: (copy form/add more rows as req'd)	10. Quantities Used	11. Workplace Exposure Limit (WEL)	12. Hazard and Precaution statements	13. Actual Potential Route of Exposure (E.g. by inhalation)	14.Datasheet Attached? Y/N
A	Bisphenol-A-(epichlorhydrin) epoxy resin.	80.0%		R36/38, R43, R51/53, H411, H315, H319, H317 – Wear correct PPE while handling.	Spills and inhalation	Y
B	Butyl glycidyl ether	10.0%		R20/22-40-68, R37, R43, R10-52/53, H226, H341, H351, H302, H332, H317, H335, H412 – Wear correct PPE while handling.	Spills and inhalation	Y
C	propylidynetrimethyl trimethacrylate	5.0%		R22, R36, R43, R51/53, H411, H302, H319, H317 – Wear correct PPE while handling.	Spills and inhalation	Y
D	1,3-bis(2,3-epoxypropoxy)-2,2-dimethylpropane	5.0%		R38, R43, H315, H317 – Wear correct PPE while handling.	Spills and inhalation	Y
E						
F						
G						
H						

15. Control Measures

1) Engineering controls – Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use or handling.

2) Personal Protective Equipment – Lab coat, safety goggles, nitrile gloves (or latex if allergic to nitrile), facemask must be worn at all time.

3) Storage – No special requirements

Now mark in the letters from the list of 'Hazardous Ingredients' above to indicate potential danger:

16. Indication of Danger				17. Route of Exposure		18. Chemical State		19. Flammability		20. Volatility		21. Dust rating	
Very Toxic		Irritant	All	Inhalation	All	Solid		Flammable	B	Low		Low	
Toxic	A, B, C	Sensitiser	A, C, D	Skin Contact	All	liquid	B	Highly flammable		Medium		Medium	
Corrosive		Carcinogen	B	Eye Contact	All	Gas/vapour	B	Extremely flammable		High		High	
Harmful	B, C	Mutagenic	B	Swallowing	All			Oxidising					
Biological Agent		Toxic to reproduction		Injection	All			Explosive					

22. First Aid Procedures (as advised from Material Safety Data Sheet)

If inhaled	If skin contact	If eye contact	If swallowed	If injected
Supply fresh air and to be sure call for a doctor. In case of unconsciousness place patient stably in side position for transportation.	Immediately wash with water and soap and rinse thoroughly.	Rinse opened eye for several minutes under running water. If symptoms persist, consult a doctor.	If symptoms persist consult doctor.	

23. Spillage Procedures:→	Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Dispose contaminated material as waste according to section 24. Ensure adequate ventilation.
24. Disposal Arrangements	

Collection	Swill down sink	Evaporation	In normal waste	Other
				Must not be disposed together with household garbage. Do not allow product to reach sewage system.

25. Are the risks adequately controlled? (Write 'Yes' or 'No'):	Yes

If you decide that the controls in Section 15. are sufficient, skip to section 27. If you decide that the risks are NOT adequately controlled (or you're not sure), then you will need to give special instructions to control the risk.

26. Special Instructions to control the risk:

27. Ensure those affected are informed of the Risks & Controls - Confirm how this will be done e.g. by issuing written instructions:

Summary information and verbal instruction will be given to those performing the procedure

Bournemouth University *COSHH Assessment Form*

1. Assessor:	2. Assessment Date:	3. Assessment Review:		
C. Arrowsmith	29/07/2019	Next Review Date:	Reviewed on: Date:	Reviewed By:

4. Summary of process or method (or make specific reference to written protocol to be used) :	
Using Epo Thin Hardener for embedding samples.	
5. Key Activity/Task (in relation to exposure potential e.g. mixing, filling, spraying, etc.):	**6. People who could come to harm** (number & roles e.g. students)
Mixing and pouring	Staff and students (2-3)
7. Duration of Exposure (minutes, hours and how often):	**8. Location and Conditions of Use** (e.g. lab, room, temp etc.)
4 hours per day	C221/C139

	9. Hazardous ingredients: (copy form/add more rows as req'd)	10. Quantities Used	11. Workplace Exposure Limit (WEL)	12. Hazard and Precaution statements	13. Actual Potential Route of Exposure (E.g. by inhalation)	14.Datasheet Attached? Y/N
A	Polyoxypropylenediamine	50.0%		Causes severe skin burns and eye damage– wear correct PPE at all times while handling.	Spills	Y
B	4-tert-butylphenol	20.0%		Irritating to eyes, respiratory system and skin – Wear correct PPE while handling.	Skin contact and spills	Y
C	M-phenylenebis	15.0%		Causes burns, Harmful if swallowed/inhaled – Wear suitable PPE while handling.	Spills	Y
D	Nonylphenol	10.0%		Causes burns, Harmful if swallowed/inhaled, possible risk of impaired fertility, possible risk of harm to the unborn child, very toxic to aquatic organisms – wear suitable PPE while handling.	Spills	Y
E	3,6-diazapctaneethylenediamin	5.0%		Causes burns, Harmful in contact with skin, may cause sensitisation by skin contact, harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment – wear correct PPE while handling.	Spills	Y

15. Control Measures

1) Engineering controls – Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use or handling.

2) Personal Protective Equipment – Lab coat, safety goggles, nitrile gloves (or latex if allergic to nitrile), facemask must be worn at all time.

3) Storage – No special requirements

Now mark in the letters from the list of 'Hazardous Ingredients' above to indicate potential danger:

16. Indication of Danger				17. Route of Exposure		18. Chemical State		19. Flammability		20. Volatility		21. Dust rating	
Very Toxic		Irritant	B,E	Inhalation	All	Solid		Flammable		Low	All	Low	
Toxic	C,D, E	Sensitiser	B,E	Skin Contact	All	liquid	All	Highly flammable		Medium		Medium	
Corrosive	A.C. D. E	Carcinogen		Eye Contact	All	Gas/vapour		Extremely flammable		High		High	
Harmful	C,D, E	Mutagenic		Swallowing	All			Oxidising					
Biological Agent		Toxic to reproduction	D	Injection	All			Explosive					

22. First Aid Procedures (as advised from Material Safety Data Sheet)

If inhaled	If skin contact	If eye contact	If swallowed	If injected
Supply fresh air and to be sure call for a doctor. In case of unconsciousness place patient stably in side position for transportation.	Immediately wash with water and soap and rinse thoroughly.	Rinse opened eye for several minutes under running water. Then consult a doctor.	Call for a doctor immediately. Drink plenty of water and provide fresh air. Call for a doctor immediately.	

23. Spillage Procedures: →	Wear protective equipment, keep unprotect persons away. Absorb with liquid-binding material (sand, diatomite, acid binders, universal binders, sawdust). Use neutralizing agent. Dispose contaminated material as waste according to section 24. Ensure adequate ventilation.
24. Disposal Arrangements	

Collection	Swill down sink	Evaporation	In normal waste	Other
				Must not be disposed together with household rubbish. Do not allow product to reach sewage system.

25. Are the risks adequately controlled? (Write 'Yes' or 'No'):	Yes

If you decide that the controls in Section 15. are sufficient, skip to section 27. If you decide that the risks are NOT adequately controlled (or you're not sure), then you will need to give special instructions to control the risk.

26. Special Instructions to control the risk:

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