Louisiana State University
LSU Digital Commons

LSU Master's Theses | Graduate School

2007

Quantitative and spatial analysis of the microscopic bone structures of deer (Odocoileus virginianus), dog (Canis familiaris), and pig (Sus scrofa domesticus)

Zoe Hensley Morris
Louisiana State University and Agricultural and Mechanical College, zmorri1@lsu.edu

Follow this and additional works at: https://digitalcommons.lsu.edu/gradschool_theses

 Part of the Social and Behavioral Sciences Commons

Recommended Citation
Morris, Zoe Hensley, "Quantitative and spatial analysis of the microscopic bone structures of deer (Odocoileus virginianus), dog (Canis familiaris), and pig (Sus scrofa domesticus)" (2007). *LSU Master's Theses*. 2088.
https://digitalcommons.lsu.edu/gradschool_theses/2088

This Thesis is brought to you for free and open access by the Graduate School at LSU Digital Commons. It has been accepted for inclusion in LSU Master's Theses by an authorized graduate school editor of LSU Digital Commons. For more information, please contact gradetd@lsu.edu.

QUANTITATIVE AND SPATIAL ANALYSIS OF THE MICROSCOPIC BONE STRUCTURES OF DEER (*ODOCOILEUS VIRGINIANUS*), DOG (*CANIS FAMILIARIS*), AND PIG (*SUS SCROFA DOMESTICUS*)

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Arts

in

The Department of Geography and Anthropology

By
Zoe Hensley Morris
H.B.Sc, University of Toronto, 2003
August, 2007

Acknowledgements

Many individuals have made my study at Louisiana State University possible with their support and encouragement.

I would like to first thank all of my committee members: Ms. Ginesse Listi, Dr. William Henk, Dr. Robert Tague, and my advisor, Ms. Mary Manhein. Had it not been for Ms. Manhein's support and interest in my histological project from our first encounter at the Forensic meetings in Dallas, I don't know where I would be.

Dr. Tague has always had an open door and helpful advice throughout this process. I have always appreciated his enthusiasm for anthropology and the push he gives all his students to improve.

Ms. Listi encouraged my project and made it seem academically valuable by showing sincere interest in the process and results. Thank you.

It is my great pleasure to have worked with the Comparative Biomedical Sciences Microscopy Center at Louisiana State University. I am indebted to Dr. Ray Wilhite for his faunal knowledge and introduction to Dr. William Henk and his laboratory. Thank you to Dr. Henk for crossing departmental lines to take the time to read an anthropology thesis and for finding the Image J program that was essential to this project. I would also like to thank Mr. Gregory McCormck for his assistance, almost every time I entered the lab, setting up the microscope and cameras.

I would like to thank Dr. Christian Crowder for introducing me to histological bone analysis, teaching me the fundamentals of slide preparation and microscopy, and for his assistance in creating this project.

I would like to thank Dr. Robert Paine for taking the time to talk me through some challenges of histological research.

For his geological expertise and guidance, I thank Mr. Rick Young. I would not have made it through my trial-and-errors in the Geology and Geosciences Rock Preparation Laboratory had it not been for Mr. Young's knowledge of the equipment and materials as well as his encouragement to try new strategies.

This research was funded in part by the Robert C. West field Research Grant from the Department of Geography and Anthropology at Louisiana State University. The Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory at Louisiana State University also funded in part this research. All specimens in this study are from the FACES laboratory comparative collection. I am sincerely grateful for the financial and academic support of both the Geography and Anthropology Department and FACES laboratory.

Many people, not directly related to my thesis, supported my decisions and assisted me in every way they could. Thank you to Dr. Joyce Jackson for her flexibility and patience. I would like to thank Dr. Heather McKillop for her time, advice, and the space to work on my thesis while in Belize.

For keeping me on my toes and pushing me to finally finish the thesis, thank you to Michelle Wydra. I would like to thank Nicole Truesdell for our theoretical debates and all the good times. The assistance of Hope Morris and Noriko Kariya was greatly appreciated and your belief in me keeps me going. For his constant support and patience through it all, I would like to thank Matthew Hardy. Thank you to my family, who may not understand histology, but have never failed to understand me.

Table of Contents

ACKNOWLEDGEMENTS …… ii

LIST OF TABLES ……. vi

LIST OF FIGURES ……..... vii

ABSTRACT …… ix

CHAPTER 1: INTRODUCTION ……... 1
1.1 Research Goals ……. 1
1.2 Significance of Species Differentiation ……... 2

CHAPTER 2: LITERATURE REVIEW …… 4
2.1 Bone Microstructure …… 4
2.2 Comparative Histology ……. 10
2.3 Histology and Forensics……. 12
2.4 Histology and Biomechanics……. 14
2.5 Histology and Archaeology…….……. 15
2.6 Histology and Non-Human Species…….. 17
2.7 Forensic and Archaeology Journal Search…… 18

CHAPTER 3: MATERIALS AND METHODOLOGY……. 20
3.1 Sample Background…….. 20
3.2 Species Selection-……. 22
3.3 Sample Selection Criteria…… 22
3.4 The Study Sample……. 25
3.5 Sampling the Specimens…… 27
3.6 Embedding, Mounting and Grinding the Specimens……. 28
3.7 Microscopy and Photography…… 30
3.8 Photo-merging…… 33
3.9 Program and Quantitative Data Collection…… 34
3.10 Qualitative Observations……. 36

CHAPTER 4: RESULTS…… 40
4.1 Plexiform Bone……..…… 40
4.2 Osteon Banding……...……..…… 41
4.3 Mid-Thoracic Rib: Secondary Osteon Area…… 42
4.4 Mid-Thoracic Rib: Haversian Canal Area…….…… 44
4.5 Mid-Thoracic Rib: Osteon Density……..……. 46
4.6 Mid-Thoracic Rib: Cortical Thickness ……. 46
4.7 Femur and Humerus: Secondary Osteon Area…….. 47
4.8 Femur and Humerus: Haversian Canal Area….……... 50
4.9 Femur and Humerus: Osteon Density……..…. 53
4.10 Femur and Humerus: Cortical Thickness ……..……. 53

CHAPTER 5: DISCUSSION…… 55
5.1 Interspecies Comparison……...……. 55
5.2 Inter-Elemental Comparison……. 56

CHAPTER 6: CONCLUSIONS……...…….... 59

REFERENCES …… 61

APPENDIX
A. FACES LABORATORY PROTOCOL FOR DNA SAMPLING..…... 66

B. RECORD SHEET FOR MID-THORACIC RIB SAMPLES.....…..… 67

C. RECORD SHEET FOR LONG BONE SAMPLES……...… 68

D. SUMMARY OF SECONDARY OSTEON AND HAVERSIAN CANAL DATA……...…… 69

VITA ...……. 70

List of Tables

2.1 Faunal Species and Bone Boolean Search of Archaeological and Forensic Publications..……..…….…. 20

3.1 Methodology Trial-and-Error Summary…… 21

3.2 Total Number of Selected and Sampled Element by Species…… 26

3.3 Age-at-Death Assessment of the Sample According to Epicondylar Fusion… 26

3.4 Pixel and Field Diameter Measures for Objectives…… 34

4.1 Percentage of Plexiform Bone Presence by Section…….. 41

4.2 Percentage of Osteon Banding Presence by Section……. 42

4.3 Mean Area (µm² x 10^4) of Secondary Osteon by Rib Section……..… 43

4.4 Mean Area (µm² x 10²) of Haversian Canals by Rib Section……..……. 45

4.5 Mean Area (µm^2 x 10^4) of Secondary Osteons by Femur and Humerus Section 48

4.6 Mean Area (µm^2 x 10²) of Haversian canals by Femur and Humerus Section... 51

4.7 Overall Secondary Osteon Mean Area* (µm² x 10^4) by Species and Element... 53

4.8 Overall Haverisan Canal Mean Area* (µm² x 10^2) by Species and Element….. 53

List of Figures

2.1 Unremodeled Lamellar Bone…… 6

2.2 Canalized Lamellar Bone……. 6

2.3 Compact and Trabecular Bone…….. 7

2.4 The Process of Bone Modeling and Remodeling……...… 8

2.5 Haversian (Secondary Osteon) System……. 11

2.6 Secondary Osteons…… 11

3.1a Adult White-Tailed Deer (*Odocoileus virginianus*) Skeleton ……. 23

3.1b Adult Domesticated Dog (*Canis familiaris*) Skeleton.…… 23

3.1c Adult Domesticated Pig (*Sus scrofa domsesticus*) Skeleton...…… 23

3.2a Dog, Deer, and Pig Femora…….. 24

3.2b Dog, Deer, and Pig Humeri ……..…… 24

3.3a Removal of the Mid-Shaft Bone Cross-Section with the Styker Saw……. 28

3.3b Pig Femur Specimen, Sample Removed…….. 28

3.4 Grinding of Histoblock Face in Preparation for Mounting..…….. 31

3.5a Loose Weighting System…… 31

3.5b Spring-Loaded Weighting System……...…… 31

3.6 Mounted and Labeled Histoblocks Ready for Re-Sectioning…… 31

3.7 Slicing of 1mm Sample from Histoblock on the Re-Sectioning Saw...…….. 32

3.8 Glass Pane with Polishing 1000 grit Silicon Carbide Powder …….…… 32

3.9 Processed and Labeled Slides Ready for Examination and Photography.. ….. 32

3.10a Graticules Pyser-SGI Ltd® Calibrating Slide 1mm/0.01 Divisions……... 33

3.10b 1mm/0.01 Calibration Image..……...……. 33

3.11 Bone Surface Differences in Processing, Preservation and Organization…… 34

3.12a Elliptical Tool Used to Measure Haversian Canals…..…….. 35

3.12b Freeform Tool Used to Measure Secondary Osteons……. 35

3.13a Divisions of Slide into 1mm Wide Sample Sections for the Rib……... 37

3.13b Divisions of Slide into 1mm Wide Sample Sections for the Long Bones..... 37

3.14 Brick-like Plexiform Bone…… 38

3.15 Example of Osteon Banding…… 39

4.1 Percentage of Plexiform Bone Presence…… 40

4.2 Percentage of Osteon Banding Presence…… 42

4.3 Mean Area (μm^2) of Secondary Osteons by Rib Section……. 43

4.4 Degraded Appearance of Some Pig Bone Cross-Section……..…… 44

4.5 Mean Area (μm²) of Haversian Canals by Rib Section…….…… 45

4.6 Whole Secondary Osteons Measured per Rib Section (mm^2)…… 46

4.7 Mean Cortical Thickness (mm) Measured per Rib Section..……. 47

4.8 Mean Area (μm^2) of Secondary Osteons by Femur and Humerus Section……. 49

4.9a Mean Area (μm²) of Deer and Dog Secondary Osteons by Femur section….. 50

4.9b Mean Area (μm²) of Deer and Dog Secondary Osteons by Humerus Section.. 50

4.10 Mean Area (μm^2) of Haversian Canals by Femur and Humerus Section..... 51

4.11a Mean Area (μm²) of Deer Haversian Canals by Long Bone Section…..….... 52

4.11b Mean Area (μm²) of Dog Haversian Canals by Long Bone Section……..... 52

4.11c Mean Area (μm²) of Pig Haversian Canals by Long Bone Section……... 52

4.12 Overall Secondary Osteon Area (μm²) by Species and Element…….. 53

4.13 Overall Haversian Canal Area (μm^2) by Species and Skeletal Element…….. 53

4.14 Whole Secondary Secondary Osteons Measured Per Long Bone Area (mm^2). 54

4.15 Mean Cortical Thickness (mm) Measured per Long Bone Section…..…...… 54

Abstract

Structure and morphology of bone are variable by species. The influence of different factors on structure and morphology is still debated. Qualifying and quantifying these differences are necessary in the evaluation of fragmentary bones in order to identify specific species. To understand the influence of species of origin on the microscopic structure of bone tissue, the developmental and biomechanical forces specific to a skeletal element must also be assessed.

This research is a preliminary analysis of the histological bone structures in terms of their area, density and spatial organization. To achieve this research goal, the cross-section of three major skeletal structures of three common quadrupeds ubiquitous across North America and commonly found in association with human remains were compared. The study analyzed the mid-shaft cross-section of six femora, five humeri, and six mid-thoracic ribs of the white-tailed deer (*Odocoileus virginianus*); six femora, six humeri, and six mid-thoracic ribs of the domestic dog *(Canis familiaris*); and five femora, four humeri, and six mid-thoracic ribs of the domestic pig (*Sus scrofa domesticus*). The cross-section of each skeletal element was divided into eight sections along anatomically recognized body planes. All histomorphometric measurements and observations were taken within these sections to explore the spatial organization of the microscopic structures across the mid-shaft cross-section.

Plexiform bone observations suggest not only species-specific presence and absence of this bone structure but a relation to the skeletal element. There was an almost complete absence of plexiform bone in the mid-thoracic rib and reduced presence in the humerus of all three species.

Secondary osteon area is larger for the pig samples compared to the other species, in all three skeletal elements, suggesting a species-specific difference in osteon development. On the

other hand, though similar in area, deer and dog showed interspecies, parallel patterns between like elements (humerus and humerus, femur and femur). Secondary osteon density followed an expected trend of increasing density associated with older animals.

The implications for this study are two-fold. First, the results suggest future avenues of research for histologically differentiating species in both forensic and archaeological contexts. Second, the results support the hypothesis that it is important to incorporate a spatial analysis of microscopic structure distribution as an additional source of information about species and bone element differences in microscopic arrangements of the bone tissue.

Chapter 1: Introduction

Fragmentary bones have complicated issues of identification for physical anthropologists and archaeologists alike. Gross morphological analysis may not be sufficient in differentiating bone specimens of mammals with similar sized skeletal elements.

Bone histology, through a microscopic comparison of bone cross-section, offers a potentially valuable tool in evaluating species when only fragments or portions of bones remain. Qualifying and quantifying differences among the femur, humerus and mid-thoracic rib cross-sections of deer (*Odocoileus virginianus*), dog (*Canis familiaris*), and pig (*Sus scrofa domesticus*) are the first steps in understanding differences in organization and structural patterns as a method of bone fragment identification and human versus non-human differentiation.

Examining the spatial organization of the bone cross-section is a major element of this study. Each bone cross-section is divided into eight sections along known body planes. All qualitative observations and quantitative measurements are reported and analyzed by section for each species and element. The methodology allows any spatially related organizational patterns to be revealed.

1.1 Research Goals

There are three major goals of this thesis project. The first goal of the research is to explore interspecies variation among mammalian quadrupeds that are common across North America in both forensic and archaeological settings. Variables including the area, densities and presence/absence of histological structures are compared among the three sample species.

The second goal of the thesis is to examine differences in microscopic bone organization between different bones of the same species. By including both of these goals in one research project, trends and patterns that are the result of phenotypic species variation may be

differentiated from biomechanical and developmental influences related to the function and growth of a particular skeletal element.

Finally, this research is an exploration of possible variables that may be used to differentiate human and non-human bone fragments. There are no human samples included in this study. However, the results of this study may be used with the work of other researchers or as part of a future project that does include human samples.

1.2 Significance of Species Differentiation

Species differentiation at a microscopic level has implications for forensic and archaeological research. Both disciplines commonly are confronted with fragmentary remains of unknown origin. Gross morphological examination and/or DNA analysis may not be possible or practical in all situations. Histomorphometrics and histological examination offer an alternative method for analyzing fragmentary remains.

"The first question in any [*forensic*] case is 'are the remains human?'" (Fairgrieve 1999:10). In situations where no other method can be used, such as gross morphological or DNA analysis, because of skeletal degradation or the cost is prohibitive respectively, bone histology offers a valuable resource. For the investigation of house fires, mass disasters, and partial skeletal recovery, the forensic anthropologist will benefit from a greater understanding of histological techniques for identifying bone fragments past the level of mammal.

For archaeologists and physical anthropologists, benefits exist for a greater understanding of bone micro-structure and species level differences in bone histology. The sub-disciplines of bio-archaeology, zooarchaelogy and paleoanthropology are challenged by fragmentary skeletal remains. According to Schultz (1997b:201), archaeologists are interested in data recovered from a site whether the material is human or faunal: "it is basically of no consequence at all whether the findings are from animals or from human beings." Faunal data provide information about a

wide range of topics of archaeological interest, including; diet, environment, domestication, seasonality, and other cultural practices. Assessing whether there are verifiable differences in the bone histology of human and non-human mammals is a primary step for histological applications of bone histology for forensic anthropologists and bio-archaeologists. Before human versus non-human differences are assessed, non-human microscopic structure and organization should be well understood. This study examines the structure and spatial layout of the bone cross-section of three non-human mammals. By examining the contribution of various influences on the architecture of bone, it may be possible to differentiate which variations are species-related, instead of being due to biomechanical or developmental influences. Understanding these influences will greatly enhance future research that attempts to differentiate human and non-human bones histologically.

Chapter 2: Literature Review

Historically, only limited research has been published with regard to the use of bone histology for species identification. This is changing as more researchers are discovering a powerful tool in the microscope. Traditionally, human bone microscopic analysis and research have focused on age-at-death estimation. In terms of species differentiation, published work has been limited in scope, analyzing the differences between humans and a single non-human species. Today, a growing number of researchers are systematically evaluating multiple species, serial bone sections, various bones, and a variety of bone structures. Researchers in several disciplines are strong advocates for the use and study of human and animal bone histology. Forensic anthropology, bio-archaeology, forensic medicine, pathology, veterinary medicine, and anatomy have made contributions to this body of knowledge. A review of the literature on bone histology provides the background for the current research.

2.1 Bone Microstructure

Microstructure of bone is well documented in histology textbooks and atlases. An and Martin (2003) and Malluche and Faugere (1986) provide two detailed guides to bone microanatomy, metabolic bone diseases, and methodology for bone histology. They include succinct sections on structural organization of the bone and bone cells, providing a basis for identifying microscopic structures. The examination of bone histology requires an ability to understand the basic microscopic building blocks of bone.

Bone is a specialized connective tissue, which functionally responds to biochemical demands and biomechanical load (Walsh et al. 2003:35). The cellular components of bone include several specialized cells.

Subtypes of bone include immature and mature bone. Immature, or woven, bone develops in utero (White 2000:26). According to Malluche and Faugere (1986:10), woven bone

is characterized by “loosely and randomly arranged collagen bundles.” Relative to mature bone, immature bone has a higher proportion of osteocytes in its matrix (White 2000:26). While immature bone is found primarily in embryonic and unremodeled bone, it may be present at pathological and fracture sites in the adult skeleton (Schultz 1997a).

As the skeleton matures, the woven bone is replaced by lamellar, or mature, bone through the process of bone formation and remodeling (Figure 2.1). Lamellar bone is the primary bone in mature skeletons and is characterized by an orderly arrangement of collagen bundles (Malluche and Faugere 1986). Throughout life the lamellar bone continues to remodel until the bone cross-section is almost completely canalized, and is covered by overlapping Haversian systems (Figure 2.2).

Both compact and trabecular bone are lamellar bone (White 2000:26). Figure 2.3 is a human rib cross-section with both the dense, compact bone and spindly, trabecular bone evident. For this thesis, only compact bone was examined histologically. Compact bone, unlike trabecular bone, is dense and heavily mineralized. Therefore, compact bone is unable to receive nutrients or excrete waste via diffusion. To compensate, compact bone is vascularized by a Haversian system that allows an exchange of oxygen, nutrition, and waste. The vascularization of compact bone is achieved through the remodeling process, altering the primary lamellar bone composition into secondary osteons as the body ages (White 2000).

According to Robling and Stout (2000:190), the “circumferential and endosteal lamellae deposited during the remodeling provide the canvas upon which discrete units of cortical remodeling leave their mark.” The theory of histological age estimation is based on the process of remodeling and assumes the formation rate of secondary osteons is predictable, therefore allowing age-at-death estimations in humans.

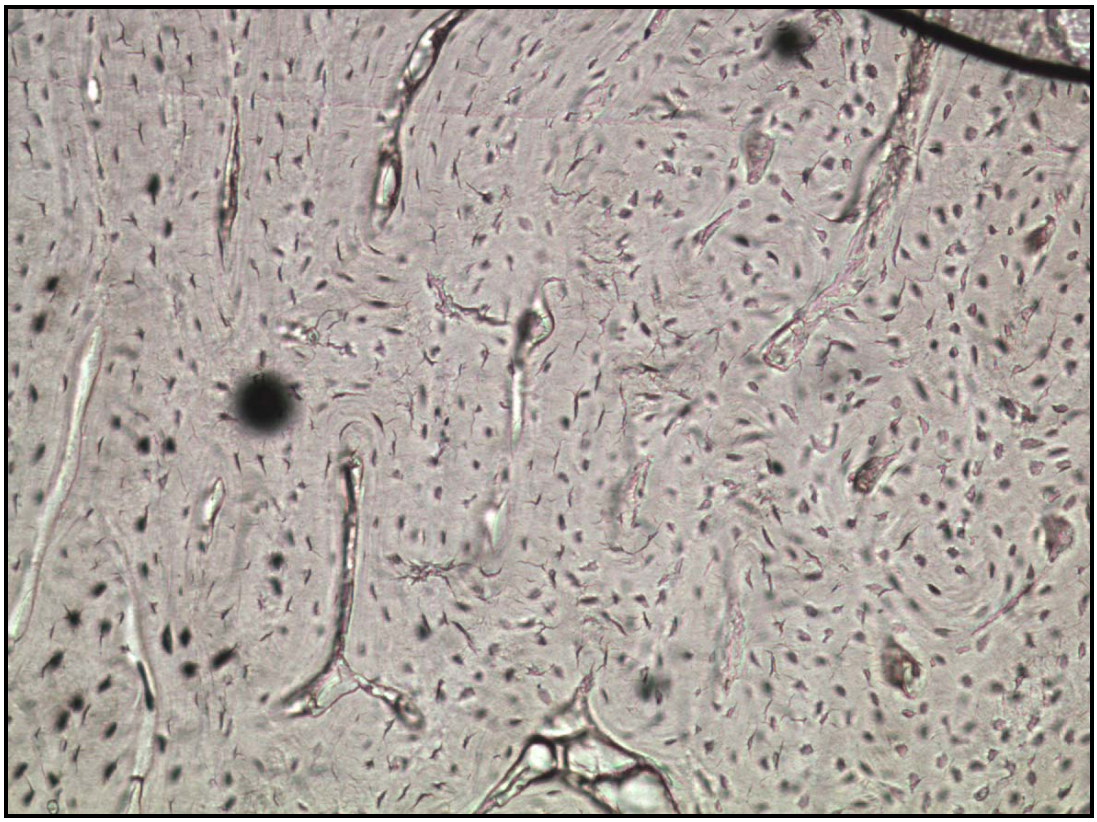


Figure 2.1: Unremodelled Lamellar Bone. (Pig humerus 2, anterior-lateral view).

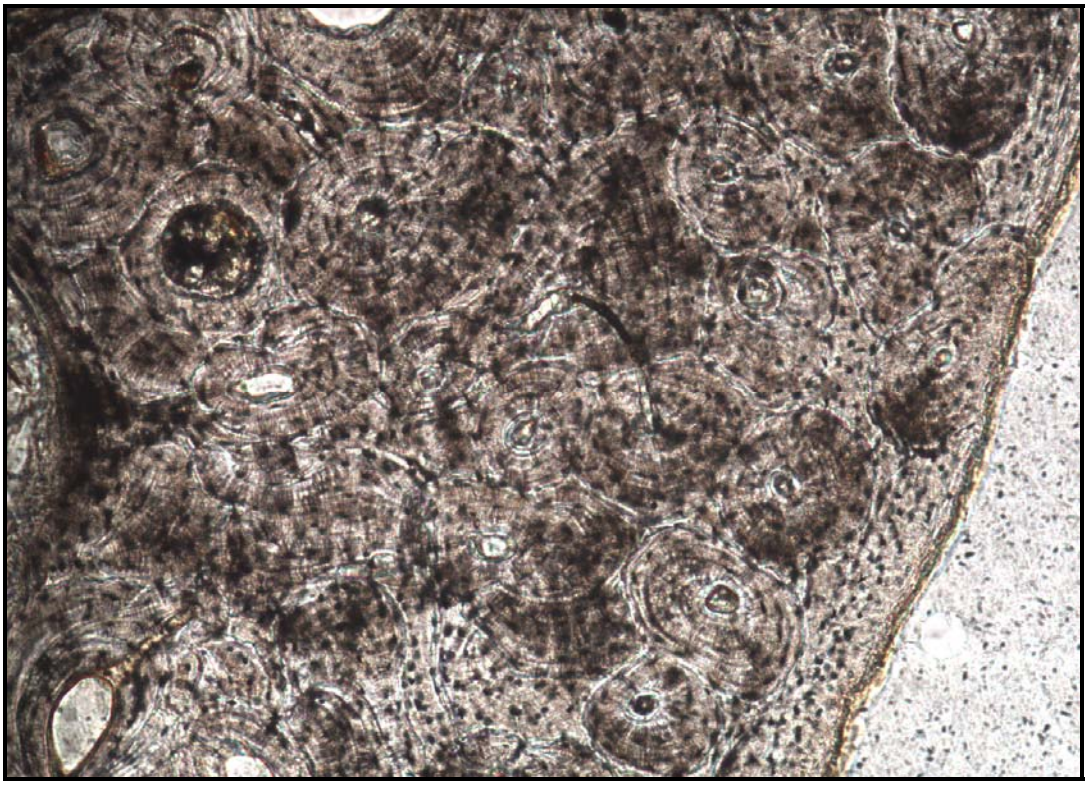


Figure 2.2: Canalized Lamellar Bone. (Dog rib 4, caudal-interior view).

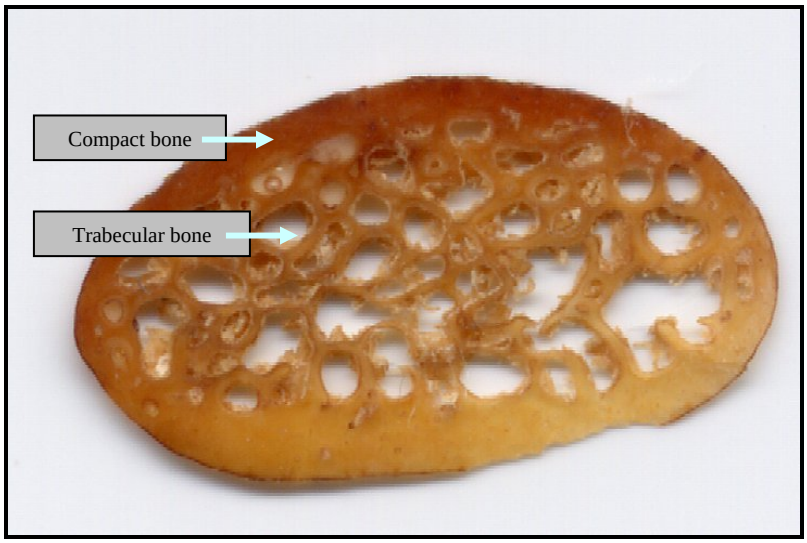


Figure 2.3: Compact and Trabecular Bone. (Human rib cross-section).

The bone formation process is similar in animals and humans, though some different tissue arrangements and structures are noted. One such structure is plexiform bone. Plexiform bone is an orderly arrangement of the bone tissue into 'rows of bricks.' Plexiform bone is not discussed in texts primarily dealing with human histology because it is infrequently present in primates. Plexiform bone is the principal bone tissue of *Bovidae* (including cattle, goat and sheep), *Suidae* (including pig) and *Cervidae* (including deer) (Owsley et al. 1985).

Osteon banding is another type of bone patterning recognized by some comparative histology authors. Mulhern and Ubelaker (2001) present evidence for the potential use of osteon banding as a means to distinguish human and non-human bone. In their study, over sixty human femoral cross-sections were compared to those of pig and sheep samples. By dividing the cross-section into quadrants and envelopes, Mulhern and Ubelaker (2001:221) were able to run a contingency table to determine if significant differences existed among the species. Their results were promising because osteon banding presence would preclude an unknown sample as human. Osteon banding, like plexiform bone, is a non-human trait. However, the absence of either plexiform or osteon banding does not conclusively indicate a species is human.

The cellular composition of compact lamellar bone is related to the modeling and re-modeling process in a collective arrangement of microscopic cells. Robling and Stout (2000:190) coined this cellular arrangement the "basic multi-cellular unit." White (2000:27) differentiates modeling as "bone sculpting during growth" from remodeling as the "process of continuous removal and replacement of bone during life."

Figure 2.4 illustrates the progression of bone modeling/remodeling. Bone modeling/remodeling process creates secondary osteons. Histological bone analysis quantifies the results of this process.

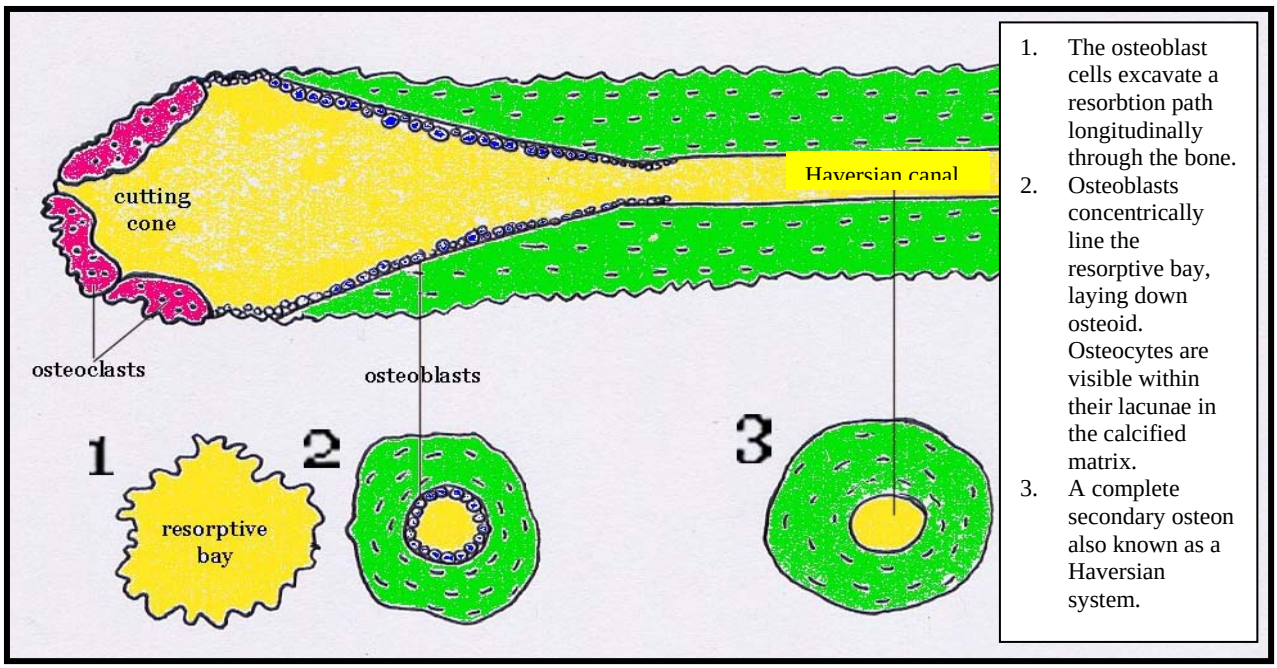


Figure 2.4: The process of Bone Modeling and Remodeling.
Adapted from Robling and Stout (2000:190)

Osteoclasts are multinucleated cells that resorb the mineralized bone matrix (Walsh et al. 2003:36). In the human skeleton, they excavate bone sections in approximately 250-300µm (Robling and Stout 2000:1980). The osteoclastically removed area of bone sets the stage for the invasion of osteoblastic, or bone forming, cells. According to Gartner and Hiatt (1994), osteoblasts are rarely captured in histological slide images of mature lamellar bone.

Osteoblasts are the principal cells involved in bone formation through synthesis and secretion (Malluche and Faugere 1986). They are often concentrated just beneath the periosteum and operate to form bone in a two-step process (White 2000). First, osteoblasts secrete matrix, or osteoid, which surrounds the osteoblast (Malluche and Faugere 1986). Then the osteoblast becomes embedded in the osteoid lacunae as the matrix is mineralized through the deposition of hydroxyapatite crystals (White 2000). Once the matrix mineralizes, the embedded osteoblast becomes an osteocyte (Walsh et al. 2003).

From each oval-sized cavity of the lacunae radiate fluid filled canals (White 2000). These canals, or canaliculi, enclose cytoplastic processes that connect isolated osteocyte cell bodies housed in the lacunae, allowing communication and exchange among the cells (Schultz 1997a). The system of canaliculi eventually opens into a Haversian canal containing blood vessels (Gartner and Hiatt 1994). The osteoncytes of an Haversian system communicate via this specialized architecture allowing "living cells to survive in a heavily mineralized environment" (White 2000:27).

The basic unit of mature, compact bone is the secondary osteon or Haversian system (White 2000). The composition of each Haversian system includes a: "Haversian canal with its surrounding lamellae of bone containing canaliculi radiating to and from the osteocytes trapped in the lacunae" (Gartner and Hiatt 1994:61). Figure 2.5 illustrates a single secondary osteon. The lamellae surrounding a Haversian canal containing blood vessels, with lacunae interconnected through their canaliculi, are apparent. The Haversian system allows the architecture of the compact bone to remain dense while maintaining the living cells of the bone.

Figure 2.6 is a microscopic image of mature dog mid-thoracic rib cross-section with several Haversian systems evident. The lacunae with radiating canaliculi are in the microscopic

image. The concentric organization of the osteocytes around a central Haversian canal are also apparent.

The number of secondary osteons increases with advancing age as bone is continuously remodeled; “As age increases the cortex becomes crowded with secondary osteons” (Robling and Stout 2000:192). An asymptote is eventually reached as the bony cortex is completely remodeled. What is preserved in the compact bone at the time of death is architecture of the bone, including the influence of age, health, and biomechanics for that individual within the limitations of phenotypic expressions of that species.

2.2 Comparative Histology

Enlow and Brown (1958), Foote (1916), Jowsley (1966), and Leake (1975) examine histological characteristics of various taxa in relation to one another. What has come out of their research are studies in the application of histological techniques beyond medical research.

Enlow (1966) provides clear descriptions of bone types and typical arrangements of bone from different taxa. While an invaluable source of comparative information, Enlow also addresses the limitations of histological species identification research: “Human bone, for example, cannot be recognized with any reasonable degree of certainty since some other mammalian forms have combinations and an organization of bone tissue types that more or less parallel that of human bone” (Enlow 1966:101). Enlow (1966) warns that some animals, such as bear, cat and monkey, may be difficult to differentiate from humans at a histological level.

Jowsley (1966) measured the Haversian system in humans and several faunal species, including dog. Results of the study indicated there were species-related differences in Haversian system size, intraspecies similarity in osteons of the rib and femur, and age-related changes in Haversian canal size.

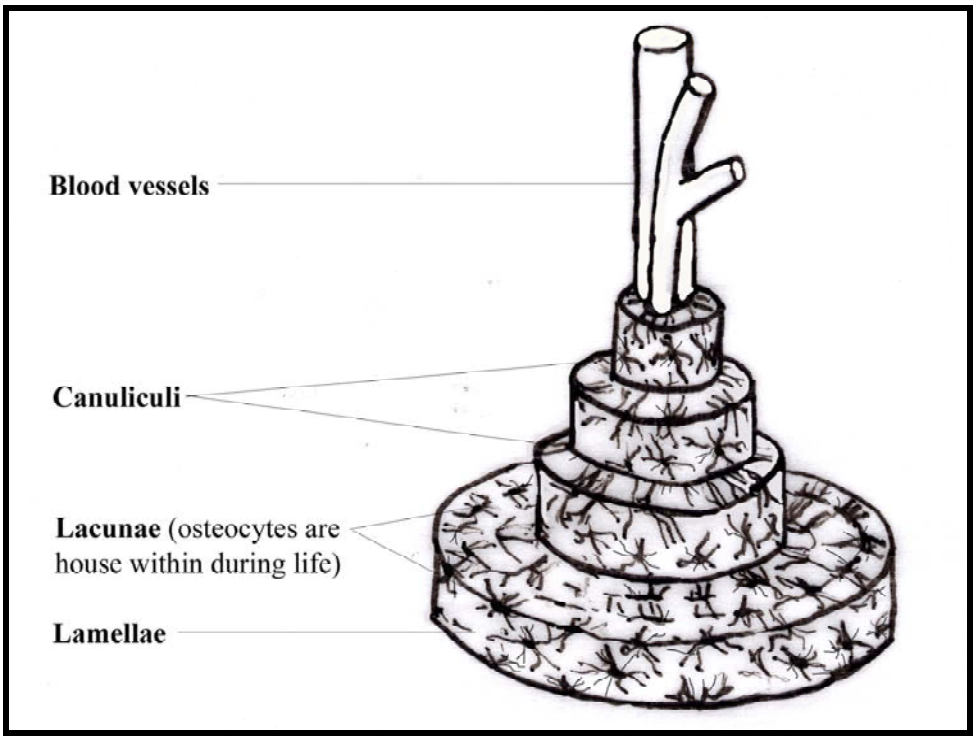


Figure 2.5: Haversian (Secondary Osteon) System.
Adapted from White and Folkens (2005: Figure 4.3)

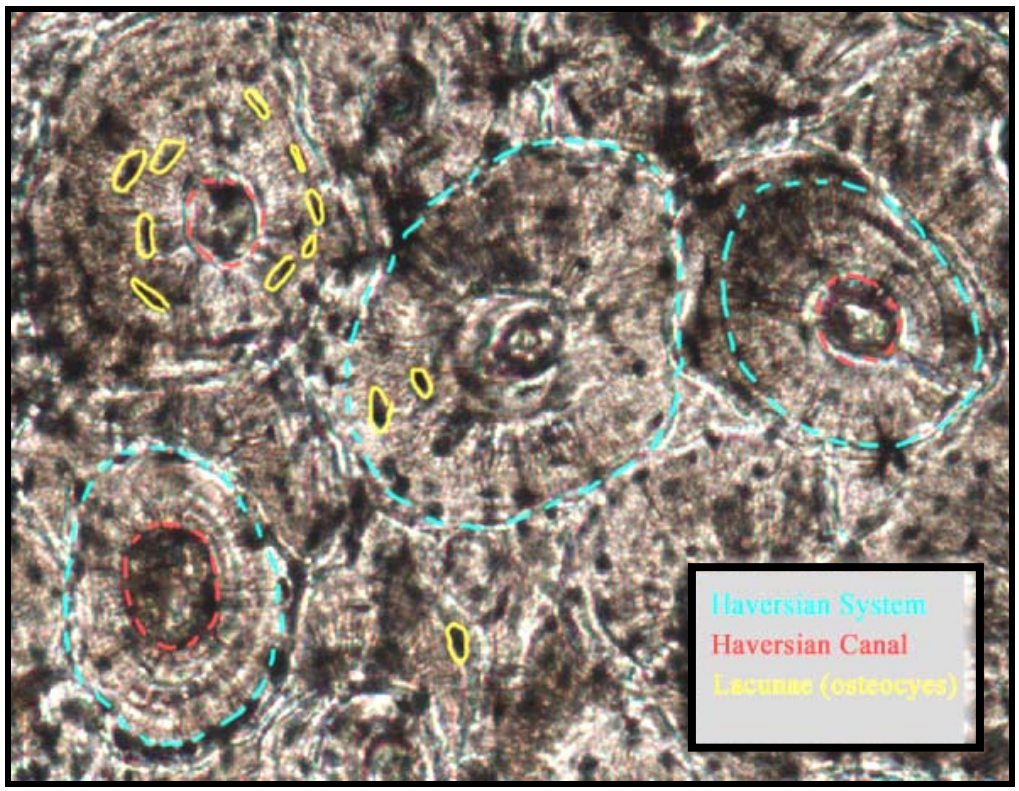


Figure 2.6: Secondary Osteons. Highlighted are complete Haversian systems (blue) with Haversian canals (red) and lacunae (yellow), which in life housed osteocytes. (Dog rib 4).

More recently, Whitman's (2004) research distinguishes between human and non-human secondary osteons in the ribs. Whitman emphasized the use of non-weight bearing bones, in particular the rib, because of the well-documented presence of secondary osteons in several species. She noted that much of the previous research focused primarily on the femora and urged further histomorphometric studies with other bones. Whitman examined ten samples from human, dog, cattle, and one bear, comparing their Haversian canal size and osteon diameters. She found cow and bear did not offer distinguishable differences in osteon size, but dog did exhibit significantly smaller osteons than humans.

Benedix (2004) examined the differentiation of fragmented bone. Benedix examined long bones from South Asian mammals including cow, deer, dog, goat, monkey, pig, and water buffalo in order to analyze both plexiform and Haversian structures. One of the significant findings by Benedix (2004:80) was a difference in Haversian cell size of humans from that of dog, monkey, and buffalo.

2.3 Histology and Forensics

Histology has an important application for forensic anthropology. In forensic situations where gross morphological differentiation is not possible and DNA analysis is difficult or cost prohibitive, bone histology offers a valuable alternative to species identification. In the case of fragmentary bone, one of the most important initial determinations is whether or not the material represented is human (White 2000). This question must be answered whether the material represented is a complete skeleton or the fragments of a single bone.

A forensic example of histological analysis of bone fragments was presented by Owsley et al. (1985) in a case involving the differentiation of deer and human bone fragments. Owsley et al. (1985) were able to demonstrate sufficient similarity between the unknown fragments and the human specimens. Fragments of bone were collected from various locations of the crime

scene and were compared with autopsied human and sampled deer bone fragments. Diagnostic characteristics, such as Haversian canal diameter and secondary osteon counts per area of cortical bone, were comparable between the unknown fragments and human autopsy specimens (Owsley et al. 1985). Owsley et al. caution that human bone may be hard to differentiate from other mammals such as primates and bears because of similar structural features. Distinguishing cow, deer, and dog is possible histologically (Owsley et al. 1985). Owsley et al. drew much of their research from Enlow and Brown's (1958) studies of comparative histology.

Evidence contributed by the histological analysis of bone fragments by a forensic anthropologist also aided in the conviction of a man who allegedly murdered his wife in 1985. The woman's vehicle was found three years after her disappearance containing cranial and post-cranial bone fragments, blood, and shotgun pellets (Dix et al. 1991; Stout and Ross 1991). The case was difficult to prosecute because the woman's body was not recovered, nor were there witnesses to the event. The analysis of skull fragments aided in the confirmation of a human victim and the conclusion that the severity of the injuries sustained to the skull was fatal (Dix et al. 1991:952).

Ubelaker (1998) is a proponent of introducing additional techniques and technologies into forensic anthropology, including microscopy. Most conventional histological methods are utilized in age-at-death approximations: "The significant involvement of microscopy in forensic anthropology traditionally has been in the area of age-at-death estimation" (Ubelaker 1998:514). Ubelaker suggests that microscopic analysis and histomorphometry have potential contributions beyond age estimation in forensic anthropology, including differentiating bone from non-bone material.

Several researchers have demonstrated that histological analysis of the skeleton is a useful and reliable methodology the age-at-death estimation. Histomorphometric analysis is

used to substantiate identity and enhance biological profiles by providing an estimate of an individual's age-at-death (Cho et al. 2002; Crowder 2005; Pratte and Pfeiffer 1999; Stout 1986, 1998; and Stout and Paine 1992)

Cattaneo et al. (1999) conducted a comparative analysis of the use of histological, immunological, and DNA techniques in the identification of burned bone fragments. The researchers' conclusions suggested that quantitative microscopy may be more reliable than some other techniques, in particular, when Haversian canal size was used as a discriminating factor.

2.4 Histology and Biomechanics

Sex, pathologies, and age are factors that affect the remodeling of bone (Lynnerup et al. 1998; Robling and Stout 2000; and Stout 1998). The influence of physical activity and biomechanics is only briefly addressed in articles regarding bone remodeling.

According to Nordin and Frankel (1989:6), biomechanically, bone is a "biphasic composite material." For bone, this means that it is a strong, stiff material embedded within a more flexible substance. Nordin and Frankel (1989) describe hypothetical bone-loading and deformation, and the various responses of bone. If failure point, the point at which bone breaks, is not reached, the bone has the ability to respond to strong, sustained and/or frequent stresses. However, to meet mechanical demands, bone has only two physiological responses: to gain or lose bone. The mechanism of bone resorption and formation is the process of microscopic architecture. "This phenomenon, in which [a] bone gains or loses cancellous and/or cortical bone in response to the level of stress sustained, is summarized as Wolff's Law, which states that bone is laid down where needed and resorbed where not needed" (Nordin and Frankel 1989:23). Laying down and resorbing of bone takes place at a cellular level, suggesting that different mechanical demands may influence bone microstructure. Robling and Stout (2000:200) conclude, "biomechanical factors affecting bone remodeling appear to be local, usually affecting

those bones being strained." Research indicates that increased activity in pigs promoted modeling, not remodeling (Robling and Stout 2000). Whitman (2004) advised researchers to study non-weight bearing bones when conducting inter-species histological studies. Whitman (2004) suggests species-level microscopic differences can be assessed more accurately from skeletal elements not affected by bone loading. Understanding the biomechanics and bone-loading forces is an important aspect of this research.

The humerus and femur are both considered weight-bearing bones for all three fauna species examined for this research. This does not suggest that the humerus and femur are considered functionally synonymous. The humerus and femur have different functional roles in locomotion and other physical activities. An example of one physiological difference is the orientation of the knee and elbow joints. In most mammals, including carnivores and ungulates, the elbow joint is oriented posteriorly and slightly lateral to the shoulder, whereas, the knee joint points anteriorly and slightly laterally. During propulsion, the humerus and femur are both involved but move differently. Further, though all three species analyzed in this study are quadrupeds, the type of locomotion used by deer, dog, and pig varies considerably among the species (Liem et al. 2001).

Hamrick's (1999) research with the opossum (*Didelphis virginia*) found differences in the humerus and femur with regard to histological development. In his research, the hind limbs of the opossum developed at a different rate histologically than the forelimbs because of different functional and behavioral influences.

2.5 Histology and Archaeology

Fragmentary bones recovered from ossuaries and middens cannot always be identified with respect to species through gross examination. Archaeologically, establishing the species represented is not just a matter of human versus non-human, as may be the goal of forensic

anthropology. Taxa identification is an imperative part of zoo-archaeological research. "The systematic attribution of specimens to taxa is essential" (O'Conner 1996:10). Histological techniques may be able to establish the species of the material. Not only would these techniques be more accurate in assessing human burials, but species identification may be extended to assist with more specific faunal analysis of a site.

Paleoanthropology is another area that would benefit from a greater understanding of the microscopic structures of bone. Human remains have always provided an important source of information about many aspects of the past (Bahn 2003:6). Enlow (1966) noted that the calcified tissues, including bone, are the only part of the body that permit histological examination of fossil tissues and therefore are of potential diagnostic use. Human bone histology may even have applications in the study of human evolution. "Comparative studies of bone tissue, including both fossil and modern forms and considering a wide variety of groups, indicate that bone histology can be of value in studies of evolution" (Enlow 1966: 105).

Bio-archaeology, an interdisciplinary field within physical anthropology, is the most obvious benefactor of histological knowledge. Bahn (2003:139) states: "The vast majority of human remains from the past take the form of purposeful burials." This phenomenon has spanned the last 250, 000 years according to some researchers. Taylor (2000:45) argues; "For at least a quarter of a million years, humans have taken great care to perform burials for their dead." It is imperative that archaeological researchers be able to recognize a purposeful burial even if only fragments remain. Burial analysis in an archaeological setting may be confirmed with our ability to identify bone fragments.

Zoo-archaeology is another area that would be enhanced with the identification of species from fragments. Zoo-archaeologists "serve to build up for archaeologists a more complete picture of our ancestors' way of life" (Davis 1987:20). In order to analyze an archaeological site

more thoroughly, “faunal remains have to be identified” (Davis 1987:32). Faunal analysis in an archaeological setting is derived almost exclusively from the bones and teeth (Davis 1987:19). Associated remains are typically fragmented due to purposeful butchery and pounding techniques, unintentional trampling, and excavation damage (Davis 1987:26).

Currently, a research team from the University of Montana at Missoula is conducting histological analysis of the fragmentary remains from the Donner party campsite. The researchers described the entire bone surface and measured a sample of the Haversian canals' diameters perpendicular to the long bones axis. The research team was able to discern several species types including deer, cow, horse, and dog (www.anthro.umt.edu/donner/default.htm, accessed April 2007).

2.6 Histology and Non-Human Species

Veterinary publications and journals were an informative resource regarding the skeletal structure of deer, dog and pig. An example of an inter-species veterinary study is Martiniakova et al. (2006). The researchers measured area, perimeter, minimal and maximum diameter of Haversian canals, Haversian systems, and the vascular canals of primary osteons of adult cows and pigs.

Anatomy and physiology texts that were consulted for the present research on pig anatomy included Currie (1988), Pond and Mersmann (2001), and Sack (1982). Dog anatomy texts included Adams (1986), Liem et al. (2001), and Olsen (1985). Olsen (1985) included information about the archaeological remains of dogs in North America. The white-tailed deer texts included Bauer (1983) and Fulbright and Ortega (2006).

The three species selected for this histological investigation were chosen because of their ubiquity in North American forensic and archaeological settings (Olsen 1964). According to Bauer (1983), the White-tailed deer (*Odocoileus virginianus*) is found in all areas of the United

States and southern Canada with the exception of dry terrain in the Southwest and California. Bauer also reported on the use of White-tailed deer in both the pre-contact and contact archaeological records by Native Americans and settlers alike.

The first pre-contact, domesticated dog (*Canis familiaris*) remains are reported by Olsen (1985) to date to between 9500 and 8400 BC in Idaho. Derr (2004:5) quotes George Catlin, the artist who depicted over 500 Native American scenes during his visits in the 1830s: "The dog, amongst all Indian tribes, is more esteemed, and more valued than amongst any part of the civilized world." Today, according to the Humane Society (www.hsus.org/pets, accessed April 2007), there are over 73 million dogs owned as pets in the United States.

The domesticated pig (*Sus scrofa domesticus*) is first recorded 7000 years ago in Persia and China (Pond and Mersmann 2001). The domesticated pig was introduced into North America by Columbus by way of the West Indies in 1492. In 1539 De Soto brought pigs to the mainland. Today, both domesticated and wild pig descendants live across North America. Pigs are an important food source in the United States, historically and currently. Pigs are also employed as animal models in biomedical research (Pond and Mersmann 2001).

2.7 Forensic and Archaeology Journal Search

Table 2.1 is a summary of the Boolean search results of three professional academic journals. The *American Journal of Archaeology* and *American Antiquity* were searched as representations of archaeological inquiry, and *Journal of Forensic Sciences* was searched for forensic references. The journals were surveyed online by entering a faunal species and the word "bone" as search parameters. The exercise was meant to determine, if according to the published literature, there is a relation between archaeological sites and/or forensic crime scenes and the selected animals for this study. The survey included several other faunal species besides deer,

dog, and pig. The three species selected for this project are among the most published animal subjects in both the archaeological and forensic journals.

The horse was another species frequently mentioned in the archaeological journals. Horses were not used for this study because relative to deer, dog, and pig, horse bones are considerably larger. Future research may consider including sheep as a possible subject of histological investigation.

Table 2.1: Search "hits" for various species in archaeological and forensic journals to suggest a possible relation between the species and the contexts.

	American Journal of Archaeology 1897-2001 and American Antiquity 1935-2003	Journal of Forensic Sciences 1972-2005
White-Tailed Deer + Bones	42 (deer + bones 546)	4 (deer + bones 32)
Dog + Bones	380	51
Pig + Bones	170	42
Cow + Bones	145	13
Horse + Bones	553	11
Sheep + Bones	348	13
Cat + Bones	177	26
Chicken + Bones	28	11
Turtle + Bones	112	3
Weasel + Bones	20	3
Black Bear + Bones	23 (bear + bones 693)	31 (bear + bones 39)

The published literature describing bone microstructure, current research and role of microscopy in forensics and archaeology is invaluable to the researchers' understanding of the process and importance of bone histology.

Chapter 3: Materials and Methods

In preparation for my thesis work, I consulted several texts regarding proper sampling techniques and methodology including Bauer and Mahovlic (2003), Malluche and Faugere (1986), Martin (1988), and Ries (2003).

I had previous exposure to histological investigation because of my work with Dr. Crowder at the University of Toronto (Crowder and Morris 2005). Crowder's (2005:301) research examined "how the selection of variables can affect the precision and, in turn, the accuracy of histological age estimation." The University of Toronto laboratory provided training in histological slide preparation as well as exposure to histological analysis and microscopic bone structure recognition.

I had personal communications with several researchers with extensive histology experience including Drs. Crowder, Paine, and Stout. The literature, the expert advice, and my previous experience volunteering with Dr. Crowder provided a guide and foundation for my methodology. However, the final techniques discussed below were developed through trial-and-error. The methodology used in this thesis was adapted to the available resources, my skills, and an on-going assessment of what techniques produced the best results. Only the final methodology is described for each section. Table 3.1 outlines the various strategies proposed and tried for each step in the process.

The skeletal material used for this study was obtained from the faunal comparative collection housed in the Forensic Anthropology and Computer Enhancement Services (FACES) Laboratory, Louisiana State University (LSU), Baton Rouge, Louisiana.

3.1 Sample Background

The FACES laboratory comparative collection is housed in Howe-Russell Geosciences Complex, LSU, and is under the direction of Ms. Mary H. Manhein. More than thirty faunal.

Table 3.1: Methodology Trial and Error Summary.

	Attempt	Result	Used
Embedding the Bone	80ml resin: 20ml hardener Tap Plastics® Super Hard Epoxy	Effective	Yes – used for all slides
	placing in the fridge	Prevent over-heating and bubbles	Yes – used for all slides over 22x30x20mm
Grinding to Prepare Histoblocks for Mounting	Ground down on steel wheel	Even surface, but grit glued to slide reducing visibility	Not used, all slides redone
	Ground down on the glass plate	Grit reduced, but some still remained visible	Not used, all slides redone
	Ground down on Hillquist ® diamond plated grind wheel	Effective - no grit, very even surface	Yes – used for all slides
Mounting	Extreme Power 5-Minute Epoxy®	Effective	Yes – used for all slides
	Hillquist Thin Section Epoxy ®	Not as clear under the microscope	Not used
Slide	2"x1" petrographic slides	Effective	Yes – all ribs and smaller humeri/femora
	3"x2" Corning 2947 slides	Slightly thinner slide resulting in breakage during mounting	All slides needed to be redone
	3"x2" geology slides (brand unknown)	Slightly thicker, no breakage	Yes - larger humeri/femora
Weighting Slides while Gluing	No weights used	Uneven adherence to surface, resulting in uneven image	Used for some initial slides
	Free weights	Some sliding but overall effective	Used for majority of slides
	Spring-loaded weighting	Effective, though limited number can be made at a time	Used for all later slides
Cutting Block	Re-sectioning Saw	Effective	Yes – used for all slides
Grinding	Grinder to .5mm	Effective but some parts to thick	Used for all slides
	Grinder to .45mm	More effective, though possible over grinding	Used for all slides when .5mm was too thick
Micro-grinding	Glass plate with 600 grit silicon carbide powder and 1000 grit silicon carbide powder	Most effective - some grit, but sonicator removes grit	Yes – used for all slides
	Tooth paste mixed with 1000 grit silicon carbide powder on slide	Somewhat effective, but time consuming- good for removing scratches	Used for some slides
	1000 grit polishing cloth	Effective	Used for some slides
Microscope	10x objective	Too many images per slide, unable to merge or too large once merged	Not used
	5x objective	Effective	Used for most ribs and humeri
	2.0x objective	Due to swiveling some cross-sections could not be photo-merged completely	Jsed for all later slides with 5x selections
Calibration slide	Graticules Pyser-SGI® 1mm calibrating slide	Photographed everyday	Yes – used for all slides
Image Type	JPEG (joints photographic experts group)	Limitations due to the type of file	Used for all later slides
	TIFF (tagged image file format)	Created file sizes that were too large	Used only for initial slides
Photoshop CS®	Photo-merge	Effective for all but initial 10x images (too many slides, file size to large)	Yes – used for all slides
Image J®	Calibration & measurements	Effective	Yes – used for all slides

species are represented in the collection. The collection was started in the early 1980s and continues to grow in terms of absolute number of elements as well as number of faunal species. The collection is composed of primarily modern samples but does include some historic animal remains. The comparative skeletal material represents almost exclusively samples collected within the state of Louisiana.

3.2 Species Selection

All deer, dog, and pig elements were selected from the FACES Laboratory comparative collection. Sample selection took place in the LSU Forensic Anthropology Laboratory. Partially complete (shaft intact) and complete samples were selected. A minimum of five of each element was selected for each species.

White-tailed deer (*Odocoileus virginianus*), the domesticated dog (*Canis familiaris*), and the domesticated pig (*Sus scrofa domesticus)* were the three species selected to be sampled from the collection. All three species are ubiquitous as both living species and archaeological remains across North America (Olsen 1964). Figures 3.1a, 3.1b and 3.1c represent the adult skeletons of each of the three species used in the study with the elements of interest highlighted. Several of the mid-thoracic ribs are highlighted to demonstrate the possible location range from which the element may have been selected.

3.3 Sample Selection Criteria

The sample selection considered several criteria. First, the species and bone element must be identifiable. The bones in the comparative collection had been previously categorized by species and element. However, I conducted an independent species, skeletal element, and siding (left/right) assessment for each bone. If species origin, skeletal element, or side were unclear, the bone was not used. Figure 3.4a and 3.4b represents the various morphological variations of the femur and humerus, respectively, for the three species used in this study.

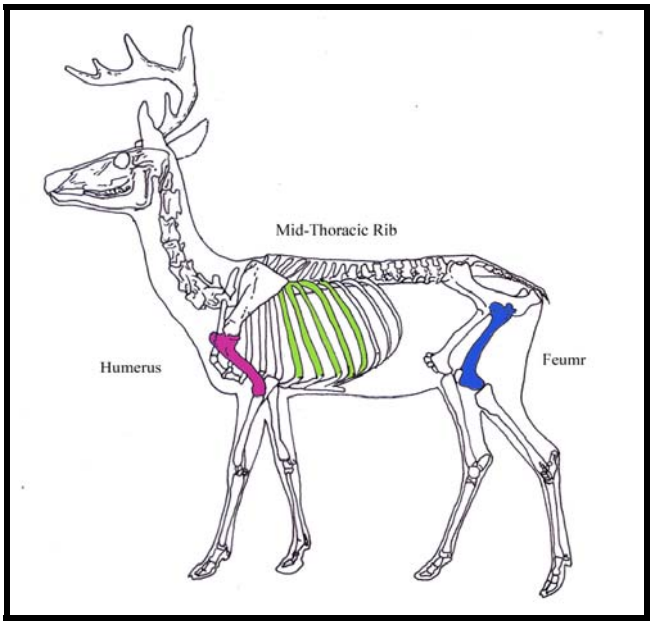


Figure 3.1a: Adult White-Tailed Deer (*Odocoileus virginianus*) Skeleton. Sampled Elements Highlighted. Adapted from Pavao-Zuckermann (2007: Figure 4).

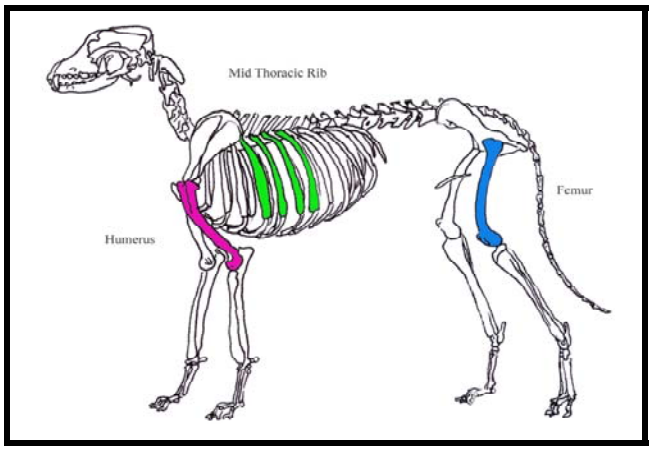


Figure 3.1b: Adult Domesticated Dog (*Canis lupus familiaris*) Skeleton. Sampled Elements Highlighted. Adapted from Olsen (1964: Figure 45).

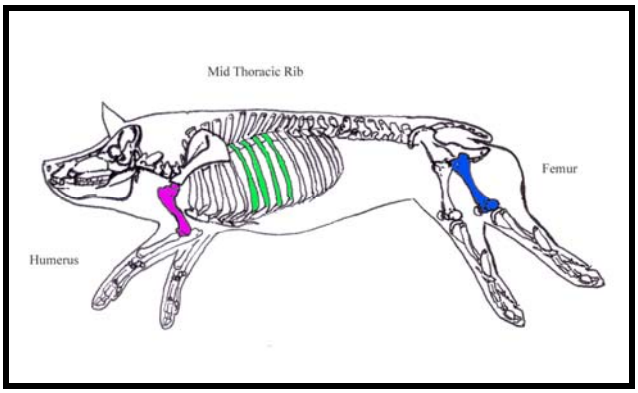


Figure 3.1c: Adult Domesticated Pig (*Sus scrofa domesticus*) Skeleton. Sampled Elements Highlighted. Adapted from Novakofski and McCusker (2001: Figure 9-1).

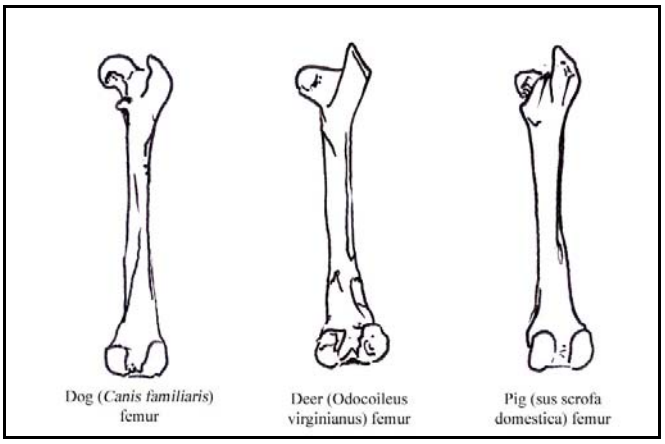


Figure 3.2a: Dog, Deer, and Pig Femora.
Adapted from Olsen (1964 Figures 20 & 92).

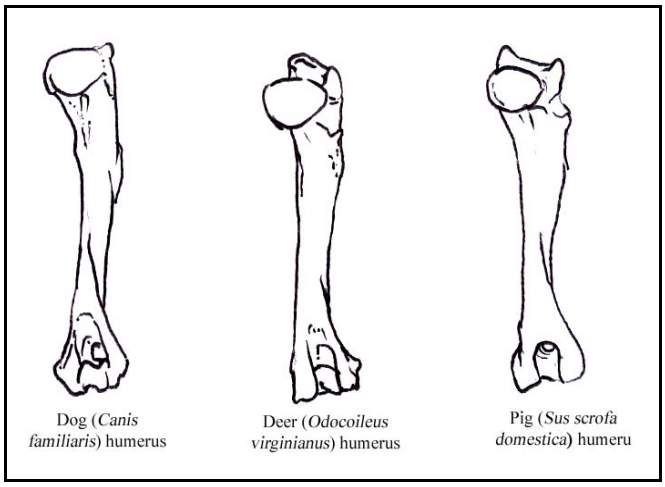


Figure 3.2b: Dog, Deer, and Pig Humeri.
Adapted from Olsen (1964: Figures 112, 114, & 115).

Second, only unpaired bones were selected for the study to prevent double representation by the same animal. This step was important because of the small number of specimens. To prevent pairing, forensic case numbers were noted and the same element was not used from any one case. Because not all elements were associated with case numbers, bones were sided and when possible only left elements were selected. Right elements were selected only in cases where the left element was not available or when the left element was damaged.

The third criteria included an assessment of damage to the bone. Damage to proximal and distal ends of the bone was considered an acceptable level of damage and was noted in the sample file. In fact, a damaged bone was selected over an undamaged bone whenever possible to

maintain the integrity of the comparative collection. Damage to the shaft was acceptable only if the middle third of the shaft was intact and species and side identification were still possible. Further, there had to be no periosteal surface damage to the mid-shaft.

The final criteria concerns observable skeletal pathology. There should be no pathology or osteological trauma associated with the bone that may affect the histology of the bone. Sex was not assessed for the species.

Each specimen was described and assigned a simple label. The labeling system described the species (De, Do or Pi), the element (Fe, Hu or Ri) and bone number (1-6). A brief assessment and description were made for each specimen, including; condition, observable taphonomy, side of origin (right or left) and general age estimation (fused or unfused epiphysis).

Unlike the femur and humerus, which were readily identifiable elements, the rib number could not always be easily assessed. Instead, any mid-thoracic rib could be selected for sampling. Siding and other observable characteristics were still noted for each specimen. The reason the 'mid-thoracic' description was used instead of a specific rib number was because of the differing number of ribs between species as well as the difficulty assigning exact rib numbers without the ability to seriate all ribs. For example, pigs have fourteen to fifteen ribs, seven sternal (true) and seven to eight asternal or false (Novakofski and McCusker 2001:455). Mid-thoracic ribs were defined as true ribs between rib numbers four through seven in any species.

3.4 The Study Sample

In total, fifty elements were selected for the study. Seventeen deer, eighteen dog, and fifteen pig bones were sampled from the FACES Comparative Collection (Table 3.2). Factors that may have affected the histological assessment include the age of the various specimen and differential preservation.

Table 3.2: Total Number of Selected and Sampled Elements by Species.

	Rib	Humerus	Femur	Total
Deer	6	5	6	17
Dog	6	6	6	18
Pig	5	4	6	15
Total	17	15	18	**50**

All pig elements were categorized as sub-adult because of their lack of complete epiphyseal fusion. The young age may be because the domesticated pig specimens represent slaughtered pigs. On the other hand, the majority of the dog elements (sixteen of eighteen) were completely fused. Only the deer elements represent various stages of fusion (Table 3.3). However, these different age-at-death estimations were considered an acceptable difference because in archaeological and forensic contexts these elements might have similar age-at-death ratios. Domesticated dogs, as pets and labor, would not die as young as domesticated pigs, as food and research subjects. Deer, as a wild animal, may be hunted or die naturally at various ages.

The second factor, different taphonomic processes, is more difficult to assess. For example, the majority of the deer samples were stained and sun bleached, having been exposed to the elements and collected from the wild. Some deer bones had associated tissue still attached.

On the other hand, the pig specimens all had a similar, slightly granular surface appearance with no staining. In some cases, the pig elements showed cut marks and burning. The pig bones may have been obtained from a slaughterhouse or butcher.

The dog bones were clean and unstained and did not have the bleached, flaky surface of the pig bones. The variability in preservation and taphonomy did affect the microscopic appearance of the specimens and was recorded for each sample.

Table 3.3: Age-at-Death Assessment of the Samples According to Epicondylar Fusion.

DEER	Fused	Partially Unfused	Unfused
Rib	2	3	2
Humerus	3	2	-
Femur	4	2	2

DOG	Fused	Partially Unfused	Unfused
Rib	6	-	-
Humerus	5	1	-
Femur	5	1	-

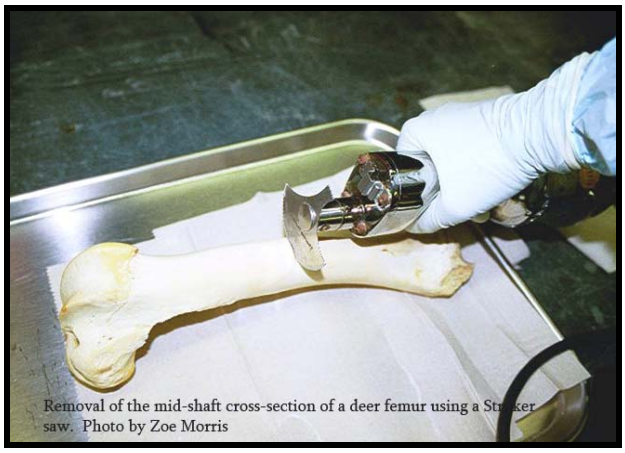
PIG	Fused	Partially Unfused	Unfused
Rib	-	-	5
Humerus	-	1	3
Femur	-	1	5

3.5 Sampling the Specimens

All sampling was conducted in the FACES laboratory. Sampling of the specimens was done in a sterile environment using DNA collection protocols established by the FACES laboratory (Appendix A).

The section to be sampled was marked in pencil prior to cutting the bone. The anterior and medial aspects of the femur and humerus were labeled directly on the bone, and the superior and external aspects of the ribs were marked on rib specimens. This step ensured that once cut the bone sample's position could be determined. The mid-shaft was defined as the approximate center of the middle one third of the bone shaft. When incomplete bones were present, the length of the bone was estimated and mid-shaft approximated.

Removing the bone cross-section was achieved with a Stryker® saw (Figure 3.3a). The entire cross-section was sampled because the spatial organization of the bone was a key element of the quantitative and qualitative analysis. All cuts were made perpendicular to the bone shaft. The 10mm bone samples were removed from the mid-shaft of each element (Figure 3.3b).



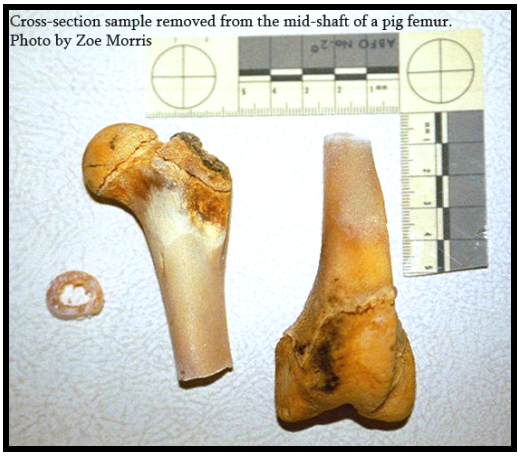


Figure 3.3a (left): Removal of the Mid-Shaft Deer Femur Cross-Section with the Stryker Saw.
Figure 3.3b (right): Pig Femur Specimen, Sample Removed.

3.6 Embedding, Mounting and Grinding the Specimens

Embedding of the bone samples was done in the Geology and Geophysics Rock Preparations Laboratory, Howe Russell Geosciences Complex, LSU, under the direction of Mr. Rick Young. The samples were not decalcified prior to embedding.

All samples were embedded in Tap® Plastics 4:1 Super Hard Epoxy in various sized Polysciences, Inc. histoblock trays. All samples were allowed to set for a minimum of 24 hours in a cool environment. Due to the exothermic reaction of the resin, it was necessary to place larger histoblocks trays, with dimensions of 22mm x 30mm x 20mm and greater, in the refrigerator while setting.

Once set, resin-embedded bone samples, or histoblocks, were removed from the trays. Each specimen was engraved with its designated label and the positional indicators. The designated label indicated the species, bone type and bone number as well as the anterior and medial aspects of the femur and humerus, and the cranial and exterior aspect of the ribs. Labeling was done with a Dremel® Electric Engraver Model #290.

Once the samples were properly labeled, the resin block-face was ground using the Hillquist® diamond plated grind wheel perpendicular to the shaft of the bone specimen (Figure

3.4). The exposed face of the block and bone was carefully smoothed on a glass pane with 1000 grit silicon carbide powder to remove surface scratches. The smoothed face of the histoblock could then be glued directly to the specimen slide. Two sizes of slides were necessary to accommodate the range of specimen sizes: 2”x1” petrographic slides were used for all ribs as well as smaller humeri and femora, and 3”x2” corning microscope slides were used for all other humeri and femora.

Resin blocks were glued to the slides using Extreme Power® 5-Minute Epoxy. In order to ensure a close seal, weights were placed on top of the specimens while the epoxy was setting. Two weighting systems were used. The first system utilized loose weights placed on the slides (Figure 3.5a). This method was convenient and allowed a large number of slides to be dried concurrently. The disadvantage to this method was that some histoblocks slid on the glass or dried slightly unevenly.

The second, more accurate method used a spring-loaded machine (Figure 3.5b). Though cumbersome and limited in the number of slides that could be set at a time, this method created more evenly and tightly glued resin blocks to the glass surface. In turn, this produced a more even specimen when ground down. The spring-loaded machine only became available part way through the gluing process; therefore, not all specimens were mounted using this technique.

Specimen slides were engraved using a glasscutter with the same specimen label and positional information as on the histoblocks. All labeling was done prior to slicing and grinding to ensure that all samples were properly tracked (Figure 3.6).

The resin blocks, glued to the slide, were sliced using the Hillquist® Re-Sectioning Saw rotating diamond rock-saw blade (Figure 3.7). This produced an approximately 1mm thick sample attached to the slide. Using the same grinding method used to expose the bone face, the sample, still attached to the slide, was carefully ground down to approximately 0.30-0.40mm.

All further grinding was done by hand on a sheet of glass. First, the slides were polished with 600 grit silicon carbide powder. The slides were then polished with 1000 grit silicon carbide powder (Figure 3.8). The polishing process ground the sample to approximately 0.10-0.30mm. This method also reduced the depth of surface scratches and appearance of surface unevenness.

Further polishing was necessary for slides that had dried unevenly or had an uneven surface due to taphonomic and/or preparational processes. For example, one area of the surface may be darker because of staining. Therefore, only that area of the slide would need to be ground slightly thinner than the other areas of the slide. Polishing paper (1000 grit) from RioGrande® 337308 was used to carefully polish down isolated areas.

The finished slide was slip covered with emersion oil, increasing visibility and helping to further eliminate scratches. At this point, the slides were prepared and ready for microscopic observation and photography (Figure 3.9).

3.7 Microscopy and Photography

All microscopic work was done in the Comparative Biomedical Sciences Microscopy Center, Louisiana State University School of Veterinary Medicine.

The Zeiss Axioplan® Transmitted Light Microscope with 10x and 5x objective and Zeiss Photoscope2® Transmitted Light Microscope with 2x objective were both used. The condenser was removed from both microscopes because of the low magnification. All images were captured using the Microfire Model #S99 808 Optical Camera by Optronics®.

All ribs were photographed with the 5x objective on the Zeiss Axioplan Transmitted Light Microscope. Humeri and femora were photographed with the 2x objective on the Zeiss Photoscope2 Transmitted Light Microscope. Sample sections from the anterior, anterior-medial, medial, anterior-lateral, lateral, posterior, posterior-medial and posterior-lateral of the humeral and femoral cross-sections were photographed on the Zeiss Axioplan with the 5x objective.

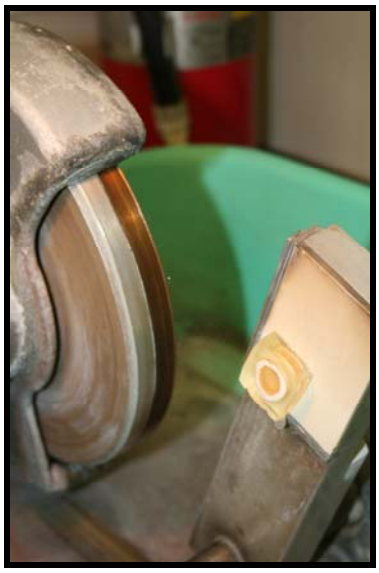


Figure 3.4: Grinding of Histoblock Face in Preparation for Mounting.

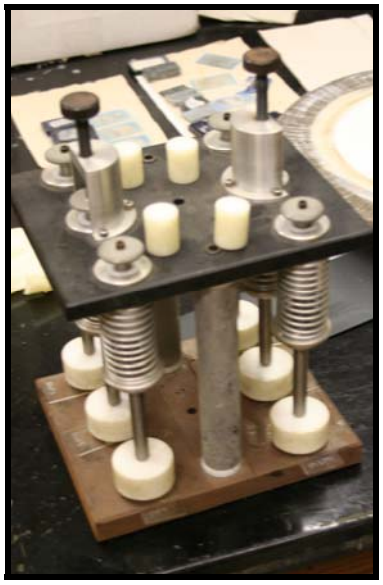


Figure 3.5a (left): Loose Weighting System.
Figure 3.5b (right): Spring-Loaded Weighting System.

.

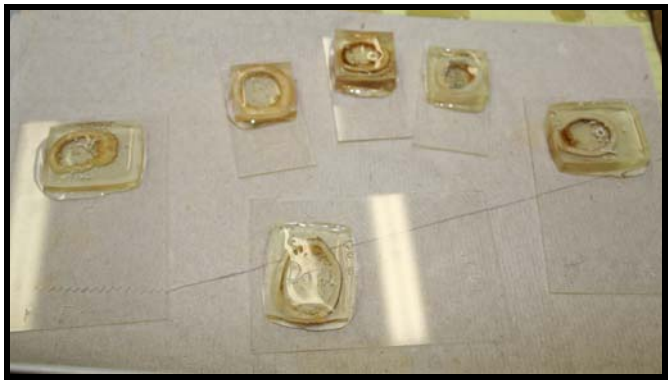


Figure 3.6: Mounted and Labeled Histoblocks Ready for Resectioning.

Figure 3.7: Slicing of 1mm Sample from the Histoblock on the Resectiong Saw.

Figure 3.8: Glass pane with Polishing 1000 grit Silicon Carbide Powder.

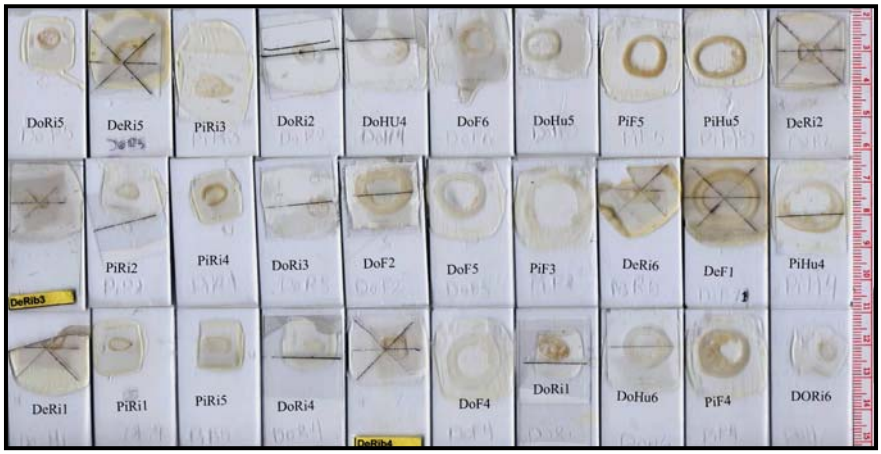


Figure 3.9: Processed and Labeled Slides Ready for Examination and Photography.

While not all slides were photographed at the same magnification, all magnifications were calibrated using a Graticules Pyser-SGI Ltd® 1mm/0.01 divisions calibrating slide (Figure 3.10a and 3.10b). To ensure that there was a consistency of results, despite the different magnifications, slides were cross checked; some slides were photographed at both magnifications, calibrated, and compared.

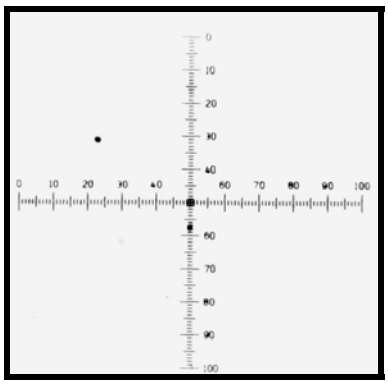


Figure 3.10a (left): Graticules Pyser-SGI Ltd ® Calibrating Slide 1mm/0.01 Divisions.
Figure 3.10b (right): 1mm/0.01 Calibration Image.

Images were captured using the program Picture Frame 2.1®. Initial settings for Auto White Balance were saved and loaded for all pictures. However, exposure was altered for each image individually to ensure optimal visualization. Figure 3.11 shows the photo-merged image of pig humerus #4 and displays an uneven cross-sectional surface due to processing challenges and surface differences in preservation. Lightening and darkening of the each slide were necessary.

3.8 Photo-merging

Photoshop CS® was used to photo-merge the images for each slide sample. All slide images were imported as jpegs into PhotoShop CS® along with a calibration slide image. While some slides had only twenty images, larger cross-sections from samples such as the femur may have well over sixty photo images to be merged. Photo-merging was only partially automated and many sections had to be merged by hand.

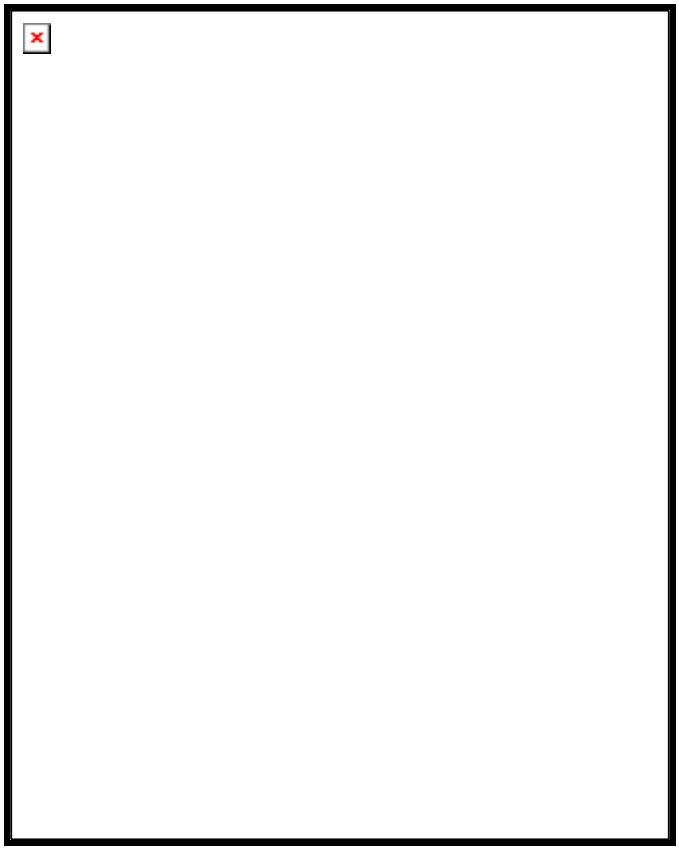


Figure 3.11: Bone Surface Differences in Processing, Preservation, and Organization. (Pig humerus 4).

3.9 Program and Quantitative Data Collection

All measurements were made on the imaging program Image J®. Images were individually calibrated within the program. Table 3.4 provides pixel and field diameter measures for each objective.

Table 3.4: Pixel and Field Diameter Measures for Objectives.

Objective	Pixels = 1µ	Field Diameter (mm)
10x	0.2740	1.1821
5x	0.6960	2.3236
2x	0.2705	3.1689

The Measurements function in Image J® allowed the user to set the program to measure several variables simultaneously. The Measurement tool was set to measure area and perimeter, as well as record X-Y coordinates. All measurements were summarized and exported to an Excel file.

Haversian canal measurements were made using the Elliptical tool. Secondary osteon measurements were made using the Freeform selection tool because of their variability in shape. Secondary osteons and Haversian canals were outlined along their reversal line or distinct outer edge (Jowsey 1966) (Figures 3.12a and 3.12b).

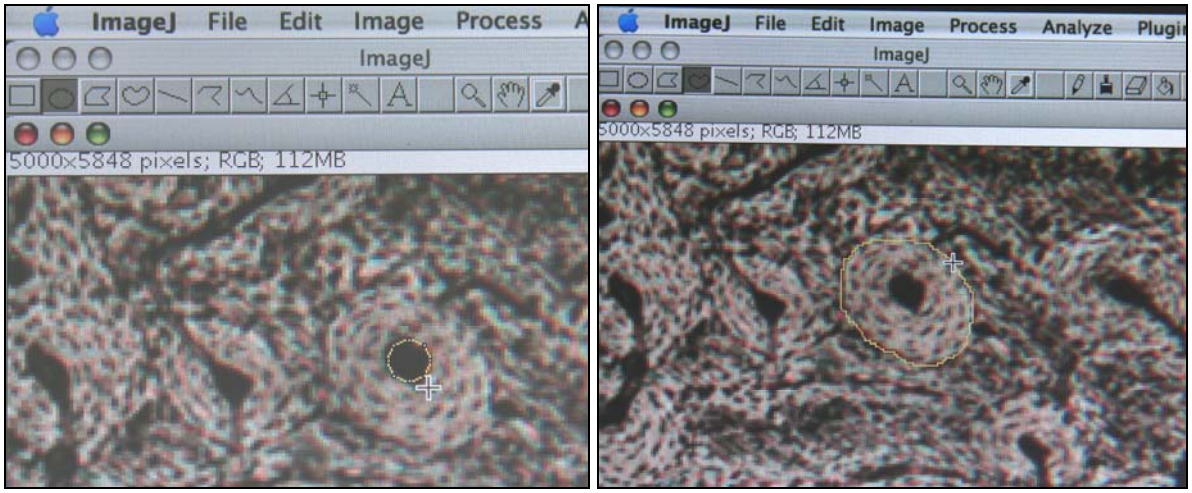


Figure 3.12a (left): Elliptical Tool Used to Measure Haversian Canals.
Figure 3.12b (right): Freeform Tool Used to Measure Secondary Osteons.

Only complete, secondary osteons were observed because of the inaccuracy of estimating the area of the partial secondary osteons.

Eight sample sections were established using the 1mm calibration slide. When the slide images were photo-merged, the calibration slide was set at the center of the completed slide image. The calibration slide was placed at the anterior-posterior-medial-lateral intersection of the femora and humeri and the intersection of the cranial-caudal-exterior-interior in the ribs. Using the 1mm calibration image as a guide, 1mm slide sample slices were established, moving clock-wise around the cross-section, in the anterior, anterior-lateral, lateral, posterior-lateral, posterior, posterior-medial, medial and anterior-medial planes for all femora and humeri and in the cranial, cranial-exterior, exterior, caudal-exterior, caudal, caudal-interior, interior, and cranial interior planes for all ribs (Figures 3.13a and 3.13b).

Only secondary osteons and Haversian canals within the sample sections of each slide were measured and recorded. For example, for one slide, there would be eight numeric files of osteon size and coordinates and eight numeric files of Haversian canal size and coordinates for each designated area: anterior, anterior-lateral, lateral, posterior-lateral, posterior, posterior-medial, medial and anterior-medial. A precedent for examining the slides by sections was set by authors such as Martiniakova et al. (2006) and Mulhern and Ubelaker (2001), who also analyzed their slides by dividing the cross-section into anterior, posterior, medial and lateral segments.

For each 1mm wide sample slice, the cortical area and cortical thickness were measured using the Polygon and Line tools, respectively. These measurements are reported in the results. Cortical thickness provides a sense of size and evenness of the cortical wall around the mid-shaft cross-section. Slice area and number of measured secondary osteons present an approximate (not absolute) gauge of secondary osteon density.

3.10 Qualitative Observations

Qualitative analysis of the sample cross-section included observations of presence or absence of defined structures and overall characteristics and patterns. The processing and preservation quality were also noted for each slide. Qualitative analysis was completed in the imaging program Picasa2®. Picasa2® permitted easy manipulation and magnification of the slide images. Observations were recorded on the Sample Record Sheet (see Appendix B and C) and included:

1) Presence or absence of banding;
2) Presence or absence of plexiform bone;
3) Processing quality;
4) Preservation quality, and;
5) Overall impressions.

Plexiform bone is characterized by its stacked, brick-like appearance. According to Benedix (2004:42), the plexiform structure is the result of a “conglomeration of woven and

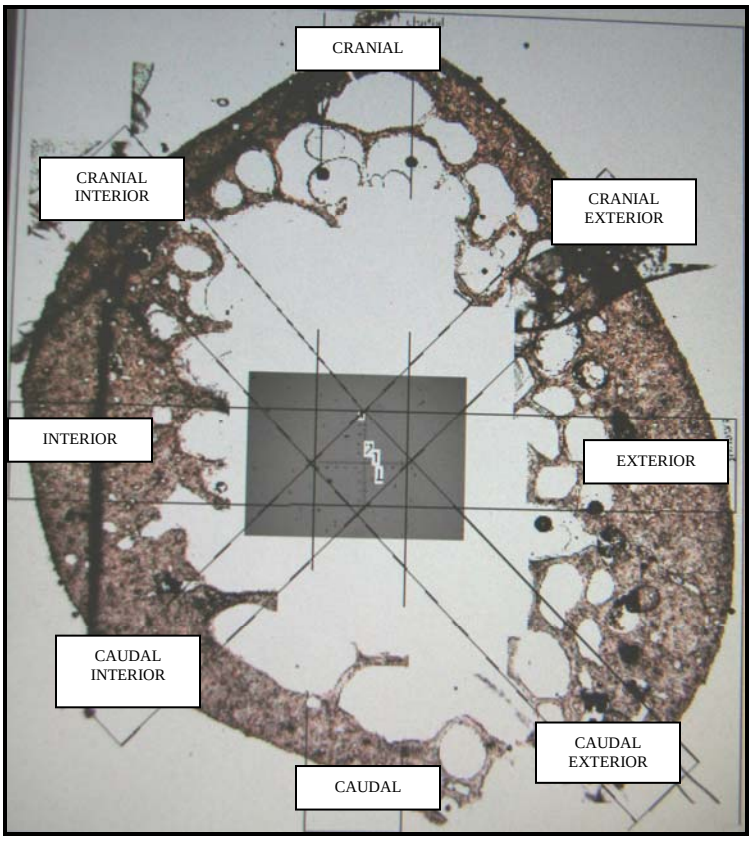


Figure 3.13a: Division of Slide into 1mm Wide Sample Sections for the Rib (Deer Rib 1).

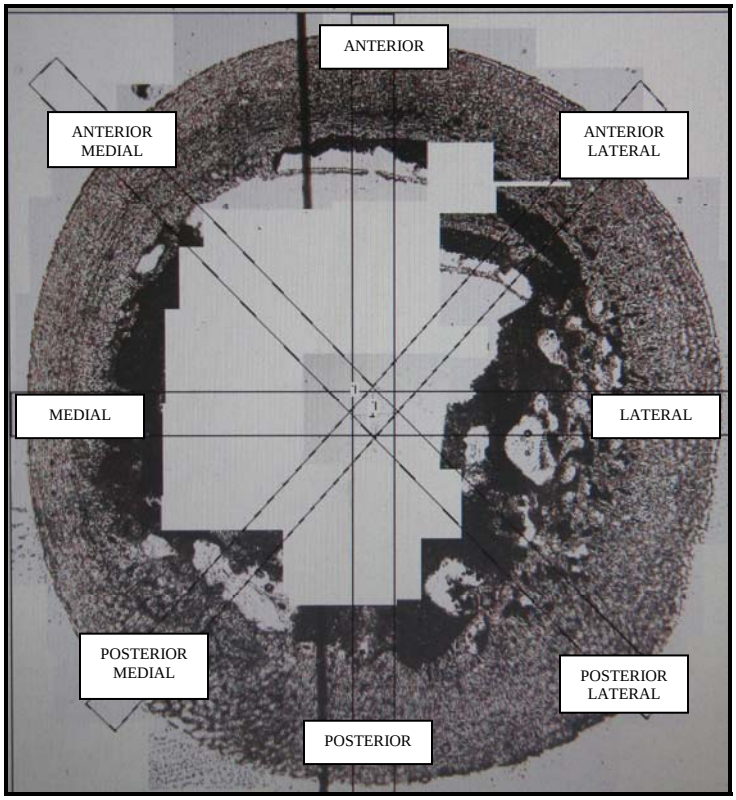


Figure 3.13b: Division of Slide into 1mm Wide Sample Sections for the long bones (Pig Femur 4).

lamellar bone at right angles to each other." As mentioned previously, plexiform bone is a defining feature on non-human bone. Figure 3.14 demonstrates the characteristic brick-like structure of plexiform bone.

Osteon banding is another histological feature associated with non-human bone. Mulhern and Ubelaker (2001:220) define osteon banding as the "arrangement of primary osteons into distinct rows or layers." Figure 3.15 illustrates an example of osteon banding.

Unlike the quantitative analysis that measured structures within the 1mm sample sections, qualitative observations were made across the entire cross-sectional surface. The cross-sectional surface was divided along the eight planes as described above.

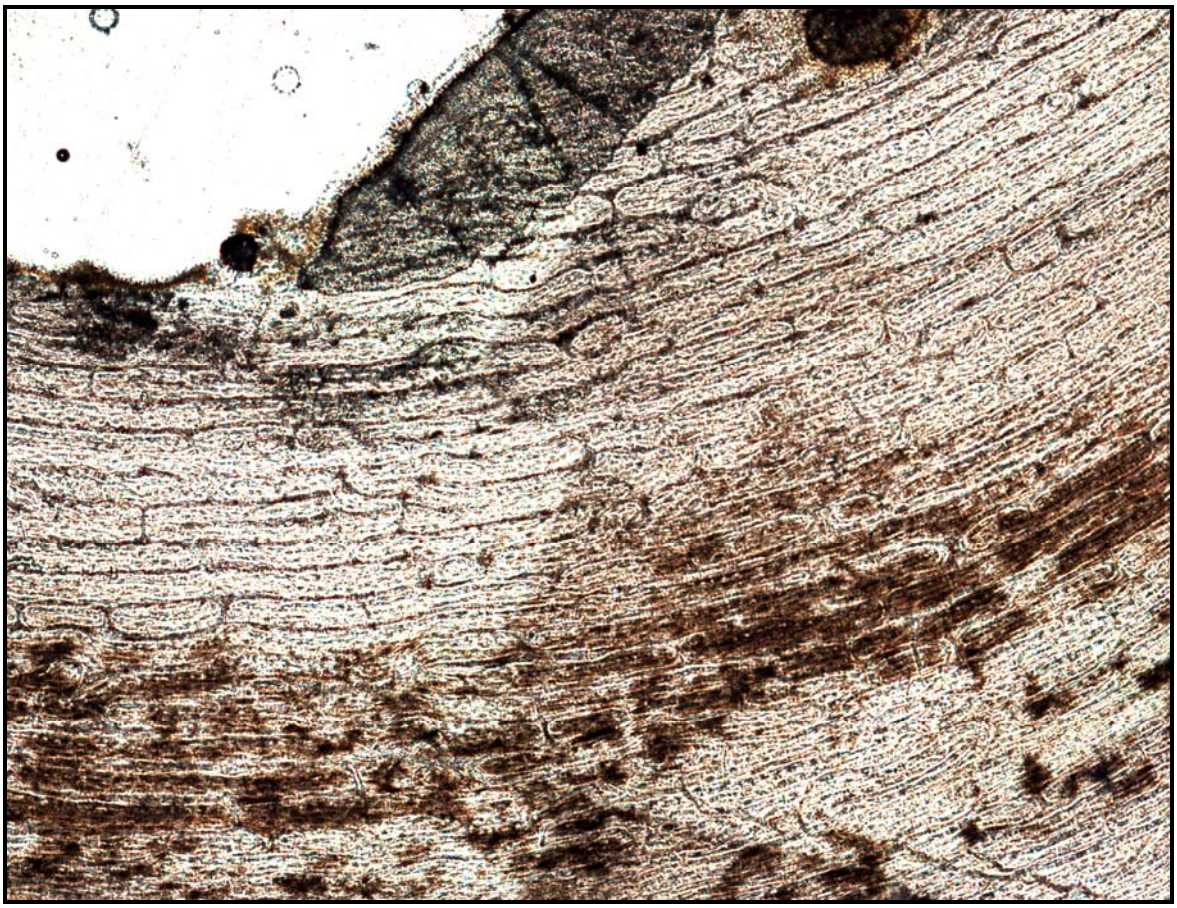


Figure 3.14: Brick-like Plexiform Bone (Posterior Section of Deer Femur 5).

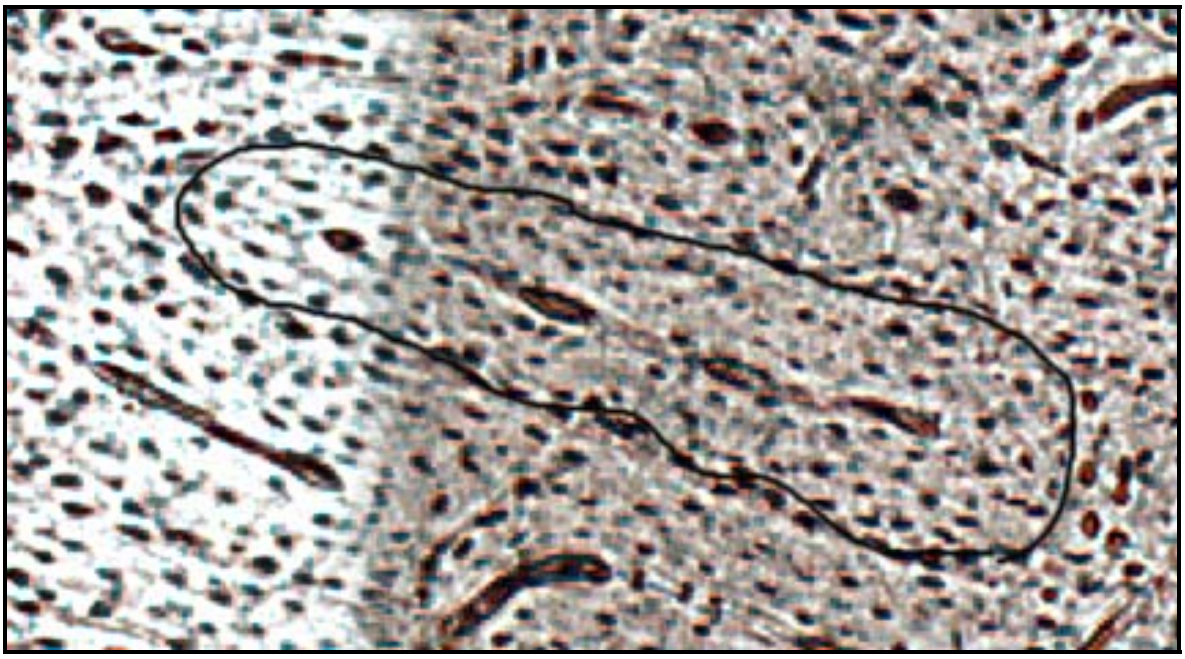


Figure 3.15: Example of Osteon Banding (Medial Section of Deer Femur 6).

Chapter 4: Results

4.1 Plexiform Bone

Plexiform bone has been used as a primary distinguishing histological feature of human and non-human bone (Benedix 2004). The results of this study indicate that 64% of all the long bones and 16.7% of the mid-thoracic ribs have plexiform bone present. Absence of plexiform bone does not indicate the bone is human, as 36% of long bones and 94% of the mid-thoracic ribs in this study did not have plexiform bone (Figure 4.1).

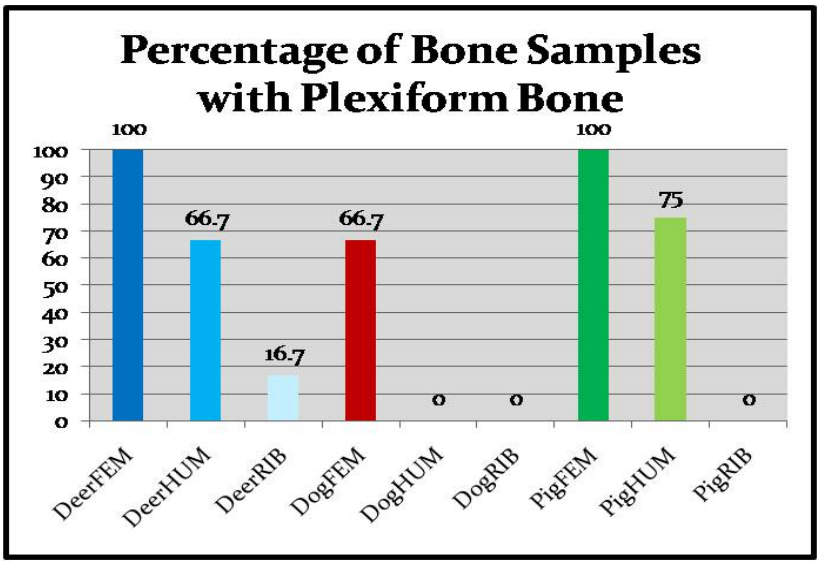


Figure 4.1: Percentage of Plexiform Bone Presence.

By species, deer and pig have the highest occurrence of plexiform bone (83% deer and 88% pig). All deer and pig femora observed have plexiform bone. On the other hand, only 33% of the dog long bones have plexiform bone. None of the dog humeri has plexiform bone. Only a single deer rib has an area of plexiform bone. The plexiform bone in the deer rib is not clearly defined structurally. No other mid-thoracic ribs have any area of plexiform bone.

There is a relatively even distribution of plexiform structures across the long bone cross-sections, but there are some fluctuations. The highest percentage of plexiform bone, 50% of total observed plexiform in long bone, is observed in the medial section of the femur and humerus.

The anterior-lateral and anterior-medial sections also have a high percentage of plexiform bone occurrences. The posterior aspect of the bone showed the lowest plexiform bone presence of only 23%. Of the one deer rib with plexiform type bone, the bone structure is present only in the interior section and cranial-interior section of the rib cross-section (Table 4.1).

Table 4.1 Percentage of Plexiform Bone Presence by Section.

	Anterior	Anterior Lateral	Anterior Medial	Lateral	Medial	Posterior	Posterior Lateral	Posterior Medial	% Samples with Plexiform Bone	% Samples with Plexiform Bone
DeerFEM	66.7%	100%	66.7%	100%	100%	66.7%	100%	100%	100%	83.0%
DeerHUM	66.7%	66.7%	66.7%	66.7%	33.3%	0	33.3%	0	66.7%	
DogFEM	0	33.3%	33.3%	0	33.3%	0	0	33.3%	66.7%	33.0%
DogHUM	0	0	0	0	0	0	0	0	0	
PigFEM	100%	50.0%	100%	25.0%	100%	0	0	75.0%	100%	88.0%
PigHUM	0	50.0%	25.0%	75.0%	50.0%	75.0%	75.0%	25.0%	75.0%	

	Cranial	Cranial Exterior	Cranial Interior	Exterior	Interior	Caudal	Caudal Exterior	Caudal Interior	% Samples with Plexiform Bone
DeerRIB	0	0	16.7%	0	16.7%	0	0	0	16.7%
DogRIB	0	0	0	0	0	0	0	0	0
PigRIB	0	0	0	0	0	0	0	0	0

4.2 Osteon Banding

The presence of osteon banding follows a different pattern in comparison to plexiform bone. Overall, only 23% of all the long bones exhibit osteon banding. Of the femur, 40% have osteon banding, while only 8% of the humeri have osteon banding. No rib samples had osteon banding (Figure 4.2). Results for the individual species indicate that deer long bones have osteon banding present in 50% of the samples. The pigs have osteon banding in 25% of the samples. No dog bones have any area of osteon banding.

The distribution of the osteon banding is distinct, with the majority of the bone sections absent of osteon banding. Osteon banding is present in 18% of the medial, long bone sections. The only other areas, with a single occurrence of osteon banding each, were the anterior section and posterior-medial section of the long bone cross-sections (Table 4.2).

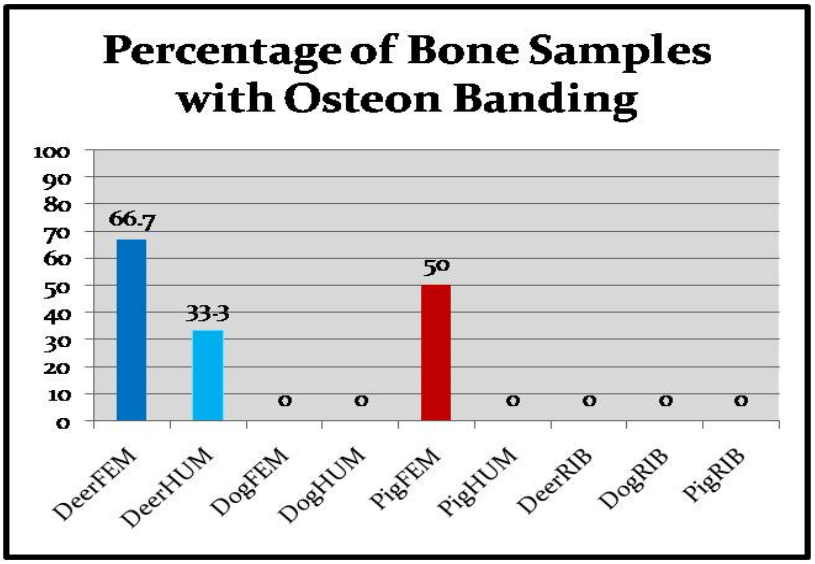


Figure 4.2: Percentage of Osteon Banding Presence.

Table 4.2 Percentage of Osteon Banding Presence by Section.

	Anterior	Anterior Lateral	Anterior Medial	Lateral	Medial	Posterior	Posterior Lateral	Posterior Medial	% Samples with Osteon Banding	% Samples with Osteon Banding
DeerFEM	0	0	0	0	66.7%	0	0	33.3%	66.7%	50.0%
DeerHUM	0	0	0	0	33.3%	0	0	0	33.3%	
DogFEM	0	0	0	0	0	0	0	0	0	0.0
DogHUM	0	0	0	0	0	0	0	0	0	
PigFEM	25	0	0	0	25.0%	0	0	0	50	25.0%
PigHUM	0	0	0	0	0	0	0	0	0	

	Cranial	Cranial Exterior	Cranial Interior	Exterior	Interior	Caudal	Caudal Exterior	Caudal Interior	% Samples with Osteon Banding
DeerRIB	0	0	0	0	0	0	0	0	0
DogRIB	0	0	0	0	0	0	0	0	0
PigRIB	0	0	0	0	0	0	0	0	0

4.3 Mid-Thoracic Rib: Secondary Osteon Area

The quantitative analysis of the Haversian system included the measurement of complete secondary osteon area and complete Haversian canal area within the 1mm wide sample of each section. Perimeter and X-Y coordinates were recorded but are not reported. The number of whole secondary osteons per measured sample area is reported and offers a general gauge of secondary osteon density. This measurement is not the true secondary osteon density, as incomplete osteons were not counted.

The mean secondary osteon area for all sections of the pig ribs is 1.13 ± 0.57 µm² x 10^4. Deer has a mean osteon area of 1.13 ± 0.59 µm² x 10^4. Dog has the smallest overall mean osteon area with an average of 1.03 ± 0.54 µm² x 10^4. The secondary osteon area of deer and pig ribs is comparable, though no spatial parallel is noted between the two species (Table 4.3).

Table 4.3: Mean Area (µm² x 10^4) of Secondary Osteons by Rib Section.

	Cranial	Cranial Exterior	Exterior	Caudal Exterior	Caudal	Interior	Caudal Interior	Cranial Interior	Overall Mean*	Overall Standard Deviation	Coefficient of Variation
Deer	1.22	1.04	1.21	1.02	1.02	1.27	1.13	0.98	1.13	0.59	0.53
Dog	1.02	0.87	0.99	1.11	0.85	1.28	1.09	1.01	1.03	0.54	0.53
Pig	1.22	1.22	1.28	1.22	1.06	1.03	0.94	1.14	1.13	0.57	0.51

*Overall mean: the mean of all measurements for the skeletal element of that species.

Examination of Figure 4.3 indicates secondary osteon means for all three species decreases in the caudal rib section, in particular dog. The results also reveal a parallel in secondary osteon area changes of deer and dog. Moving clock-wise around the rib cross-section, osteon area decreases in size from the cranial section to the cranial-exterior section, followed by increase in osteon size. From the caudal through caudal-interior section, both deer and dog osteon area increase in size, followed by a sharp decrease. Even though the pig ribs' overall secondary osteon area is similar to deer, inspection of the data indicates the pig's secondary osteon area, for most sections, follows a different pattern relative to the other species.

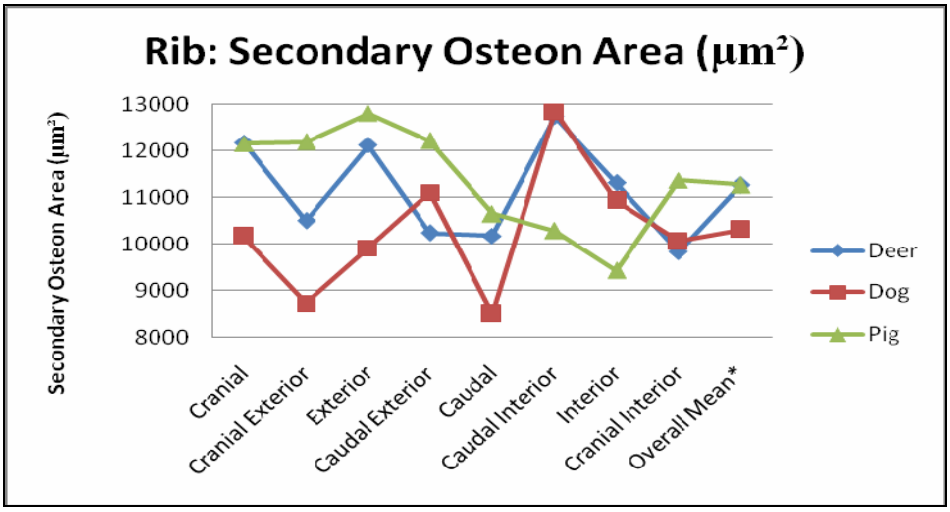


Figure 4.3: Mean Area (µm²) of Secondary Osteons by Rib Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

4.4 Mid-Thoracic Rib: Haversian Canal Area

An observation made regarding the preservation quality for some pig samples should be noted at this time. Histologically, the surface of some pig sections appeared 'scraped out'. The surface degradation may be related to processing of the bone. The surface appearance made it difficult to measure some structures. It was necessary to be more selective of measurements within the pig cross-section compared to the other species because it was difficult to assess whether a Haversian canal area was the true histological structure or degraded hole in the bone (Figure 4.8).

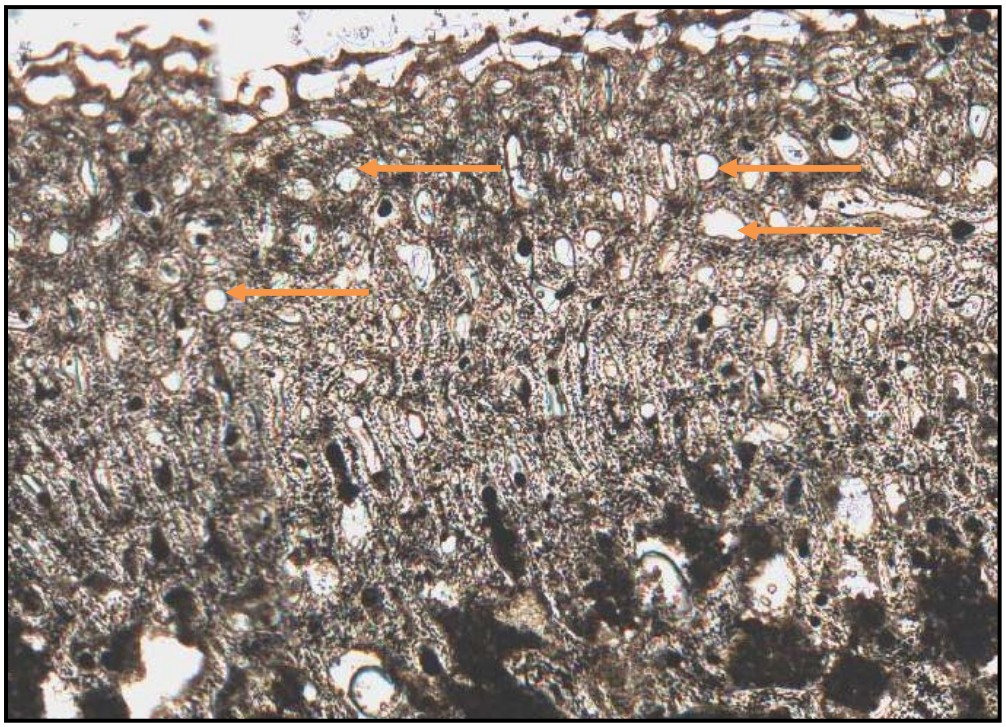


Figure 4.4: Degraded Appearance of Some Pig Bone Cross-Sections. Arrows indicate Haversian canals that appear to be larger due to processing than they may have been in life.

The Haversian canal area of the ribs did not demonstrate a similar pattern to the secondary osteon area. Deer has a relatively small overall mean canal area compared to the other species with an average of 2.45 ± 1.64 $\mu m^2 \times 10^2$. Dog Haversian overall canal area is 3.92 ± 2.59 $\mu m^2 \times 10^2$. Pig had a distinctly higher overall Haversian canal area than the other two species, with a mean of 6.02 ± 4.69 $\mu m^2 \times 10^2$ (Table 4.4).

Table 4.4: Mean Area (µm² x 10^2) of Haversian Canals by Rib Section.

	Cranial	Cranial Exterior	Exterior	Caudal Exterior	Caudal	Interior	Caudal Interior	Cranial Interior	Overall Mean*	Overall Standard Deviation	Coefficient of Variation
Deer	2.47	2.41	2.19	2.23	2.25	2.53	2.52	3.37	2.45	1.64	0.67
Dog	4.45	3.27	4.54	3.74	3.83	3.51	3.92	4.04	3.92	2.59	0.66
Pig	5.84	7.96	6.85	7.49	7.99	4.12	4.00	5.23	6.02	4.69	0.78

*Overall mean: the mean of all measurements for the skeletal element of that species.

Deer and dog samples' mean section areas remain relatively constant when examining the area changes moving clock-wise around the cross-section. Both dog and pig canal area decreases at the interior rib section, followed by an increase in osteon area along the caudal-interior aspect of the rib (Figure 4.5).

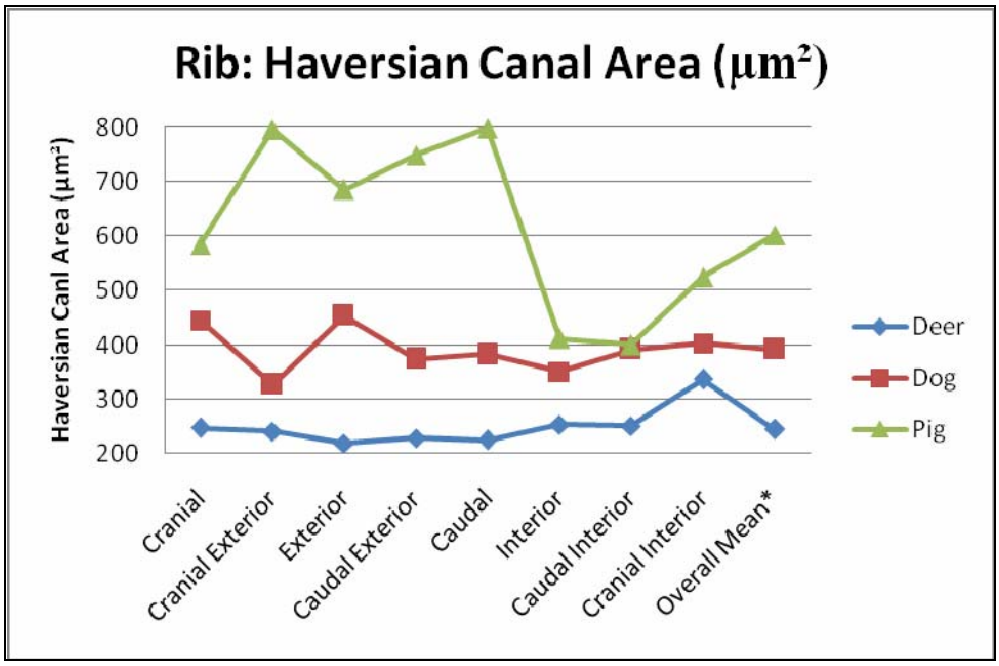


Figure 4.5: Mean Area (µm²) of Haversian Canals by Rib Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

Pig Haversian canal area is larger than deer and dog canal area for most of the rib sections. Pig canal area is more than double that of deer canal area. The pig Haversian canal area fluctuates in a similar ratio to the pig osteon area. Canals on the exterior of the rib cross-section are larger than canals on the pig samples' interior rib cross-section. This may indicate that the entire Haversian system size is changing around the rib cross-sections.

4.5 Mid-Thoracic Rib: Osteon Density

Dog has a mean density (osteons measured/area mm) of 28.326 ±5.035. Deer and pig have very similar densities of 15.538 ±4.107 and 19.425 ±4.033, respectively. The number of whole secondary osteons per area suggests that dog ribs have relatively higher density secondary osteons per area relative to deer or pig (Figure 4.6). During analysis, I noted that the dog bones were visually distinct from the deer and pig bones because of the high density of similar sized osteons across the dog's cross-sectional surface. No inter-species spatial relation is visible by mid-thoracic rib section.

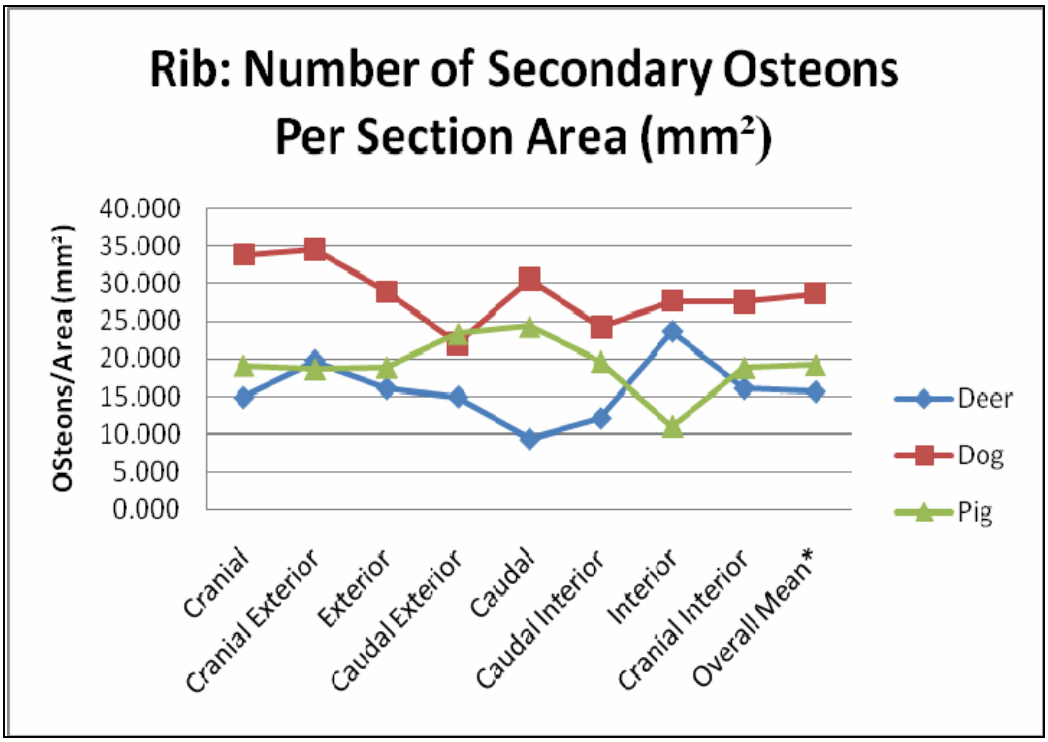


Figure 4.6: Mean number of Whole Secondary Osteons Measured per Rib Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

4.6 Mid-Thoracic Rib: Cortical Thickness

A pattern emerges across all three species in terms of cortical thickness. An increase in cortical thickness is evident along the cranial section through to the exterior section of the rib cross-section. There is a distinct decrease in cortical thickness along the caudal margin for all

three species. For all three species, the thinnest bone section is the caudal margin. The decrease along the caudal margin is followed by an increase in cortical thickness along the interior of the rib. For all three species, their thickest section is along the interior or caudal-interior of the rib. The cortical thinning along the caudal border may correspond with the costal groove on the ribs. Overall, pig cortical bone is the thinnest compared to the other two species. Deer has thicker cortical bone, overall and for every section, relative to pig and dog (Figure 4.7).

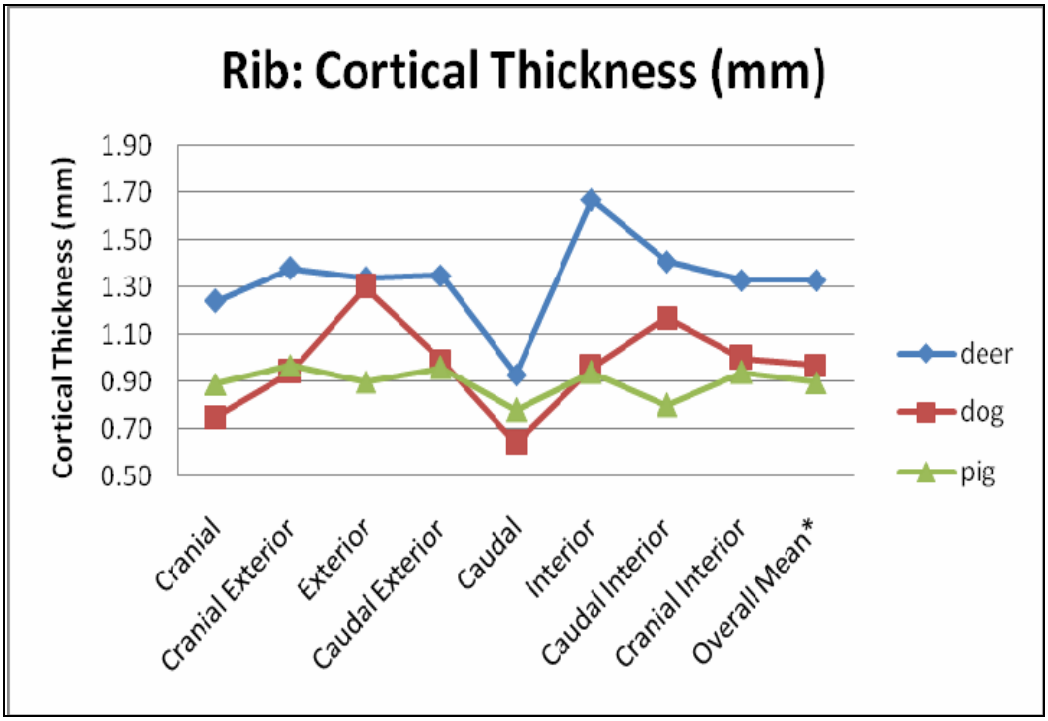


Figure 4.7: Mean Cortical Thickness (mm) Measured per Rib Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

4.7 Femur and Humerus: Secondary Osteon Area

Instead of individually analyzing the femur and humerus, the results for both long bones are reported together. Patterns that emerge within the same species are simultaneously examined with patterns that occur between like skeletal elements of different species.

Overall, pig humeri and femora have larger mean secondary osteon area compared to deer and dog. Pig humeri demonstrated the greatest variability in osteon area according to the coefficient of variation. For the femur, the pig's mean secondary osteon area is 1.97 ± 0.87 μm^2

x 10^4. The mean secondary osteon area for the pig humerus is 2.51 ± 1.66 μm² x 10^4. These osteon area measurements are in sharp contrast to the smaller osteon area for both dog and deer. Deer have a mean secondary osteon area of 1.39 ± 0.65 μm² x 10^4 for the femur and 1.47 ± 0.60 μm² x 10^4 for the humerus. Dogs have a femur mean of 1.56 ± 0.67 μm² x 10^4 and humerus mean of 1.49 ± 0.74 μm² x 10^4. Secondary osteon area is most similar between the humerus and femur within each species. However, the relationship between intraspecies humeri and femora is not identical for all three species. For deer and pig, the osteon area of the humerus is larger than that of the femur, while for dogs, the osteon area is overall larger for the femur than the humerus (Table 4.5).

Table 4.5: Mean Area (μm² x 10^4) of Secondary Osteons by Femur and Humerus Section.

	Anterior	Anterior Lateral	Lateral	Posterior Lateral	Posterior	Posterior Medial	Medial	Anterior Medial	Overall Mean*	Overall Standard Deviation	Coefficent of Variation
DeerFEM	1.12	1.34	1.70	1.56	1.51	1.48	1.09	1.25	1.39	0.65	0.47
DeerHUM	1.59	1.58	1.54	1.66	1.46	1.46	1.41	1.22	1.47	0.60	0.41
DogFEM	1.60	1.73	1.57	1.59	1.59	1.60	1.40	1.42	1.56	0.67	0.43
DogHUM	1.39	1.37	1.76	1.73	1.45	1.47	1.41	1.46	1.49	0.74	0.49
PigFEM	1.99	1.54	1.76	2.35	2.37	1.58	1.79	1.78	1.97	0.87	0.44
PigHUM	2.30	2.42	2.82	1.99	2.76	2.25	2.75	2.82	2.51	1.66	0.66

*Overall mean: the mean of all measurements for the skeletal element of that species.

Despite the observation that osteon area appears to be species related, examining the cross-sectional change in osteon size across the bone surface reveals another pattern. Parallels between the spatial changes in mean osteon size of deer and dog long bone cross-sections appear to follow skeletal element (dog femur and deer femur, dog humerus and deer humerus), as opposed to species. Pig humerus follows a separate trend relative to pig femur and the bones of the other species, with no obvious relation to any other bone (Figure 4.8).

On closer examination of the deer and dog osteon area, a spatial trend emerges. Following the deer femur and dog femur osteon area clock-wise around the long bone cross-section, we see an increase in osteon areas along the anterior through anterior-lateral sections.

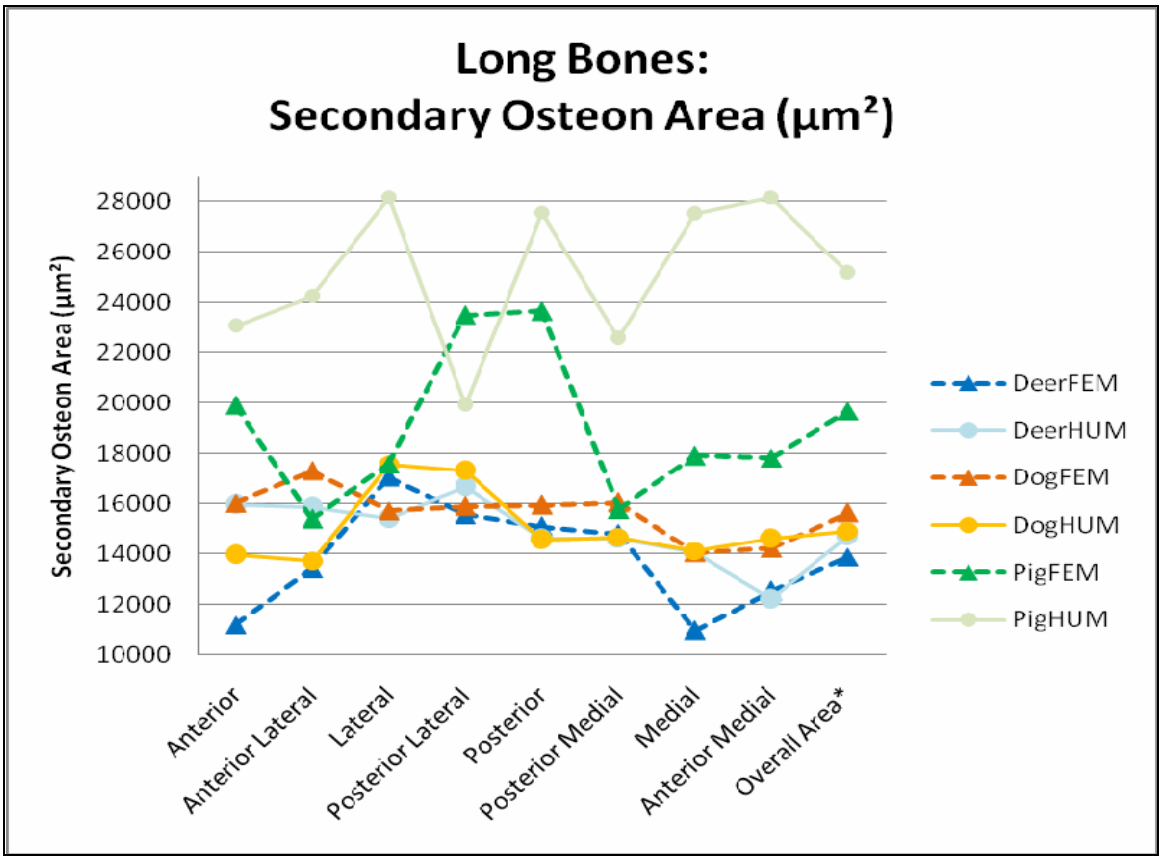


Figure 4.8: Mean Area (µm²) of Secondary Osteons by Femur and Humerus Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

Mean osteon area remains relatively stable along the posterior-lateral through posterior-medial sections of the bone. A sharp decrease in osteon area, followed by a slight increase, is noted along the anterior-medial aspect of both the deer and dog femora's cross-sections (Figure 4.9a).

On the other hand, deer and dog humerus follow a different path. Figure 4.9b indicates humeral osteon area is relatively stable along the anterior and anterior-lateral sections. Osteon area increases along the lateral margin, followed by a decrease in mean osteon area along the posterior section and again, along the medial section. Not only do the deer and dogs' secondary osteon areas of the humerus parallel one another, the absolute areas are similar for all sections from the posterior-lateral to medial aspect of the bone. The lateral and anterior-medial sections are the exceptions to this trend. At these sections the two species diverge from one another in terms of their osteon area.

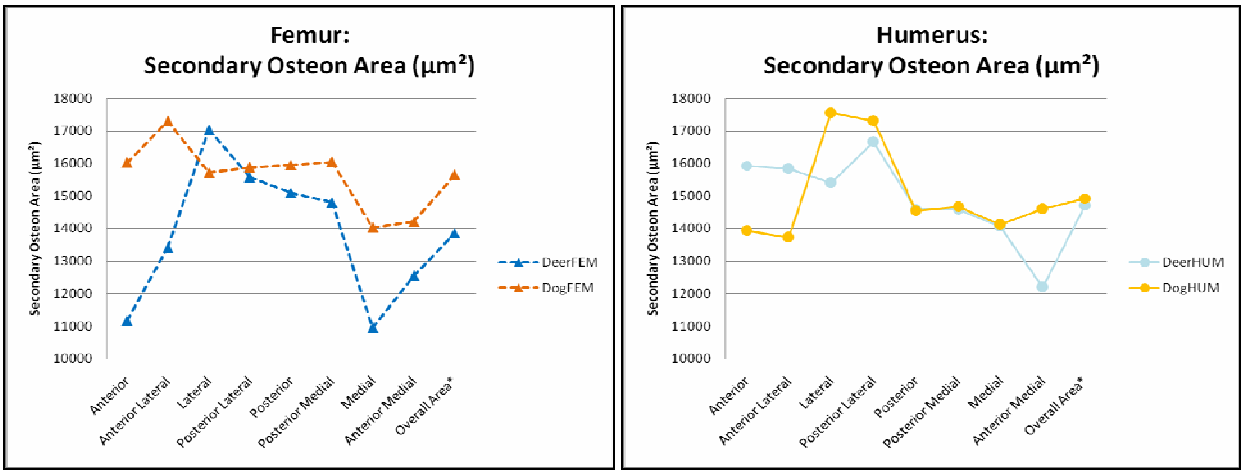


Figure 4.9a (left): Mean Area (µm²) of Deer and Dog Secondary Osteons by Femur section.
Figure 4.9b (right): Mean Area (µm²) of Deer and Dog Secondary Osteons by Humerus Section.
*Overall mean: the mean of all measurements the skeletal element of that species.

4.8 Femur and Humerus: Haversian Canal Area

In a similar pattern to the secondary osteon area, pig Haversian canal area is larger and fluctuates more than both deer and dog. All pig sections, except the medial section of the pig femur, have larger mean canal sizes than deer and dog. Pig overall mean femoral Haversian canal area is 6.45 ± 3.41 µm² x 10^2. The mean humeral Haversian canal area for pig is 7.75 ± 5.60 µm² x 10^2. Deer femora have a mean canal area of 3.87 ± 2.05 µm² x 10^2. The mean deer humerus canal area is 4.01 ± 1.86 µm² x 10^2. Dog femora have a mean of 4.32 ± 3.14 µm² x 10^2 and for the dog humerus the mean is 3.14 ± 2.27 µm² x 10^2. Overall, the entire pig Haversian system, both osteons and canals, is larger than the other two species. More specifically, the pig's humeri samples have larger Haversian systems than the pig's femora samples. Larger humeral Haversian systems are also noted in the results for the dog; however, the difference in overall means is not as distinct (Table 4.6).

There are not the same patterned spatial trends between like skeletal elements evident in the Haversian canal data as there are for the secondary osteon area, although there are some similarities between deer and dog humeri, and deer and dog femora (Figure 4.10).

Table 4.6: Mean Area (µm² x 10^2) of Haversian canals by Femur and Humerus Section

	Anterior	Anterior Lateral	Lateral	Posterior Lateral	Posterior	Posterior Medial	Medial	Anterior Medial	Overall Mean*	Overall Standard Deviation	Coefficent of Variation
DeerFEM	3.52	3.90	3.80	3.97	4.78	3.92	3.01	3.34	3.87	2.05	0.53
DeerHUM	3.56	4.72	4.75	4.04	3.78	4.13	3.51	3.92	4.01	1.86	0.46
DogFEM	4.38	3.78	4.44	4.09	4.59	4.06	4.23	4.35	4.32	1.82	0.42
DogHUM	3.69	3.62	3.05	3.34	2.84	2.58	2.85	3.02	3.14	2.27	0.72
PigFEM	7.73	5.90	6.30	7.86	5.60	5.42	5.48	6.97	6.45	3.41	1.20
PigHUM	6.74	8.04	5.90	8.63	8.56	6.09	8.58	9.87	7.75	5.60	0.72

*Overall mean: the mean of all measurements for the skeletal element of that species.

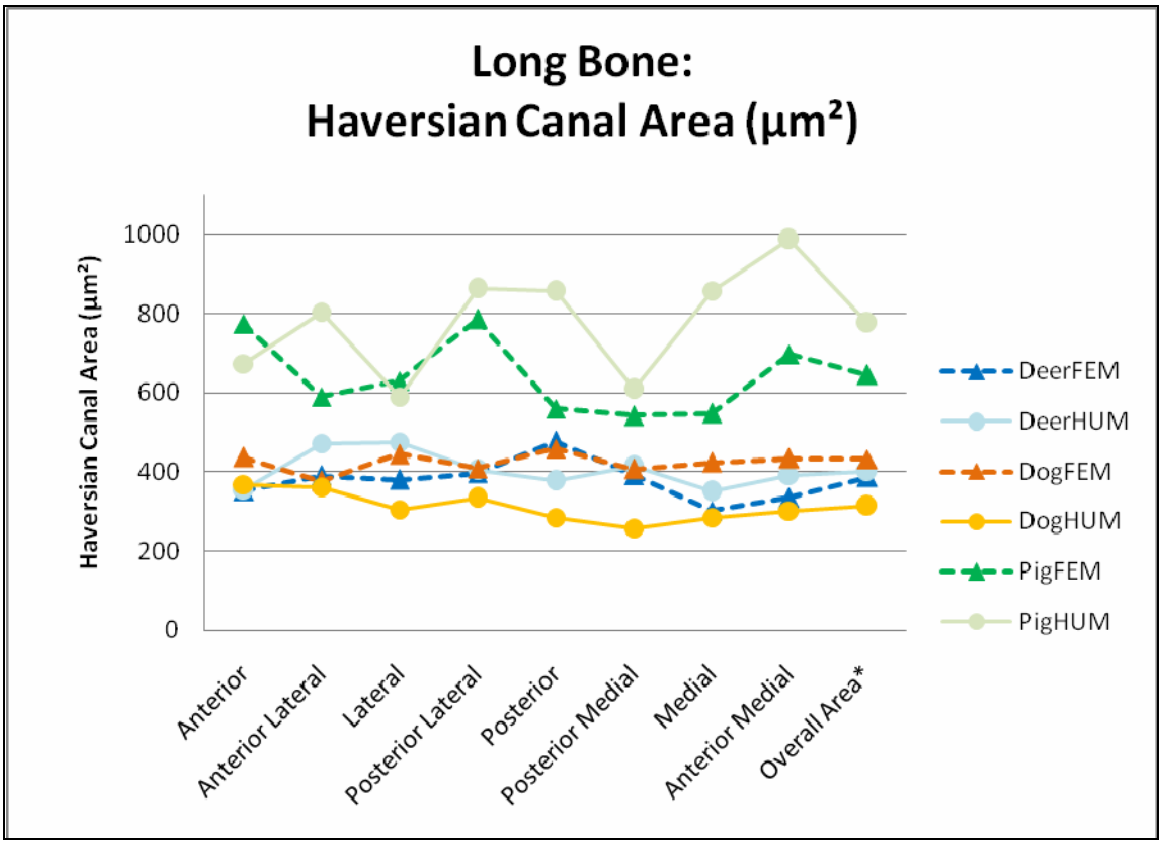


Figure 4.10: Mean Area (µm²) of Haversian Canals by Femur and Humerus Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

Haversian canal areas are most similar between like species. The humerus and femur of each species canal area changes around the cross-section in a similar pattern within species. The parallel changes in the femur and humerus within each species is strongest along the medial side of the long bones' cross-section (Figure 4.11a and Figure 4.11b). Pig Haversian canal means

follow a similar trend for the humerus and femur around the entire cross-section, even though the means are distinctly different for the two elements (Figure 4.11c).

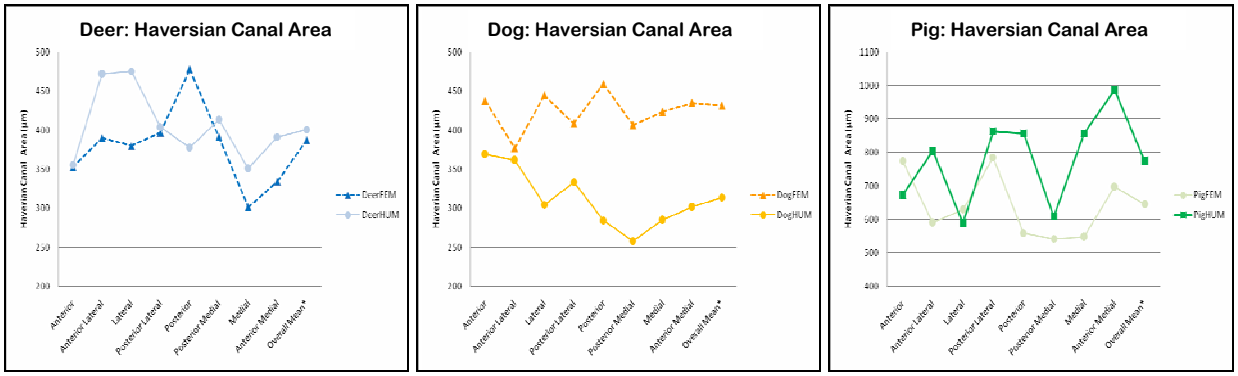


Figure 4.11a (left): Mean Area (µm²) of Deer Haversian Canals by Long Bone Section.
Figure 4.11b (center): Mean Area (µm²) of Dog Haversian Canals by Long Bone Section.
Figure 4.11c (right): Mean Area (µm²) of Pig Haversian Canals by Long Bone Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

Examining the osteon and Haversian canal areas for all skeletal elements, several patterns emerge. First, pig Haversian canal areas are all distinctly larger than the corresponding areas of deer and dog (Table 4.7 and Table 4.8). For many of the mid-thoracic rib cross-sections, pigs' Haversian systems are larger than that of deer and dog.

Second, pig long bone Haversian canal area fluctuates more for each element compared to both deer and dog, according to its co-efficient of variation. Pigs' humeri also demonstrate greater variability than the humeri of deer and dog. Deer and dog are not distinctly different in terms of the area of their long bone osteon or Haversian canal. On the other hand, dog mid-thoracic ribs have distinctly smaller areas relative to the other species. For a more detailed examination of the overall statistics of the species, see Appendix D.

Secondary osteon and Haversian canal areas of deer and dog remain relatively similar for all elements. For secondary osteon area there is a parallel increase in osteon area from rib to humerus for the three species. The humerus has the greatest overall area for pig and deer. Pig samples have relatively large humeral Haversian canals compared to their femoral Haversian

canal areas. This oberservation may be associated with lower plexiform bone presence in the humerus versus the femur of both deer and pig (Figure 4.12 and Figure 4.13).

Table 4.7: Overall Secondary Osteon Mean Area* ($\mu m^2 \times 10^4$) by Species and Element

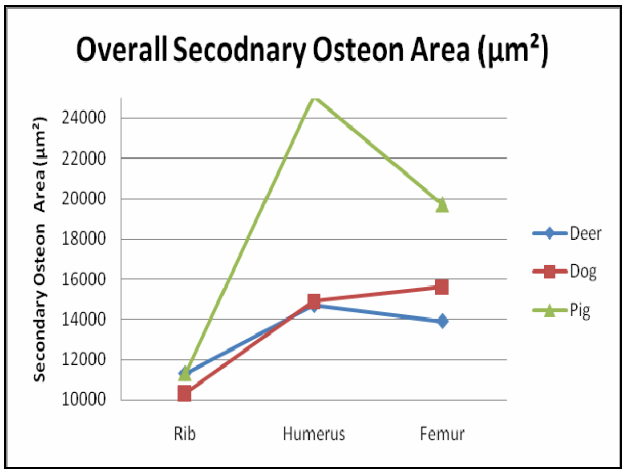
	Rib	Humerus	Femur
Deer	1.13	1.47	1.39
Dog	1.03	1.49	1.56
Pig	1.13	2.51	1.97

*Overall mean: the mean of all measurements for the skeletal element of that species.

Table 4.8: Overall Haverisan Canal Mean Area* ($\mu m^2 \times 10^2$) by Species and Element

	Rib	Humerus	Femur
Deer	2.45	4.01	3.87
Dog	4.00	3.14	4.32
Pig	6.02	7.75	6.45

*Overall mean: the mean of all measurements for the skeletal element of that species.



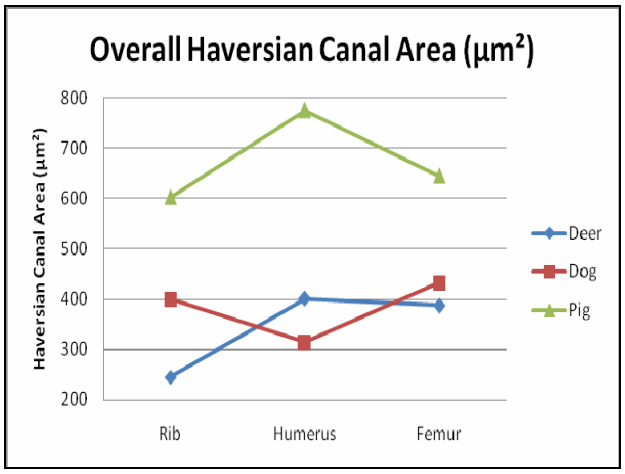


Figure 4.12 (left): Overall Secondary Osteon Area* (μm^2) by Species and Element
Figure 4.13 (right): Overall Haversian Canal Area* (μm^2) by Species and Element
*Overall mean: the mean of all measurements for the skeletal element of that species.

4.9 Femur and Humerus: Osteon Density

The dog long bone samples have the densest secondary osteon per cortical area for every section. All the humerus dog samples, within every section, showed greater secondary osteon density than the dog femur samples. This trend is not seen in the other species. Overall, pigs have the lowest secondary osteon density by area (Figure 4.14).

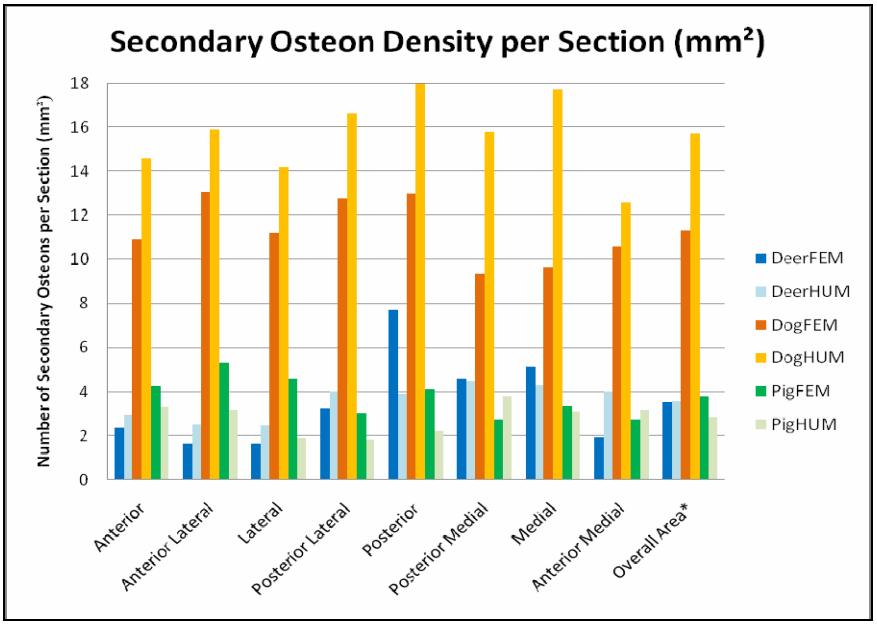


Figure 4.14: Whole Secondary Osteons Measured per Long Bone Area (mm²)
*Overall mean: the mean of all measurements for the skeletal element of that species.

4.10 Femur and Humerus: Cortical Thickness

Cortical thickness follows an expected trend: thicker cortical bone in the femur (generally the larger of the two bones) versus the humerus. One exception to this observation is the thicker cortical area in the posterior of the pig humerus relative to the pig femur (Figure 4.15).

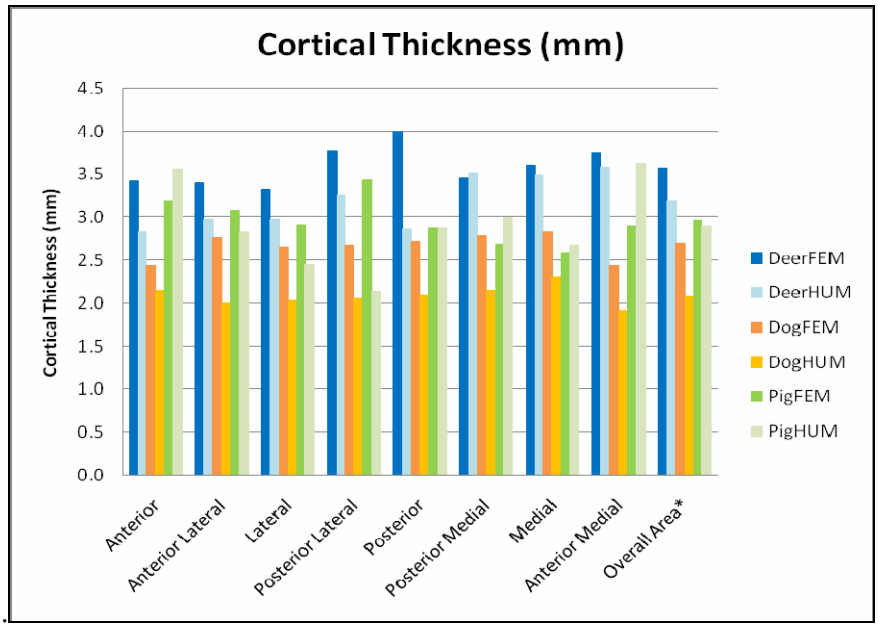


Figure 4.15: Mean Cortical Thickness (mm) Measured per Long Bone Section.
*Overall mean: the mean of all measurements for the skeletal element of that species.

Chapter 5: Discussion

At the onset of the research, three goals were set forth. I collected a wide range of data including measurements, presence/absence observations, and spatial relationships to fulfill the three objectives. The results of this study address all three of the following goals:

1) The quantitative and qualitative comparison of three different species' microscopic skeletal structures and the histological spatial organization of these structures;

2) The quantitative and qualitative comparison of different skeletal elements in terms of their microscopic structures and the histological spatial organization of these structures, and;

3) The contribution that quantitative, structural, and organizational information provides for species differentiation, including human versus non-human comparisons.

5.1 Interspecies Comparison

Deer, dog, and pig were quantitatively and qualitatively compared. Overall, structural differences are noted among the three species in this project. The quantitative comparison suggests there may be histomorphometric variations in the area of Haversian canals and secondary osteons for the species tested in this study. Further studies are needed to confirm these results.

The presence/absence results for plexiform bone and osteon banding show a higher percentage of these structures in deer and pig relative to dog. These results indicate differences in the microscopic structure and organization of bone between different species. The presence/absence of plexiform bone corroborates other researchers' observations that plexiform bone is typical among *Suidae* and *Cervidae* (Owsely et al. 1985). The relatively low frequency of plexiform bone among the dog samples suggests a different structural pattern for this species. These results reinforce the importance of quantitative analysis when looking for a methodology to differentiate between species because presence/absence observations are not enough to differentiate among species or human versus non-human skeletal remains.

Mulhern and Ubelaker (2001) noted other researchers had found osteon banding in dog fragments. In this study, there is a complete absence of osteon banding in the dog specimens. This inconsistency is unclear, but may be related to the small sample size.

The results for secondary osteon and Haversian canal area reveal some important patterns. First, pigs have a larger mean Haversian canal area compared to deer and dog samples. One possible explanation is the preservation quality of the bone. As noted in the results, the pig microscopic surface did not appear consistent, possibly as a result of bleaching or boiling the bone. The quality of the bone morphology made it difficult to assess certain microscopic structures. Larger Haversian canals may be over-represented from these slides.

Another factor that may have affected the pigs' osteon and Haversian canal area results was the relatively young age of the sample. Differences in maturation rates of the skeletal elements may result in areas of variable osteon and Haversian canal size. This assumption is supported by research such as Hamrick's (1999) study that reports histological differences that are dependent on developmental stage of the species.

The incomplete secondary osteon density analysis was presented as an estimate of the true secondary osteon density. The patterns seen in the results suggest a more focused density analysis should be conducted. The low, overall secondary osteon density for the pigs is likely due to the fact that all pigs were sub-adults, as evidenced by their unfused long bones. On the other hand, the dog sample was older overall, with complete long bone fusion; this may have resulted in the higher secondary osteon density noted in dog samples for this study.

5.2 Inter-elemental Comparison

The results for the comparison of the mid-thoracic rib, humerus, and femur were unexpected. Parallels were seen between like elements of different species, supporting the idea that microstructure of bone may be influenced by biomechanical or functional variables.

For all faunal species, the femur had a higher percentage of plexiform bone compared to the humerus. This observation, despite the fact that both bones are weight-bearing, may indicate intraspecies differences in the histological organization of bone within the body. Benedix (2004) suggested that growth rate may be a factor in plexiform bone formation. Differences in plexiform bone distribution in the same individual may be interpreted as a result of different growth rates or growth patterns. The differences in the dog humerus and femur may be the result of functional difference in the bones, as all the dogs in the study were mature at their time of death. As Liem et al. (2001) note, there are locomotor differences in the role of the humerus and femur during a stride.

Plexiform bone may also have an influence on density analysis. Areas of bone with plexiform bone present had relatively low secondary osteon densities. This phenomenon may explain why dog humeri have a higher secondary osteon density than dog femora. No dog humeri had any plexiform bone, while 33% of dog femora had plexiform bone.

The medial section of the femur had a higher percentage of plexiform bone than the corresponding sections of the humerus of all the species examined. There is a higher percentage of osteon banding in the femur compared to the humerus of pig and deer. This phenomenon is another indication of spatial differences in long bone organization. Again, these differences may relate to developmental or functional differences in the bone. Benedix (2004) took plexiform observation a step further, measuring the plexiform band width. Combining his quantitative approach with the spatial analysis of plexiform bone may help to extrapolate structural differences in bone.

A final point regarding the structural organization of the bones is the almost complete lack of plexiform bone and osteon banding in the ribs for all three species. The single observed incident of plexiform bone in the deer rib is a small area of indistinct plexiform organization

relative to the compact, distinct, brick-like pattern observed in the long bones. One possible explanation is the difference in bone function. The nature of the long bones as weight bearing could necessitate a different developmental pattern from the relatively stable pressures exerted on the ribs.

The interspecies spatial similarities for the deer and dog long bone secondary osteon area could indicate that microscopic structural development may be a response to biomechanical or functional influences and not soley determined by species. The similar trend for secondary osteon area change is evident for both the femur and the humerus between the deer and dog. This is excellent evidence to suggest that biomechanical and/or developmental influences may be a factor in bone formation. This information is important as it ultimately will aid in the understanding of species differences and the histological differences between bones of the same species.

Non-human species appear to demonstrate a similar increasing secondary osteon density with age, as in humans. At this time, I know of no study that has quantified the age-related changes in non-human species to create a predictive age-at-death equation. While this may not prove to be a functional and applicable exercise, understanding different development rates may assist further in species differentiation. The differing secondary osteon density between the femur and humerus of the same species is more evidence for differences in development or biomechanics between bones.

One trend I had expected to see but did not was differences in the bone structure related to muscle attachment sites. There is no conclusive evidence from my study that there are any structural changes in size or density of microscopic bone features.

Chapter 6: Conclusions

Three objectives were presented at the onset of this thesis. The first goal of the research was to compare presence/absence of structures, osteon and Haversian canal area, density and cortical thickness of three species: deer (*Odocoileus virginianus*), dog (*Canis familiaris*), and pig (*Sus scrofa domesticus*). The three species represented mammals that are ubiquitous across North America and frequently associated with forensic settings and archaeological sites. Information discovered in the research will be useful for species differentiation and may also be used in future human versus non-human comparisons.

Evident from the results and discussion, certain trends appear to be species specific. The pig is histomorphometrically isolated from both the deer and dog in terms of the Haversian system size, thought further exploration of the data is necessary. In particular, examination of the intraspecies variance and spatial distribution of cellular structures is warranted. Deer, for example, have considerable variability in its osteon area, while dog has relatively stable osteon area for all three skeletal elements. I would like to explore the variation in osteon and Haversian canal size within each species.

The second goal of the project was to compare the different skeletal elements: the mid-thoracic rib, the humerus, and the femur. The ribs are considered non weight-bearing bones while both the humerus and femur are weight-bearing in the quadrupeds selected for this study. The hypothesis is that non weight-bearing bones would exhibit differences related to species, not functional or biomechanical influences. Attributes including presence/absence of structures, osteons and Haversian canal area, density, and cortical thickness were compared quantitatively and spatially. There appear to be distinct differences between skeletal elements. In particular, the presence of plexifom bone and osteon banding is different between skeletal elements. Haversian system areas are also variable between the different elements within a species.

Future interspecies histoical research must be conducted in order to support any of the hypotheses discussed with regard to biomechanical and functional influence on the spatial organization of bones. The parallel changes in osteon area of the deer and dog humerus and femur are good evidence for this hypothesis though more research is necessary. The research should include serial sections from the same bone and a larger sample population. Serial sections would allow greater exploration in the differences between bones and the factors influencing microscopic bone structure development.

The final goal investigated how these variables may contribute to human versus non-human microscopic skeletal differentiation. No human samples were used; however, the results suggest that some organizational and histomorphometric differences may be species specific. The spatial analysis of the bones' mid-shaft cross-sections revealed some important trends that would not have been evident had the surface been measured as a single unit. I believe the division of the cross-section into eight distinct slices was a valuable tool in understanding the structure and organization of the bone.

The current investigation, comparing the femora, humeri, and mid-thoracic ribs of deer, dog, and pig requires further study. Identification of species from fragmentary remains has implications for both archaeology and forensic anthropology. For archaeology, there are many advantages to a technique that would allow detailed information to be collected regarding diet, past environments, and cultural practices. For forensics, histology may aid in more accurate differentiation of human and non-human fragments when gross morphological examination and/or DNA analysis are neither possible nor practical. Cataloging the differences in structural and spatial organization of bones also adds to the understanding of bone and its response to the influences of biomechanical, functional, and genetic influences. The study serves as an important basis for future research differentiating North American fragmentary faunal remains.

References

Adam, D.R.
1986 Canine Anatomy: A Systemic Study. Iowa State University Press: Ames.

An, Y.H. and Martin, K.L.
2003 Handbook of Histology Methods for Bone and Cartilage. Humana Press: Jersey City.

Bahn, P.
2003 Written in Bones: How Human Remains Unlock the Secrets of the Dead. Firefly Books: Toronto.

Bauer, E.A.
1983 Edwin Bauer's Deer in Their World. Outdoor Life Books: New York City.

Bauer, T.W. and Mahovlic, D.
2003 Cutting and Grinding Methods for Hard-Tissue Histology. *In* Handbook of Histology Methods for Bone and Cartilage. Eds. Yuehuei H. An and Kylie L. Martin. Humana Press: Jersey City. Pp. 233-242.

Benedix, D.C.
2004 Differentiation of Fragmented Bone from South East Asia: The Histological Evidence. Ph.D. dissertation, University of Tennessee, Knoxville, Department of Anthropology.

Cattaneo, C., DiMartino, S., Scali, S., Craig, O.E., Grandi, M. and Sokol, R.J.
1999 Determining the Human Origin of Fragments of Burnt Bone: A Comparative Study of Histological, Immunological and DNA Techniques. Forensic Sciences International. 102(2-3):181-191.

Cho, H., Stout, S.D., Madsen R.W. and Streeter, M.A.
2002 Population Specific Histological Age-Estimating Techniques: A Model for Known African-American and European American Skeletal Remains. Journal of Forensic Sciences. 47(1):12-18.

Crowder C.M.
2005. Evaluating the Use of Quantitative Bone Histology to Estimate Adult Age at Death. Ph.D. dissertation, University of Toronto, Department of Anthropology.

Crowder C.M. and Morris, Z.M.
2005. Reducing Intra- and Inter-observer Error through Histomorphometric VairableSelcetion. Poster presented at the Annual Meeting of the American Academy of Forensic Sciences, New Orleans, February 20th.

Currie, W.B.
1988 Structure and Function of Domestic Animals. CRC Publishing: Boca Raton.

Davis, S.J.
1987 The Archaeology of Animals. Yale University Press: London.

Derr, M.
2004 A Dog's History in America: How Our Best Friend Explored, Conquered, and Settled a Continent. North Point Press: New York City.

Dix, J.D., Stout, S.D. and Mosley, J.
1991 Bones, Blood, Pellets, Glass and No Body. Journal of Forensic Sciences 36(3):949-952.

Department of Anthropology at the University of Montana at Missoula
2004 Donner Party Archaeological Project, Electronic document, www.anthro.umt.edu/donner/default.htm, accessed April 30, 2007.

Enlow D.H.
1966 An Evaluation of the Use of Bone Histology in Forensic Medicine and Anthropology. *In* Studies on the Anatomy and Function of Bone and Joints. Ed. F.G. Evans. Springer-Verlog: New York Cit. Pp. 93-112.

Enlow, D.H. and Brown, S.O.
1958 A Comparative Histological Study of Fossil and Recent Bone Tissues. Part III. Texas Journal of Science. 10(1):187-230.

Fairgrieve, S.I.
1999 Of Beasts and Humans: A Case of Recognition. *In* Forensic Osteological Analysis: A Book of Case Studies. Ed Scott I. Fairgrieve. Charles C. Thomas Publisher Ltd.: Springfield, Illinois. Pp. 10-16.

Foote, J.S.
1916 A Contribution of the Comparative Histology of the Femur. Smithsonian Institution: Washington, D.C.

Fulbright, T. E. and Ortega-Santos J. A.
2006. White-Tailed Deer Habitat: Ecology and Management on Rangelands. Texas A&M University Press: College Station.

Gartner, L.P. and Hiatt, J.L.
1994 Color Atlas of Histology 2nd Edition. Williams and Wilkins: Baltimore.

Hamrick, M.W.
1999 Development of Epiphyseal Structure and Function in *Didelphis virginiana* (Marsupiala, Didephidae). Journal of Morphology. 239(3):283-296.

Humane Society of the United States
2007 U.S. Pet Ownership Statistics, Electronic document, http://www.hsus.org/pets/issues_affecting_our_pets/pet_overpopulation_and_ownership_statistics/us_pet_ownership_statistics.html, accessed April 30, 2007.

Jowsey, J.
1966 Studies of Haversian Systems in Man and Some Animals. Journal of Anatomy. 100(4):857-864.

Leake, L.D.
1975 Comparative Histology: An Introduction to the Microscopic Structure of Animals. Academic Press: London.

Liem, K.F., Bemis, W.E. Walker Jr., W.F. and Grande, L.
2001 Functional Anatomy of the Vertebrates: An Evolutionary Perspective 3rd Edition. Thomsen Learning - Brooks/Cole: Belmont, Califoria.

Lynnerup, N., Thomsen, J.L. and Frohlich, B.
1998 Intra- and Inter- Observer Variation in Histological Criteria Used in Age at Death Determination Based on Femoral Cortical Bone. Forensic Science International. 91(1):219-230.

Malluche, H.H. and Faugere, M.C.
1986 Atlas of Mineralized Bone Histology. Karger: London.

Martin D.L.
1988 Thin Sections. *In* Methods in Biological Anthropology Lab Manual: Microstructural Analysis of Bone and Teeth. Hampshire College, Amherst, Massachusetts. Pp. 40-48.

Martiniakova, M.,Grosskopf, B., Vondrakova, M., Omelka, R. and Fabi, M.
2006 Differences in Femoral Compact Bone Tissue Microscopic Strcutre between Adult Cows (*Bos taurus*) Pigs (Sus scrofa domestica). Anaomia, Histologia, Embryologia: Journal of Veterinary Medicine Series C. 35(3):167-170.

Mulhern D.M. and Ubelaker, D.H
2001 Differences in Osteon Banding Between Human and Nonhuman Bone. Journal of Forensic Science. 46(2):220-222.

Nordin, M. and Frankel, V.H.
1989 Basic Biomechancis of the Musculoskeletal System 2nd Edition. Lea and Febiger: Philadelphia.

Novakofski, J.E. and McCusker, R.H.
2001 Skeletal and Muscular Systems. *In* Biology of the Domestic Pig. Eds. W.G. Pond and H.J. Mersmann. Cornell University Press: Ithaca. Pp. 454-501.

O'Conner, T.P.
1996 A Critical Overview of Archaeological Animal Bone Studies. Zooarchaeology 28(1):5-19.

Olsen, S.J.
1964 Mammal Remains from Archaeological Sites: Part 1- Southeastern and Southwestern United States. Peabody Museum: Cambridge.

1985 Origins of the Domestic Dog; The Fossil Record. University of Arizona Press: Tucson.

Owsley D.W., Mires A.M. and Keith M.S.
1985 Case Involving Differentiation of Deer and Human Bone Fragments. Journal of Forensic Science. 30(2):572-578.

Pavao-Zuckermann, B.
2007 Deerskins and Domesticates: Creek Subsistence and Economic Strategies in the Historic Period. American Antiquity. 72(1):5-33.

Pond, W.G. and Mersmann, H.J.
2001 General Characteristics. *In* Biology of the Domestic Pig. Eds. W.G. Pond and H.J. Mersmann. Cornell University Press: Ithaca. Pp. 1-40.

Pratte D.G. and Pfeiffer S.
1999 Histological Age Estimation of a Cadaveral Sample of Diverse Origins. Canadian Forensic Science Journal. 32(4):155-167.

Ries, W.L.
2003 Techniques for Sectioning Undecalcified Bone Tissue Using Microtomes. *In* Handbook of Histology Methods for Bone and Cartilage. Eds. Yuehuei H. An and Kylie L. Martin. Humana Press: Jersey City. Pp. 221-232.

Robling, A.G. and Stout, S.D.
2000 Histomorphometry of Human Cortical Bone: Applications to Age Estimation. *In* Biological Anthropology of the Human Skeleton. Eds. M. Anne Katzenburg and Shelly R. Saunders. Wiley-Liss, Inc: New York. Pp. 187-214.

Sack, W.O.
1982 Essentials of Pig Anatomy. Veterinary Textbooks: Ithaca, New York.

Schultz, M.
1997a Microscopic Structure of Bone. *In* Forensic Taphonomy; The Postmortem Fate of Human Remains. Eds. William D. Haglund and Marcella H. Sorg. CRC Press: Baco Raton. Pp. 187-199.

1997b Microscopic Investigation of Excavated Skeletal Remains: A Contribution to Paleopathology and Forensic Medicine. *In* Forensic Taphonomy; The Postmortem Fate of Human Remains. Eds. William D. Haglund and Marcella H. Sorg. CRC Press: Baco Raton. Pp. 201-222.

Stout S.D.
1986 The Use of Bone Histomorphometry in Skeletal Identification: The Case of Francisco Pizarro. Journal of Forensic Sciences 31(1):296-300.

1998 The Application of Histological Techniques for Age at Death Determination. *In* Forensic Osteology: Advances in the Identification of Human Remains. Ed Kathleen J. Reichs. Thomas Books: Springfield, Illinois. Pp. 237-252

Stout, S.D. and Paine, R.R.

1992 Brief Communication: Histological Age Estimation Using Rib and Clavicle. American Journal of Physical Anthropology 87(1):111-115.

Stout, S.D. and Ross, L.M.
1991 Bone Fragments a Body Can Make. Journal of Forensic Sciences. 36(3):953-957.

Taylor, R.
2000 Death and the Afterlife: A Cultural Encyclopedia. ABC-CLIO: Santa Barbara.

Ubelaker, D.H
1998 Evolving Role of Microscope in Forensic Anthropology. *In* Forensic Osteology: Advances in the Identification of Human Remains. Ed. Kathleen J. Reichs. Thomas Books: Springfield, Illinois. Pp. 514-532.

Walsh, W.R., Walton, M., Warwick, B., Yan, Y., Gillies, R.M. and Svehla, M.
2003 Cell Structure and Biology of Bone and Cartilage. *In* Handbook of Histology Methods for Bone and Cartilage. Eds. Yuehuei H An and Kylie L. Martin. Humana Press: New Jersey. Pp. 35-58.

White, T.D.
2000 Human Osteololgy, 2nd Edition. Academic Press: San Diego.

White, T.D. and Folkens, P.A.
2005 The Human Bone Manual. Elsevier Academic Press: Oxford.

Whitman, E.J.
2004 Distinguishing between Human and Non-Human Secondary Osteons in Ribs. Paper presented at the Annual Meeting of the American Academy of Forensic Sciences, Dallas, February 20th.

Appendix A: FACES Laboratory Protocol for DNA Sampling

When taking a DNA sample this protocol will be followed by FACES Lab personnel to protect personnel and to minimize contaminations.

Apparel

Protective clothing, including a mask, face shield, sleeves, booties, smock or apron, double gloves, and a hair cap or hat shall be worn by all personnel involved in taking the DNA sample.

Equipment

Equipment used in taking the samples shall include a Stryker Saw and/or pliers.

Sampling Protocol

1) Samples will be taken underneath the fume hood in Howe-Russell Building, room E129AA
2) The case from which the sample is taken shall be the only one in E129AA during sampling
3) The area in which the sample is taken shall be thoroughly cleaned with a 50/50 bleach/water solution prior to taking each sample.
4) All equipment used to take the sample shall be thoroughly cleaned with a 50/50 bleach/water solution prior to taking each sample.
5) FACES personnel shall put on a clean pair of gloves prior to handling each sample.
6) Two samples shall be taken from each case.
7) Depending on the availability, samples shall consist of two of the following:
    a. A plug of bone (approximately, one to two inch squared) from the anterior distal thir of the left femur or tibea (the right side shall be used if the left is not available)
    b. A virgin molar or premolar
    c. A section of rib
    d. A plug of bone from the cranium (preferably parietal)
8) The element from twhich the sample is taken shall be photographed prior to sampling.
9) The sample itself shall be photographed prior to being placed in the bag.

Sample Storage

1) Every sample shall be placed in its own paper bag. Every bag shall be labeled with the date, case number, element sampled, and who took the sample.
2) Bagged Samples from the same case shall be stored together in one large paper bag that is labeled with the case number.

Appendix B: Record Sheet for Mid-Thoracic Rib Samples

SAMPLE NAME HERE

Bone Description:

Age-at-Death Estimation:

Source:

Add slide photo here

QUANTITATIVE	# of whole 2ndary Osteons	Osteon Area mm^2	Osteon Perimeter mm	# of Haversian Canals	Haversian Canal Area mm^2	Haversian Canal Perimeter mm	Area Measured mm^2	Cortical Thickness mm	Total Cross Section
Cranial		±	±		±	±			
Cranial-Exterior		±	±		±	±			
Cranial-Interior		±	±		±	±			Average Osteon Area
Exterior		±	±		±	±			±
Interior		±	±		±	±			
Caudal		±	±		±	±			Average Canal Area
Caudal-Exterior		±	±		±	±			±
Caudal-Interior		±	±		±	±			

QUALITATIVE	Processing Quality	Preservation Quality	Osteon Banding	Distribution Pattern	Plexiform Bone	Distribution Pattern	Osteon Shape	Distribution Pattern
Cranial								
Cranial-Exterior								
Cranial-Interior								
Exterior								
Interior								
Caudal								
Caudal-Exterior								
Caudal-Interior								

Appendix C: Record Sheet for Long Bone Samples

SAMPLE NAME HERE

Bone Description:

Age-at-Death Estimation:

Source:

Add slide photo here

QUANTITATIVE	# of whole 2ndary Osteons	Osteon Area mm^2	Osteon Area mm^2	# of Haversian Canals	Haversian Canal Area mm^2	Haversian Canal Area mm^2	Area Measured mm^2	Cortical Thickness mm	Total Cross Section
Anterior			±			±			
Anterior-Lateral			±			±			
Anterior-Medial			±			±			Average Osteon Area
Lateral			±			±			±
Medial			±			±			
Posterior			±			±			Average Canal Area
Posterior-Lateral			±			±			±
Posterior-Medial			±			±			

QUALITATIVE	Processing Quality	Preservation Quality	Osteon Banding	Distribution Pattern	Plexiform Bone	Distribution Pattern	Osteon Shape	Distribution Pattern
Anterior								
Anterior-Lateral								
Anterior-Medial								
Lateral								
Medial								
Posterior								
Posterior-Lateral								
Posterior-Medial								

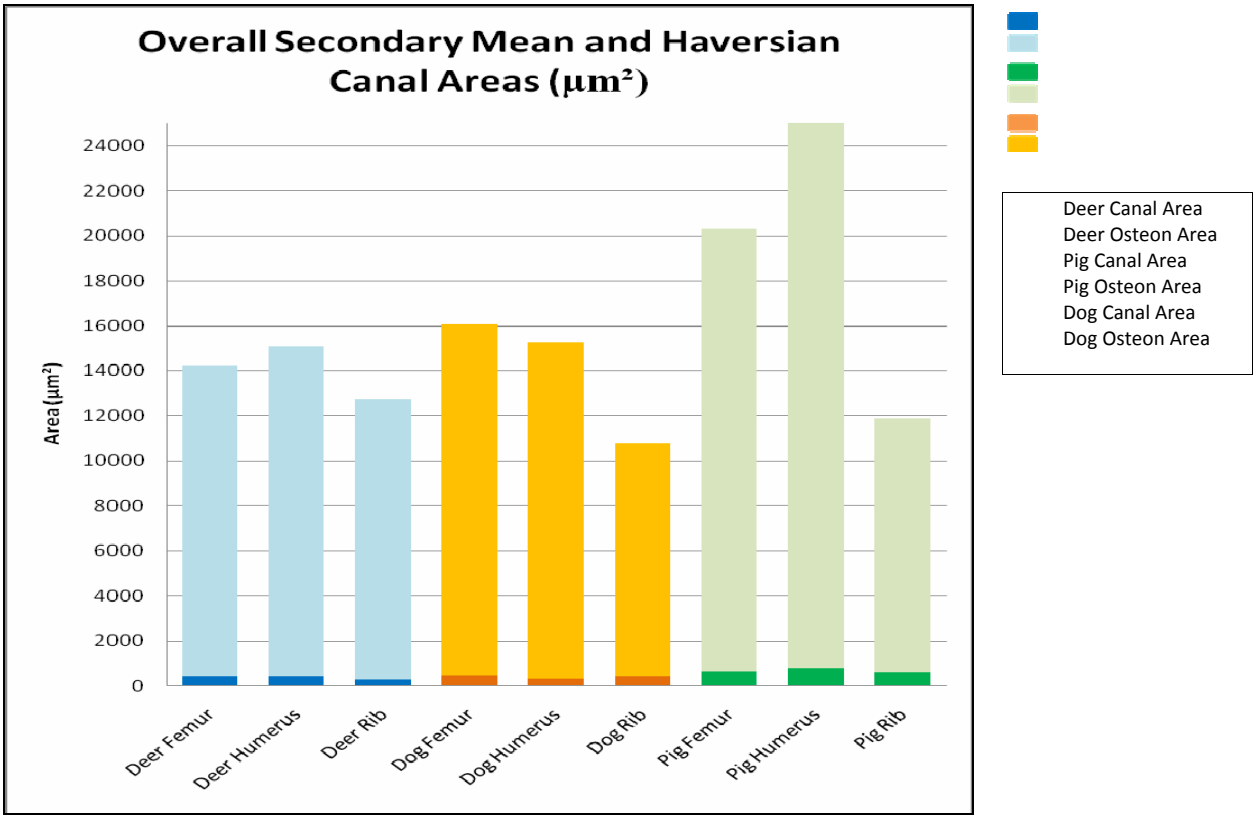
Appendix D: Summary of Secondary Osteon and Haversian Canal Data

Summary of Secondary Osteon Data by Species and Skeletal Element

	DeerFEM Osteons	DeerHUM Osteons	DeerRIB Osteons	DogFEM Osteons	DogHUM Osteons	DogRIB Osteons	PigFEM Osteons	PigHUM Osteons	PigRIB OSTEONS
Mean ($\mu m^2 \times 10^4$)	1.39	1.47	1.13	1.56	1.49	1.03	1.97	2.51	1.13
StDev ($\times 10^4$)	0.65	0.60	0.59	0.67	0.74	0.54	0.87	1.66	0.57
Maximum ($\mu m^2 \times 10^4$)	3.91	4.64	8.10	5.41	5.70	4.80	5.06	7.80	4.08
Minimum ($\mu m^2 \times 10^4$)	0.38	0.32	0.022	0.29	0.40	0.017	0.53	0.40	0.19
Count	271	287	795	630	311	1134	252	205	562

Summary of Haversian Canal Data by Species and Skeletal Element

	DeerFEM Canals	DeerHUM Canals	DeerRib Canals	Dog FEM Canals	DogHUM Canals	Dog RIB Canals	PigFEM Canals	PigHUM Canals	PigRib Canals
Mean ($\mu m^2 \times 10^2$)	3.87	4.01	2.45	4.32	3.14	3.92	6.45	7.75	6.06
StDev ($\times 10^2$)	2.05	1.86	1.64	1.82	2.27	2.59	3.41	5.60	4.81
Maximum ($\mu m^2 \times 10^2$)	12.31	12.21	18.80	17.51	30.00	20.00	20.58	34.98	33.20
Minimum ($\mu m^2 \times 10^2$)	0.80	0.97	0.68	0.76	0.47	0.38	1.43	0.63	0.80
Count	302	331	988	815	389	1102	254	208	655



Vita

Zoe H. Morris was born in Kelowna, British Columbia, Canada, and grew up traveling between the west coast of British Columbia and Southeast Asia. She attended West Vancouver Secondary School in West Vancouver, British Columbia, receiving an International Baccalaureate Diploma. In 2003, she graduated from the University of Toronto, with an Honours Bachelor of Science with a Specialist in anthropological sciences and major in archaeological sciences.

Zoe spent the summer of 2002 in northwest Belize at the University of Calgary archaeological field school. The following summer, Zoe worked on a Tsimshian archaeological site on the northwest coast of British Columbia. Upon returning from the fieldwork, she volunteered for, then Ph.D. candidate, Dr. Christian Crowder. Her role in Dr. Crowder's project included the preparation of microscopic slides and analysis of the slides to test inter-observer error in histological age-at-death estimation. Zoe co-authored a poster presentation for the 2004 American Academy of Forensic Sciences meeting with Dr. Crowder. In spring 2007, Zoe accompanied Dr. Heather McKillop and her archaeological field team to Payne's Creek National Park, Belize. Dr. McKillop and Zoe co-authored a paper describing the demography of skeletal remains of two coastal Maya sites at the 2007 Society for American Archaeology meetings.

Zoe has also presented her cultural research on the role of Vietnamese-American youth activists from New Orleans' East Village at several conferences including the 2005 Society for Applied Anthropology meetings, 2005 American Folklore Society meetings, and the 2007 American Ethnological/Canadian Anthropology Society meetings.

Zoe begins the doctoral program at the University of Western Ontario in the fall of 2007. She hopes to continue to be involved in anthropology at all levels, including life-long student, teacher, and researcher.