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UNIVERSITY OF CALIFORNIA, SAN DIEGO

Avian Wing Bones

A Thesis submitted in partial satisfaction of the requirements for the degree
Master of Science

in

Engineering Sciences (Mechanical Engineering)

by

James Huai Kiang

Committee in charge:

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2013

The Thesis of James Huai Kiang is approved and it is acceptable in quality and form for publication on microfilm and electronically:

Chair

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2013

Table of Contents

Signature Page.....	iii
Table of Contents	iv
List of Figures.....	vi
List of Tables	xi
List of Equations.....	xi
Acknowledgements	xii
Vita	xiii
ABSTRACT OF THE THESIS	xiv
Chapter 1: Introduction.....	1
Ch. 1.1: Introduction to Biomimetics.....	1
Chapter 2: Background	6
Ch. 2.1: Structural and Mechanical Properties of Bone.....	6
Chapter 3: Methods and Experimental Procedures.....	18
Ch. 3.1: Sample Preparation and Procedures.....	18
Ch. 3.1.1: Cleaning the Samples	18
Ch. 3.1.2: Sectioning Samples.....	24
Ch. 3.1.3: Sample Preparation.....	29
Ch. 3.2: Facilities and Equipments.....	35

Chapter 4: Results and Discussions.....	40
Ch. 4.1: Structural Characterization and Optical Observation of the of the Bones in each Species.....	40
Ch. 4.2: Mechanical Properties of the Bones from each Species	77
Chapter 5: Summary and Conclusions.....	84
Chapter 6: Future Work.....	89
References.....	91

List of Figures

Figure 1: Schematic representation of characteristic constraints/components in the study of biological systems [4].....	3
Figure 2: General skeletal structure of a human body. All major bones in the body are labeled [6].....	7
Figure 3: Image of a typical long bone that shows both the cortical or compact bone and the trabecular or cancellous bone [9].....	8
Figure 4: Image of a human long bone [11].....	9
Figure 5: Diagram of a long bone that illustrates an osteon [15].	11
Figure 6: Schematic diagram of the microstructure of cortical bone region [16].	11
Figure 7: Hierarchical structural organization of bone. From left to right: Cortical and Trabecular bone; Osteons; Lamellae; Collagen fiber bundle that consists of many collagen fibrils; Bone minerals, collagen molecules, and non-collagenous protein [17].	13
Figure 8: General skeletal structure of a bird body. All major bones and the location of the fused bones in the body have been labeled [19].....	14
Figure 9: Means and 95 percent confidence intervals for cranial, humeral, and femoral density in a) birds, b) bats, and c) rodents with the sample sizes in parentheses. The stars for each species indicate the weighted means of the bone density for each group [25].....	16
Figure 10: Left and right wing of the California Gull.....	19
Figure 11: Left wing of the Turkey Vulture. The flesh and feathers were not attached to the bones upon receiving the specimens.....	22
Figure 12: Left wing of the raven. The wing was received without the flesh and feathers attached.....	23

Figure 13: a) The humerus bone from the left wing of a California Gull. The length of this humerus bone is approximately 13cm. b) The humerus bone from the right wing of a Pekin Duck. The length of this humerus bone is approximately 9.5cm. c) The humerus bone from the right wing of the Turkey Vulture. The bone was already damaged when it was received. The length of the humerus bone is approximately 14cm. d) The humerus bone from the right wing of the Common Raven. The length of the humerus bone is approximately 7.5cm..... 24

Figure 14: Length-wise cross section of the gull humerus bone. This section is located at the middle of the bone. 26

Figure 15: The duck humerus bone after being sectioned into 5mm portions.27

Figure 16: A section of the duck humerus bone. 28

Figure 17: The right humerus of the vulture after sectioning. Where the bone attaches to the body or other wing bones is labeled respectively..... 29

Figure 18: SamplKup® with a 5mm section of the vulture humerus prior to filling with epoxy solution. Once filled with epoxy solution, the setup is left alone for 24hrs to allow for the epoxy to dry and harden..... 30

Figure 19: Exposed surface of the humerus bones from different species after grinding and polishing. a) Common Raven, b) California Gull, c) Pekin Duck, d) Turkey Vulture..... 31

Figure 20: a) A portion of the length-wise cross section of the gull humerus bone. b) Zoomed image of the internal structures of the gull humerus bone.. 42

Figure 21: Approximate location of the struts found within the gull humerus bone. 42

Figure 22: a) and b) show two different struts found within the humerus bone of the gull using the Keyence 3-D optical microscope. 43

Figure 23: Strut (b) from Figure 22. a) Shows a high resolution image of the excised strut from the humerus bone of the gull using the Keyence 3-D optical microscope. b) Shows a top down view with dimensions. c) Shows a side view with dimensions.....	44
Figure 24: Gull strut (a) from Figure 22 after the deproteinization process under the SEM.....	45
Figure 25: a) Fractured surface of the deproteinized strut from the gull humerus. b) Shows the microstructures within the strut. c) Shows one of the many microstructures within the strut	46
Figure 26: Cross sectional image of a section of the gull right humerus cortical bone showing the microstructures such as osteons (O), vascular channels (Va), lacuna spaces (La), and Volkmann's canals (VC).	47
Figure 27: Optical image of untreated bovine cortical bone. Some of the microstructures can be seen in the yellow oval. Osteons (O); Lacuna spaces (La); Vascular channels (Va); Volkmann's canals (VC).....	48
Figure 28: Fractured surface of a deproteinized section of the gull humerus bone. a) through d) shows increasing magnification of the microstructures that were seen in the bone.....	49
Figure 29: Cross sectional view of the duck humerus bone, taken from the most proximal end of the bone not including the proximal epiphysis, showing the trabecular bone with the marrow still inside.....	50
Figure 30: The duck humerus bone after sectioning. The trabecular portions of the bone are found in the ends of the bone while the middle section is hollow.	51
Figure 31: High resolution of the trabecular portion of the duck humerus bone.	52
Figure 32: a) Optical image of a cross section of the duck humerus bone. Also shows the different regions within the cross section of the duck humerus bone. b) Higher magnification of the duck humerus bone showing the microstructures. Osteons (O); Vascular channels (Va); Lacuna spaces (La); Volkmann's canal (VC).....	53

Figure 33: Cross sectional image of young bovine bone showing the outer sheath.....	54
Figure 34: a) SEM image of the deproteinized duck trabecular bone cut out from the duck humerus bone section shown in Figure 30. b) and c) are higher magnification images of the structures within the trabecular portion.	55
Figure 35: The reinforcing structures or ridges found in the ulna bone of the vulture.....	57
Figure 36: a) Top down view of the struts inside the right ulna bone of the vulture. b) Angled view of the struts. c) Stitched SEM images of the struts....	58
Figure 37: Cross sectional image of the vulture's right ulna bone showing structures similar to that of the gull and duck bones. Osteons (O); Lacuna spaces (La); Vascular channels (Va); Volkmann's canals (VC).....	59
Figure 38: Entire cross section of the vulture's right ulna bone. This optical image also shows the ridges that provide support to the bone.....	60
Figure 39: a) Section #4 of the vulture's right humerus bone showing struts within. b) Section #5 of the vulture's right humerus bone showing a ridge along one side of the inner circumference.	61
Figure 40: a) Image of the ulna of the vulture's left wing. b) μ CT scan from the middle portion of the ulna. c) A top down view of the ulna. d) A cutaway view revealing the struts within the ulna.	63
Figure 41: A cutaway showing that the small bumps, or quill knobs, on the ulna bone are mostly hollow on the inside.	64
Figure 42: a) Image of the ulna of the vulture's left wing. b) μ CT scan of the end portion of the ulna. c) Top down view of the ulna. d) Cutaway view of the ulna that shows the internal structures found in the bone.....	65
Figure 43: a) Image of the carpometacarpus and phalanx of the vulture's left wing. b) μ CT scan of the end portion of the carpometacarpus. c) Top down view d) A cutaway view that reveals the struts and ridges.	66

Figure 44: Image of the metacarpal bone from a vulture's wing taken from Thompson's book [62, 63].	67
Figure 45: a) Image of the metacarpal and phalanx of the vulture's left wing. b) μ CT scan of a portion of the phalanx. c) A cutaway view revealing the struts and ridges inside. d) Top down view of the phalanx bone.	68
Figure 46: Cross sectional image of the raven's right humerus bone showing similar microstructures to that of the gull, duck, and vulture. Osteons (O); Lacuna spaces (La); Vascular channels (Va); Volkmann's canal (VC).....	69
Figure 47: General location of where the internal structures were found in each bone with the exception of the raven bone. a) gull humerus, b) duck humerus, c) vulture humerus, d) raven humerus.....	71
Figure 48: Microstructure comparison for each bone. a) vulture, b) raven, c) duck, d) gull.	72
Figure 49: Chart showing the water and ash content of all the avian species studied and compared with that of the bovine and young bovine [57].	76
Figure 50: Diagram of the Vickers Pyramid Diamond Indenter [73].....	79
Figure 51: Average micro-hardness value of the humerus bones from each species and compared to the micro-hardness values of the long bones in the bovine and young bovine [57].....	80
Figure 52: Diagram of the forces that act upon the wing of a bird.	81

List of Tables

Table 1: Equipment found at the Nano3 facility in CaliT2	36
Table 2: Equipment found at the research laboratories of Professor McKittrick and Meyers.....	37
Table 3: Equipment found in the Undergraduate Research Laboratory	38
Table 4: Equipment found at the Pediatrics Diabetes Research Center at UCSD's School of Medicine	39
Table 5: Table of avian species used in this study. The flying styles, wingspan, interior structures, and the structure locations within the bone are also listed.	72

List of Equations

Equation 1: Vicker's Pyramid Diamond hardness number (HV) equation. F is the applied load in kgf while d is the average length of the diagonal left by the indenter in mm [73].....	79
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ABSTRACT OF THE THESIS

Avian Wing Bones

by

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Master of Science in Engineering Sciences (Mechanical Engineering)

University of California, San Diego, 2013

Professor Joanna McKittrick, Chair

Nearly all species of modern birds are capable of flight; therefore mechanical competency of appendages and the rigidity of their skeletal system must be optimized. Birds have developed extremely lightweight skeletal systems that help aid in the generation of lift and thrust forces as well as helping them maintain flight over a long period of time. The wing bones of different species of birds, the California Gull, Pekin Duck, Turkey Vulture, and Common Raven have been analyzed. The structures found within these bones vary from species to species depending on how a particular species utilizes its wings. The California Gull and Turkey Vulture both exhibited struts and/or ridges within their wing bones while the wing bones of the Common Raven were complete hollow. In addition, from optical and Scanning Electron

Microscope observation, the wing bones contain similar micro-structure to those of other mammalian long bones. These micro-structures are arranged in osteons located in the compact region of the whole bone. Mineral content analysis was done on the wing bones of each species studied and the data showed that the mineral content in all species studied, except for the Pekin Duck, were similar to the mineral content found in bovine long bone. Furthermore, looking at the hardness testing data for the gull, vulture, and raven, and comparing it with the flying habits and size of these birds, it can be seen that the struts and ridges may be playing a role in helping to reinforce the gull and vulture wing bones. This work is funded by the NSF, Ceramics Program Grant 1006931.

Chapter 1: Introduction

Ch. 1.1: Introduction to Biomimetics

Mechanical engineering is a disciplinary field that encompasses a variety of studies such as physics and material science. By understanding and using the core concepts behind these studies, mechanical engineers are able to analyze, design, manufacture, and maintain mechanical systems. However, biomimetics is emerging as a new area of interest that opens up a completely different view on mechanical engineering. This field, biomimetics, is the study of the formation, structure, and/or function of biologically produced substances and materials and biological mechanisms and processes as models for the design and synthesis of similar products by artificial methods [1].

In nature, there are many excellent examples of engineering solutions. These engineering solutions have been perfected over many years by a process called natural selection. Natural selection provides ample lessons from which engineers from all fields can learn and understand from. By studying and understanding these lessons from nature as well as the properties and structural relationships in biological materials, new or better designs of materials or structures can be made that are stronger and/or lighter and are able to last beyond their design capabilities.

In biomimetics, it is important to have a clear understanding of biology. The anatomy, physiology, and lifestyle all determine the development of an

animal's structural components just as how an engineer must understand how a mechanical system will be used as well as the environment it is used in. Taking this into mind, biological systems have developed mechanical properties that rival that of modern day systems [2]. These biological systems are developed from inorganic materials as well as protein. When they are combined, they form a structure that is ordered and strong, sometimes creating structures that are far stronger than they appear to be [3]. But, these systems have constraints that are unique to each design and structure. These constraints or components can be seen in **Figure 1** [4].

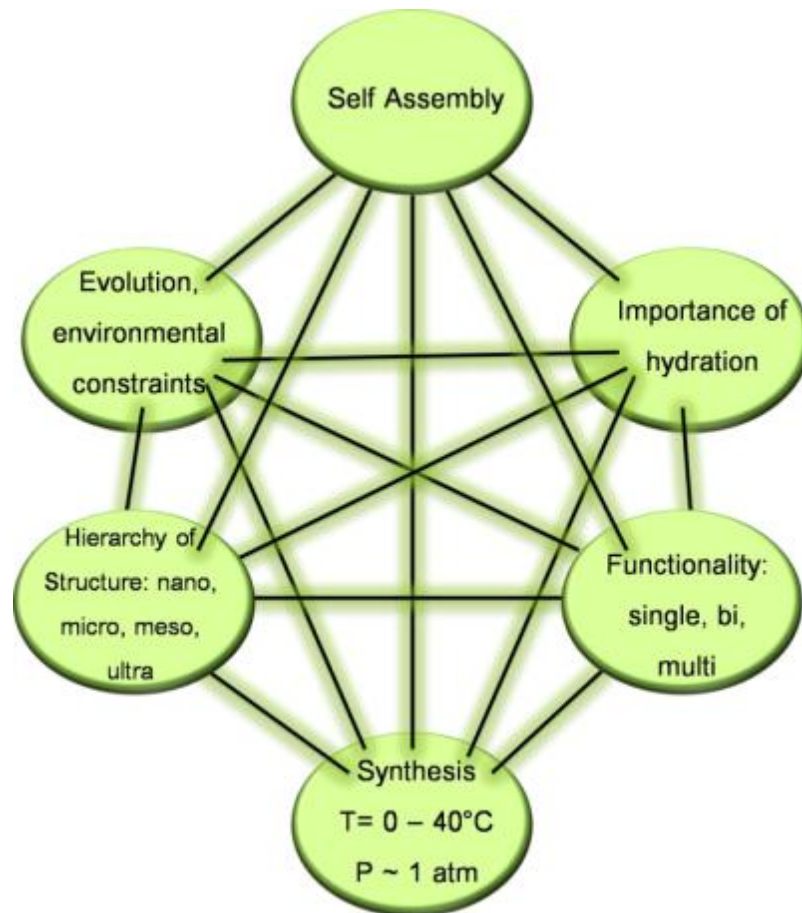


Figure 1: Schematic representation of characteristic constraints/components in the study of biological systems [4].

The components are explained below [4].

1. Self Assembly – Structures are assembled from the bottom up instead of top down. This is necessary because during the growth process there is no way to nullify the scaffold that is already present.
2. Hydration – The amount of water in the system or structure is important to its properties.
3. Functionality – More often than not, components in a biological system are used for more than one purpose.

4. Synthesis – The synthesis of these biological structures or materials generally takes place at Standard Temperature and Pressure (STP) and a somewhat moist environment, depending on the biological system.
5. Hierarchy of Structure – Each scale level of the structure has different and distinct properties. Biological systems and structures are organized from bottom up, beginning with the nano to the micro and finally to the macro level.
6. Evolution – The limited supply of materials directs the development of biological systems and structures as well as its properties. Materials such as copper, aluminum, and/or other types of metals are not present in biological systems.

These components and/or constraints can be seen in variety of natural materials, one of which is the bone. Bone is of interest because many organisms rely on the bone to create their skeletal structure. On top of giving shape and form to the organism, the bone also supports and protects vital organs and structures within the organism's body. Combined with the muscles in the body, the bone also helps generate motion, which can be seen in organisms that use legs to move and birds that use wings to fly. The wings of the avian species are of interest because by enabling the animal to achieve and maintain flight, the wings are subjected to a variety of different forces. These forces are different when compared to the forces acting on the legs of land animals. Therefore this study is focused on investigating the structure of

avian wing bones, taking into account the different flying styles of each avian species used in this study. In addition, some mechanical testing is performed on sectioned portions of the wing bones from each species and then correlated to how each species flies and uses its wings.

Chapter 2: Background

This chapter combines previous relevant and fundamental work in order to present information that pertains to and aids this study. These topics will provide all the necessary information to allow for a more comprehensive understanding of the results.

Ch. 2.1: Structural and Mechanical Properties of Bone

In animals such as an elephant, a bird, or a human, the bones and their joints serve as the most basic structure that shapes the way the organism looks, moves, and how it performs a variety of actions, all the while protecting the various soft tissues and organs that are found within the body. There are many bones that make up the structure of an organism but only a few are put through rigorous exercises day in and day out. In a human, for example, there are 206 bones that constitute the body [5]. Some of these bones, along with their names, can be seen in **Figure 2**, and since the human body is symmetrical in nature many of the bones are labeled only once [6]. In general, the bones in the human body that are exercised the most daily are those that constitute the legs and feet. These bones are the femur (thighs), tibia (legs), fibula (legs), tarsal (ankles), metatarsal (feet), and phalanges (toes). These bones are used for movement day in and day out. In addition to being used for movement, these bones must also support the entire weight of a human over the course of the human's natural life.

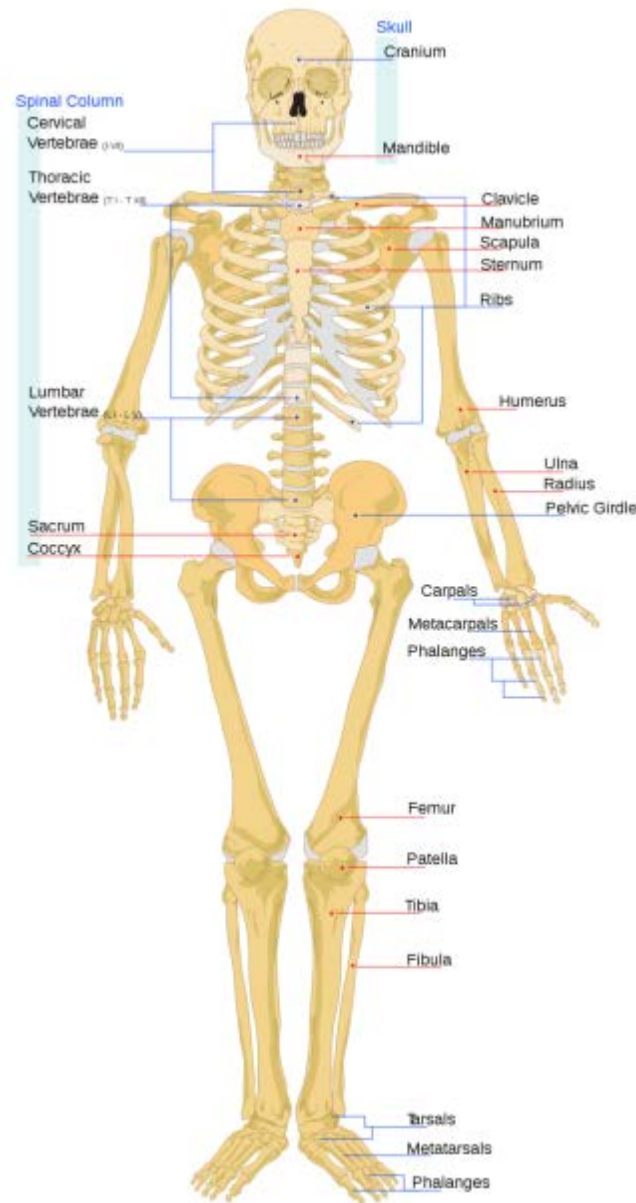


Figure 2: General skeletal structure of a human body. All major bones in the body are labeled [6].

Because these bones are required to support so much weight as well as be generally the most highly exercised bones in the human body, they have developed over time to be some of the strongest bones in the body. Studies have shown that practicing exercise at a young age helps to maximize the

mineral density of the bones and maintaining the level of exercise as one grows older helps minimize bone loss later in life. Also, changes in lifestyle and diet are further preventions that can lead to stronger bones and a significant decrease in bone fracture rates [7].

Looking further into how the bone is formed, it can be seen that the density alone does not give rise to the strength that we observe. The structure, thickness, area moment of inertia, and the microstructures found within the bone are the main factors that contribute to the mechanical competence of the whole bone [8]. A typical long bone has two distinct types of bone tissues, the cortical or compact bone and the trabecular or cancellous bone, which are shown in **Figure 3** below.

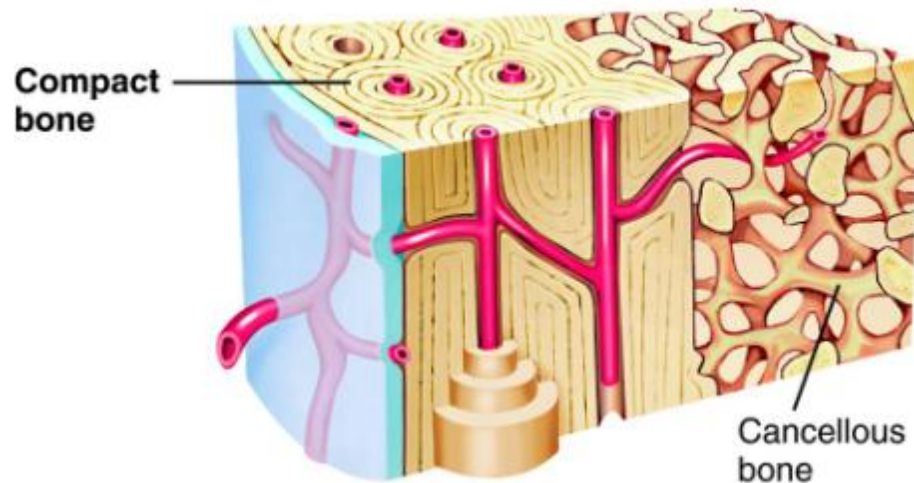


Figure 3: Image of a typical long bone that shows both the cortical or compact bone and the trabecular or cancellous bone [9].

Each of these types of bone has their own particular function. The cortical bone for instance, aids the bone's main function, which is to support the body,

protect the organs, and store chemical elements such as calcium. The cortical bone also forms the “outer shell” of the bone and generally makes up roughly 80% of the weight of a human skeleton [10] as seen in **Figure 4**.

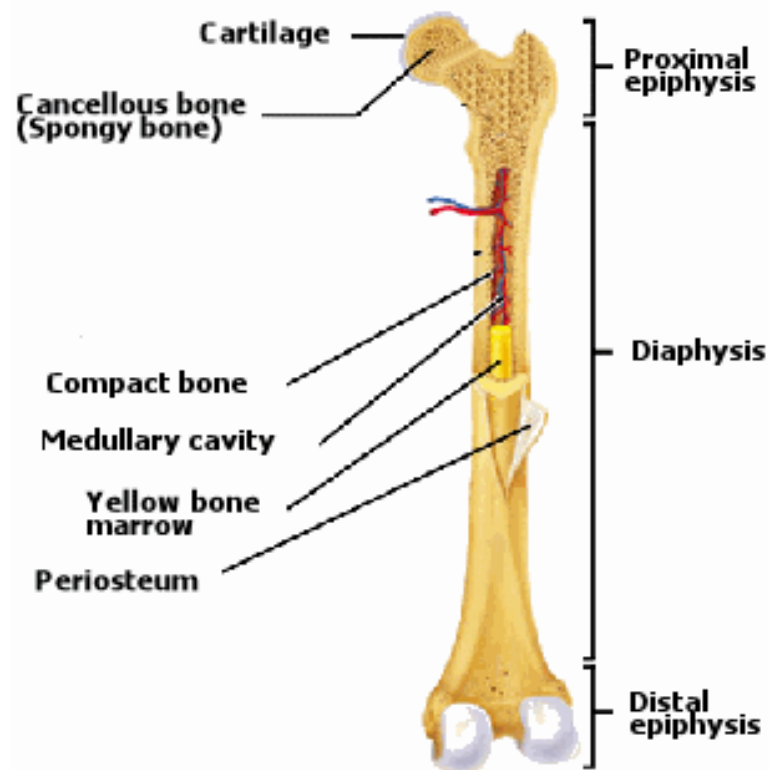


Figure 4: Image of a human long bone [11].

The trabecular bone is the other type of tissue that forms the bone first seen by Martinez [12]. However, the trabecular bone, which has a spongy look to it, only forms in the ends of the long bone, closest to the joints, and is surrounded by a cortical bone shell. The trabecular bone has a higher surface area when compared with the cortical bone and therefore a great location for the exchange of calcium ions. Additionally, trabecular bone is generally where bone marrow is found within the bone, and is also the site where red blood cells are produced [13]. Also, the trabecular bone is capable of withstanding

stresses that are applied from many different directions without increasing the weight of the bone and thus allowing the muscles to have an easier time moving the bone. However, when compared to the trabecular bone, it can be seen that the cortical bone is by far harder, stronger, and stiffer. This strength is generated by the most basic building block of the bone, the osteon or Haversian system. The osteon is cylindrical in shape and consists of concentric layers of cortical bone with a canal centered in the middle, the Haversian canal, seen in **Figure 5** and **Figure 6**. This canal contains all the nerves and blood supply for the bone. The osteon is actually the product of bone remodeling which takes place at a steady rate throughout life. So as the age of the animal increases, the osteonal area increases as a proportion of the cross-sectional area of cortical bone. Additionally, it has been found that the inorganic content of cortical bone also increases with the age of the animal. However, the number of osteons found per unit cross-sectional area in the cortical bone does not depend on the age of the animal [14].

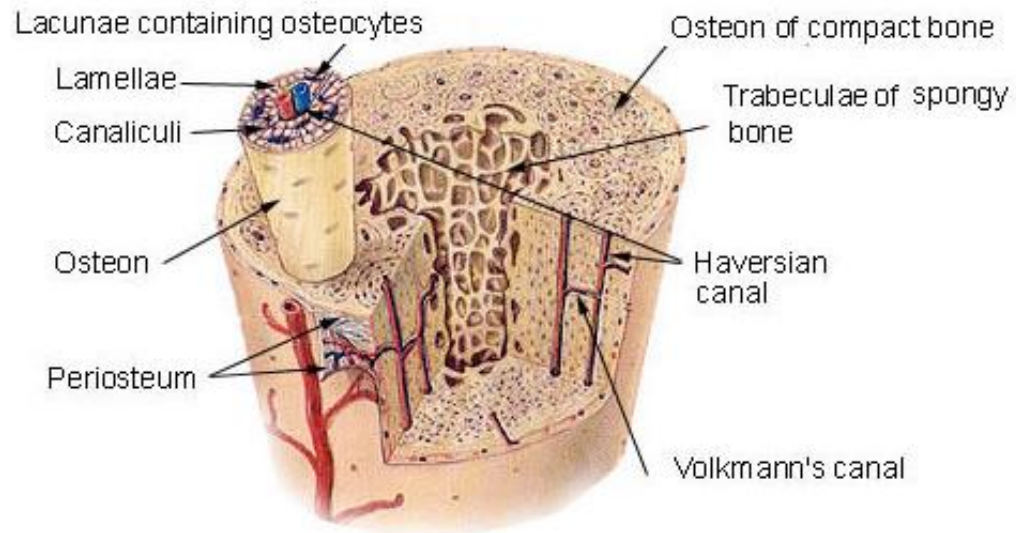


Figure 5: Diagram of a long bone that illustrates an osteon [15].

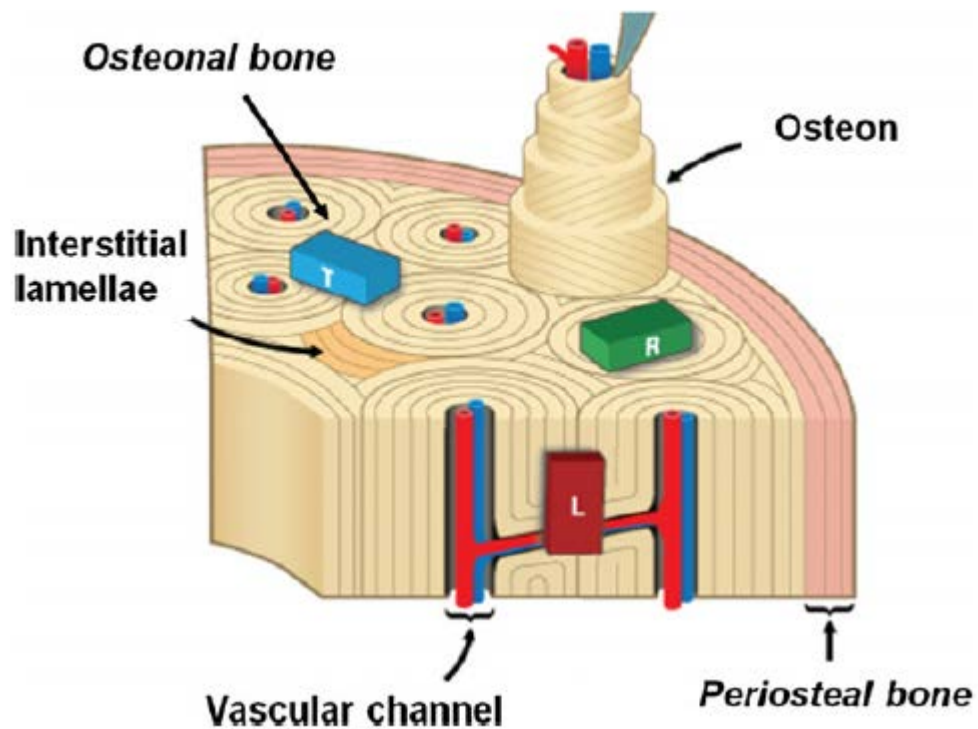


Figure 6: Schematic diagram of the microstructure of cortical bone region [16].

Even though the osteon is the basic building block of cortical bone, and thus the long bone, it is only on the micro level of the structural hierarchy of

the bone. These levels of the hierarchical structure of the bone starting from the nano scale are detailed below [17] and can be seen in **Figure 7**.

1. The sub-nanostructures which consists of the constituent elements such as the minerals, collagen molecules, and proteins. These structures are generally under a few hundred nanometers in size.
2. The nanostructures which consists of collagen fibrils and collagen fiber bundles. These structures range from a few hundred nanometers to 1 μ m in size.
3. The sub-microstructures which consists of the lamella or the concentric layers of cortical bone that surround the osteon. Lamella consistent of collagen fibers that form a spiral in order to add to the strength and resiliency of the bone. These structures range in size from 1 to 10 μ m.
4. The microstructures which consists of the osteons and a single trabecula. These structures are roughly 10 to 500 μ m in size.
5. The macrostructures which consists of the cortical and trabecular bone. These range in size depending on the animal that the long bone is taken from.

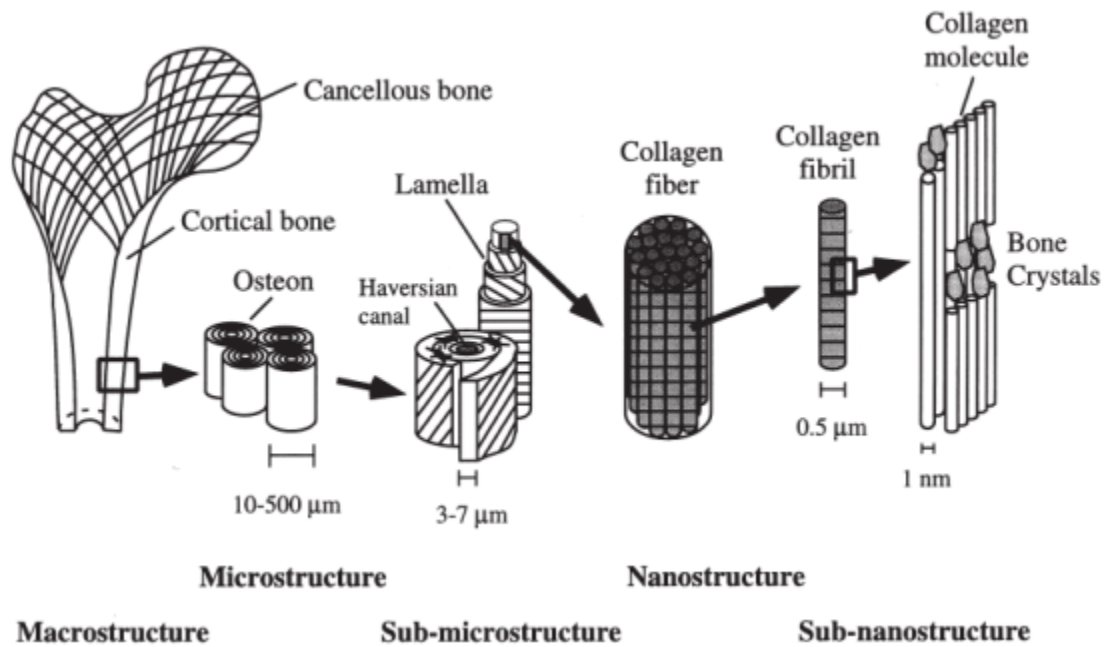


Figure 7: Hierarchical structural organization of bone. From left to right: Cortical and Trabecular bone; Osteons; Lamellae; Collagen fiber bundle that consists of many collagen fibrils; Bone minerals, collagen molecules, and non-collagenous protein [17].

Moving the focus from human long bones to the bones of the avian species, it has been observed that the bones have adapted over time to allow the birds to achieve and maintain flight. One adaption is the fusing of bones into single ossifications. By fusing the bones, the total number of bones found within a bird's skeletal system is far less than that of other land based animals, or terrestrial vertebrates [18]. By fusing some bones together, the bones achieve higher rigidity, while maintaining the mobility needed on other parts of the body. Also, by fusing some of these bones together, the bird's skeleton becomes much more lightweight as well as sturdy. The bones found in the wing of a bird are a prime example of this. The carpals, metacarpals, and phalanges, all the types of bones that can be found in a human hand, were fused together to form the carpometacarpus and phalanges bones thereby

reducing the number of bones in the body while maintaining the necessary structure and strength the bird needs to sustain flight. Other bones in the bird skeleton have been fused together as well to help make the skeleton lightweight as well as sturdy. The following figure, **Figure 8**, shows the general skeleton of a bird with the majority of the bones labeled as well as points out where some of the fused bones are located.

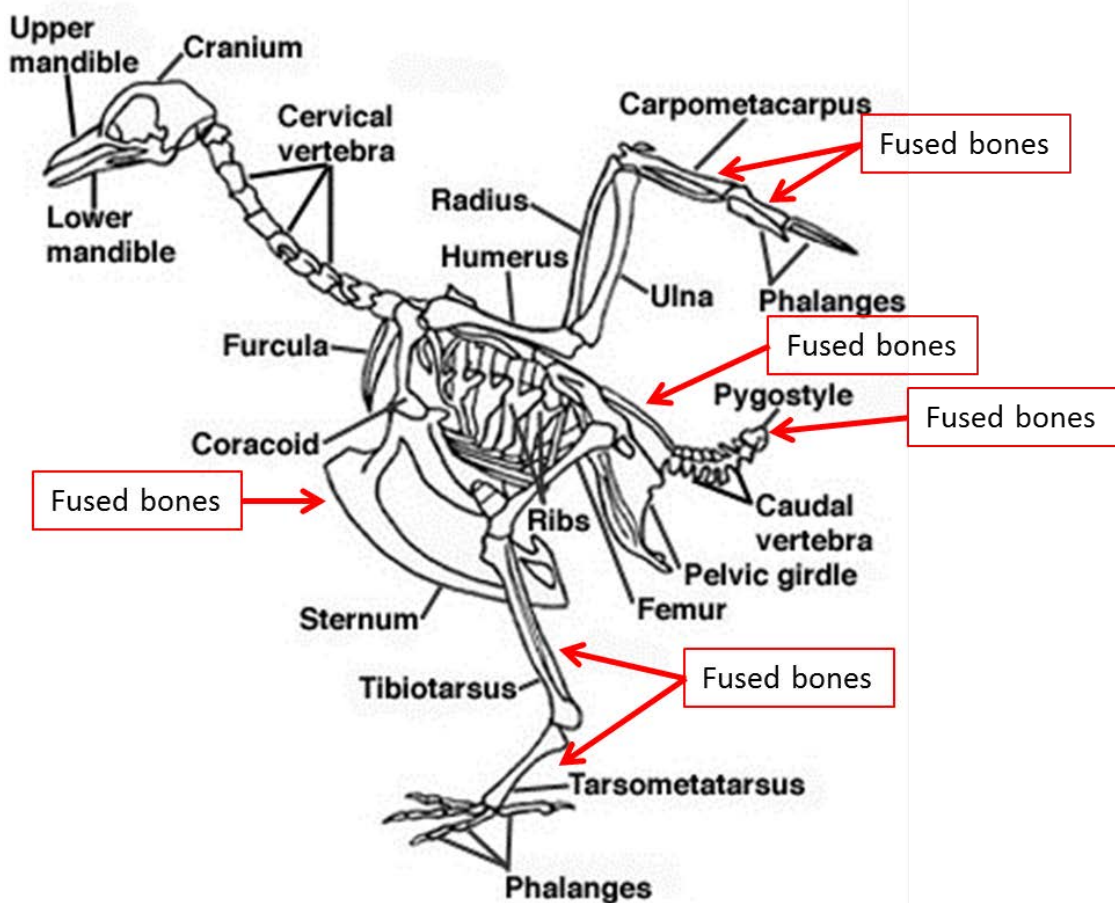


Figure 8: General skeletal structure of a bird body. All major bones and the location of the fused bones in the body have been labeled [19].

Another adaption for flight that birds have developed is that many of the bones that are found within the bird's body are hollow or semi-hollow. These hollow bones are pneumatic which means that the hollow air spaces within the bones

are integrated with portions of the air sacs that are a part of the “flow-through ventilation” system that the avian species uses to move air through their lungs. This creates an enhanced respiratory system, thus allowing the bird to have a more efficient breath [20-22]. The hollow bones within the bird’s body also exist to help it offset the high energy cost of flight [23]. Additionally, the cortical bone and its structural hierarchy seen in human long bones as well as other mammalian long bones are also seen in the long bones of various avian species [24]. But yet, even with these structural hierarchies and the adaptations of fused and hollow bones, a bird’s skeleton does not weight less than the skeleton of a similarly sized mammal. A study was done by Durmont in which she explained how a bird skeleton can look so delicate and still be heavy. She started by measuring the densities of dry crania, humeri, and femora from twenty families of perching birds, eleven families of rodents, and thirteen families of bats. She selected these bones due to the fact that they are the largest skeletal elements that contribute to the animal’s locomotion and feeding. In addition to selecting these three bones from the body, she limited her species samples to those that weighed less than 400g to achieve a dataset that would encompass similar body sizes. By analyzing the bone density data, she found that bird bones on average were denser than the bones of similarly sized mammals. Looking at the data, she concluded that bird skeletons have a higher strength-to-weight ratio as well as a higher stiffness-to weight ratio due to their denser bones [25]. This can also be seen in **Figure 9**.

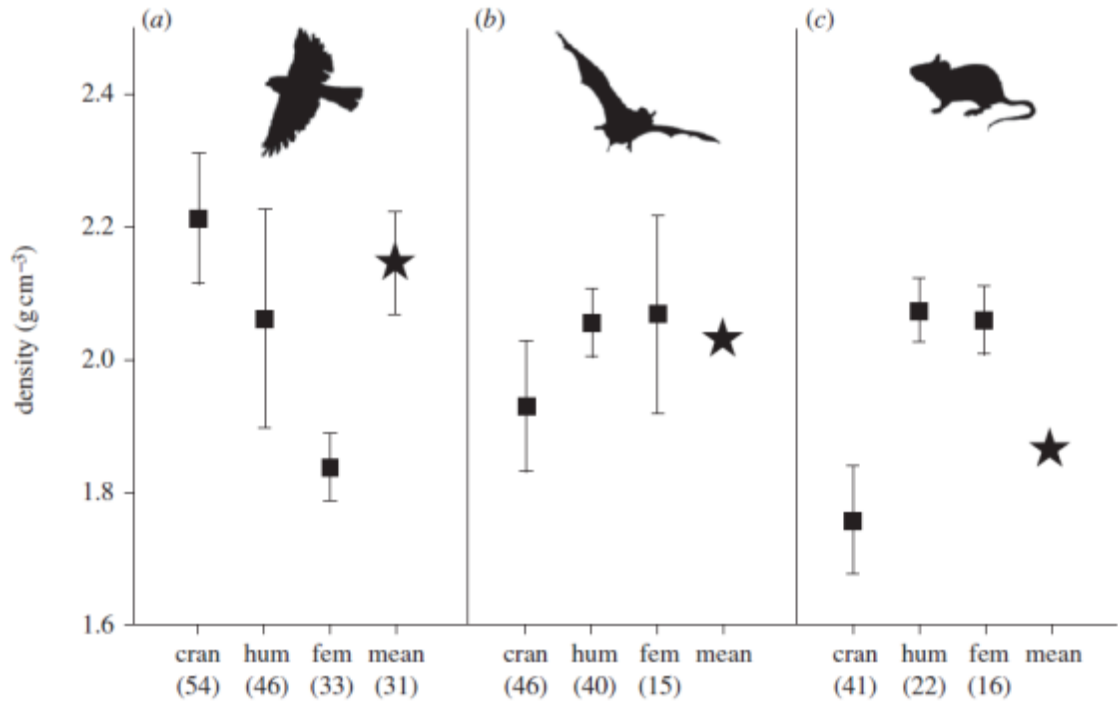


Figure 9: Means and 95 percent confidence intervals for cranial, humeral, and femoral density in a) birds, b) bats, and c) rodents with the sample sizes in parentheses. The stars for each species indicate the weighted means of the bone density for each group [25].

In addition, Cubo and Casinos attempted to study the mechanical properties and the chemical compositions of avian long bones in order to form a correlation between the two. They utilized the three-point bending test to calculate the mechanical parameters such as maximum load, stiffness, bending strength, and flexural Young's modulus. They tested the humerus, radius, ulna, femur, tibiotarsus, and tarsometarsus of 46 adult specimens that belonged to twenty-nine different species of birds. Whole bones were used for their three-point bend tests with the loading applied to the sagittal plane at the mid-point of the length and using a span in accordance with the sagittal diameter so that the shear deformation would be limited. They found that the mechanical behavior of the bone as a whole structure depends on both its

shape and material, which was also seen by Martin [26]. From their testing, Cubo and Casinos found that in bones other than the ulna and radius of the wing bones, the bending strength and flexural Young's modulus appears to decrease with body mass. Along with these observations, they also saw that magnesium and phosphorus appear to be the most important inorganic elements to providing an understanding of the mechanical behavior of avian long bones. However, since Cubo and Casinos tested whole bones, the geometrical effect was not canceled and thus produced a scaling effect on the strength and Young's modulus of the whole bone [27]. This resulted in a disagreement between their results and the finds of Biewener [28] who found that the bending strength was not correlated with body mass in his experiments. Additionally, Cubo and Casinos summarized that if pressures caused by flight acts on the strength or elasticity of bone as a whole, it would most likely affect the geometry rather than the material properties. This was also seen in previous works done by Cubo and Casinos [29-31].

Chapter 3: Methods and Experimental Procedures

The methods, procedures, experimental equipment, and facilities utilized in this study will be described here.

Ch. 3.1: Sample Preparation and Procedures

To characterize the structural properties of each avian species, different methods of preparations were required due to the condition of the bird when it was obtained.

Ch. 3.1.1: Cleaning the Samples

This section will detail the methods used to clean each bone sample that was obtained. The avian species that were used in this study included a California Gull (*Larus californicus*), Pekin Duck (*Anas Peking*), Turkey Vulture (*Cathartes aura*), and Common Raven (*Corvus corax*).

Ch. 3.1.1.1: California Gull

The first avian species that was obtained was the California Gull [32]. Professor Meyers, a professor at the University of California, San Diego (UCSD), initially found a deceased California Gull while driving and proceeded to section off the left and right wing of the bird and thereby provided the wing specimens to begin the study. These wings can be seen in **Figure 10** below.



Figure 10: Left and right wing of the California Gull.

Firstly, the primary feathers on both wings were removed and labeled so that they could be used in a separate study on the structural and mechanical properties of feathers. After the feathers were removed, the meat, muscles, and tendons of the gull were crudely cut and removed using scissors so as to minimize the amount of work needed to fully clean the bones. Next, with a scalpel, the remaining portions of meat, muscles, and tendons that were left attached the bones, were carefully cut away so as to not damage the bones. The remaining bits of flesh that were attached to the bone were scraped off using the backside of the scalpel similar to the process of peeling a potato. Once all the tissue was removed, the bones themselves were separated from each other and cleaned so that they would not mold and decompose. After photographing each bone of the wing, they were placed inside a bag and vacuum-sealed to prevent any unwanted smells from emitting. A second California Gull was again obtained and provided by Professor Meyers. This gull was processed the exact same way as the previous one.

Ch. 3.1.1.2: Pekin Duck

The next avian species used in this study was a duck that was obtained from a local grocery store. Since the label on the product did not specify the species of the animal, it was assumed that the species of duck sold was the Pekin Duck [33] due to it being the most popular domestic breed used primarily for their eggs and meat production. Two ducks were purchased for this study since it was so readily available. Because the duck was purchased at a supermarket it had already been de-feathered, therefore the separation of the wings from the body was the first step to cleaning the bones. The left and right wings from the first duck were cleaned in the same way that the gull was cleaned with the exception of the feather removal. Scissors were used to remove as much flesh as possible and then a scalpel was used to clean off the remaining portions that were left attached to the bones. The duck bone was considerably softer near the joints as compared to the gull bone so there were some parts at the end of the bone that were mistakenly cut into and removed because it was assumed to be cartilage. The wings from the second duck however were cleaned with a different method in order to try and reduce the amount of damage that would be done to the bone as compared to the first cleaning process. The wings were first separated from the body and then a method called maceration [34, 35] was used to attempt to remove the tissue from the bones. Maceration starts out by first removing as much of the flesh as possible by hand, then placing the bones in a container of water that is kept at a constant temperature of normally 35°C but no warmer than 50°C. Because

the structure of the bones was to be studied later on, the water temperature was kept at 35°C. Since there was no controllable hot plate, hot water from the sink, at 45°C, was mixed with room temperature water, 25°C, in a large beaker to maintain the 35°C temperature. A thermometer was placed inside the beaker to monitor the water temperature and then a lid was placed on the beaker to somewhat seal it off. This water was replaced often over the period of five days to maintain the water temperature and each time it was replaced a large portion of the “old” water was reused so as to prevent the bacteria culture from dying out. Due to the controlled putrefaction, the maceration process was performed under the fume hood where the smell could be maintained. After five days, the bones were removed from the beaker and the flesh was removed from the bone. Most of the meat that was still attached to the bone was easy to remove but since the temperature of the water was able to fluctuate over time, the maceration process was not allowed to fully complete and therefore there were bits of flesh that were extremely difficult to remove from the bone. These bits had to be dried for twenty-four hours in order for them to be removed.

Ch. 3.1.1.3: Turkey Vulture

The vulture was provided by Professor Meyers, which he obtained during his trip to Baja, California. He noted that the vulture was already deceased when he found it so no animal was shot in order to acquire it. Since the vulture was already dead and with only the bones to identify the species, it

was difficult to determine which vulture it actually was so it was assumed that the wings that Professor Meyers' provided were from the Turkey Vulture [36] because this species is the most widespread of the seven species found in the Americas. Upon receiving the vulture wings, it was seen that the right wing was already damaged so therefore the left wing was immediately set aside so that it could be imaged later using a micro-computed tomography machine (μ CT scan). The intact left wing can be seen in **Figure 11** below.



Figure 11: Left wing of the Turkey Vulture. The flesh and feathers were not attached to the bones upon receiving the specimens.

After setting aside the left wing, the right wing was prepared for cleaning. The cleaning process differed slightly from that of the gull and duck since there was no meat or feathers on the vulture wing that needed to be removed. However, there were plenty of dried portions of meat and tendons as well as blood spots that needed to be scraped off. The backside of a scalpel was again used to scrape off the dried meat and tendons while a nail was used to get into and scrape the more difficult parts where the scalpel could not reach without scratching or damaging the bones. The scalpel was also used to cut the cartilage and tendons that held the bones together.

Ch. 3.1.1.4: Common Raven

Again, as with the vulture wing samples, Professor Meyers provided the raven wings. However, there were no identifying features on the raven wing bones, so it was difficult to determine which species it had originated from. Therefore, since it was mentioned that these wings were from a raven, it is assumed that these bones belonged to that of the Common Raven [37]. The left wing of the raven can be seen in **Figure 12** below.



Figure 12: Left wing of the raven. The wing was received without the flesh and feathers attached.

The procedure to clean these raven wing bones was the same procedure that was used for the vulture wing bones. A comparison of the humerus bones from each of the species used in this study can be seen below in Figure 13.



Figure 13: a) The humerus bone from the left wing of a California Gull. The length of this humerus bone is approximately 13cm. b) The humerus bone from the right wing of a Pekin Duck. The length of this humerus bone is approximately 9.5cm. c) The humerus bone from the right wing of the Turkey Vulture. The bone was already damaged when it was received. The length of the humerus bone is approximately 14cm. d) The humerus bone from the right wing of the Common Raven. The length of the humerus bone is approximately 7.5cm.

Ch. 3.1.2: Sectioning Samples

This section details the various sectioning methods used to collect samples from the wing bones of each avian species.

Ch. 3.1.2.1: California Gull

The gull wing bones were the first specimens that were cut into, starting with the humerus bone. **Figure 13a** shows the gull humerus bone prior to sectioning. The right humerus was cut length-wise with a diamond cutting wire [38], mounted in a hacksaw frame. Diamond cutting wire was used since regular hacksaw blades would most likely damage the interior structures, if any, during the cutting process. The hacksaw blade would also leave a fairly large kerf, which was undesirable since the diameter of the humerus bone was small. Cutting the humerus bone in half took some time since the cutting wire, with a kerf of 0.28mm, had a tendency to flex and bend while cutting and therefore cause a change in the cutting line. After cutting the humerus bone in half length-wise, the marrow was cleaned out using the backside of a scalpel as well as a Waterpik® [39]. By cutting the humerus bone in half length-wise, any internal structures found within could be immediately located and imaged. A small portion of half the humerus bone can be seen in **Figure 14**. The rest of the images of the humerus bone will be shown later on.

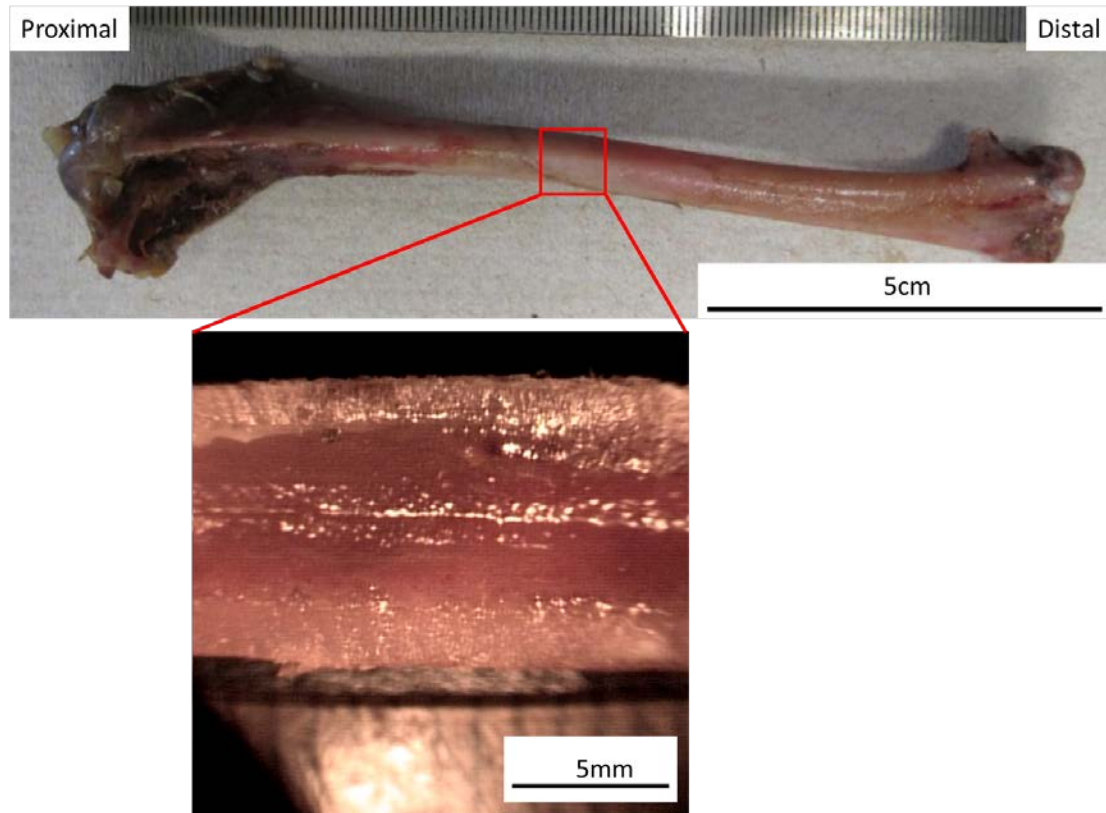


Figure 14: Length-wise cross section of the gull humerus bone. This section is located at the middle of the bone.

Next, the right ulna bone of the gull wing was carefully documented while 5mm sections were marked out on the bone; the length dimension taken from Hamed et al [40]. This length gave reasonably large enough samples to work with, either for optical characterization or mechanical testing, as well as enough samples for the various characterization works. Each 5mm section was cut using the Ryobi 9in band saw and labeled starting with number one being the most proximal. The most proximal section of the ulna bone was the side where the ulna connects to the humerus bone minus the proximal epiphysis, labeled zero, which was cut off before the 5mm sections were marked out. All other sample dimensions from the paper were not used due to

the size of the samples that were being sectioned. The kerf of the Ryobi band saw blade was 0.4953mm and since these sections were not cut on a precision machine, the actual length varied with each section.

Ch. 3.1.2.2: Pekin Duck

The duck wings bones were the next ones that were sectioned. Since cutting the bone length-wise required too much effort, supplies, and time it was decided that, that process would be skipped. **Figure 13b** shows the duck humerus before sectioning began. Again, 5mm sections were marked out on the humerus bone starting with number one being the most proximal without cutting into proximal epiphysis, labeled as zero, of the bone. The entire humerus bone was sectioned, which can be seen in **Figure 15** while a close up of a section can be seen in **Figure 16** below. The ulna of the duck was sectioned in the same way as the humerus bone with the proximal epiphysis of the bone being the side that connects to the humerus bone.



Figure 15: The duck humerus bone after being sectioned into 5mm portions.

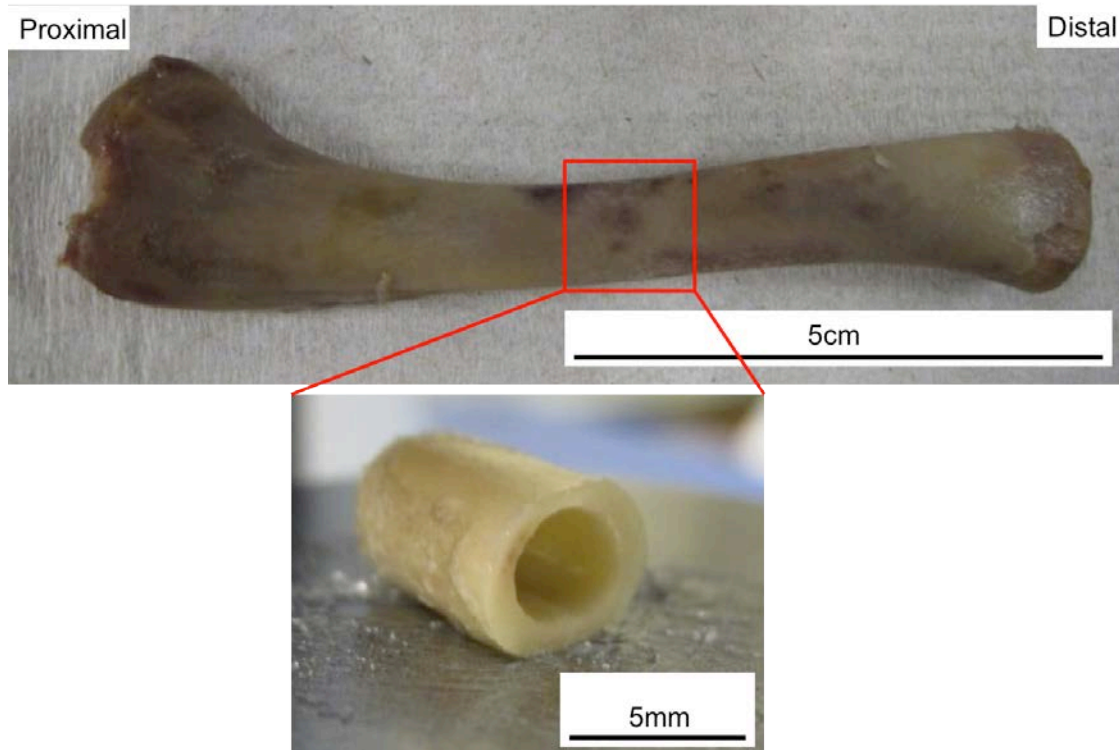


Figure 16: A section of the duck humerus bone.

Ch. 3.1.2.3: Turkey Vulture

Next, the right vulture wing bones were sectioned. Since there were limited quantities of each wing bone, and since the bones were obtained without the flesh attached, it was decided that the sectioning would start with the ulna bone so as to avoid any possible mistakes or damage to the bones that could arise in cutting the humerus bone. The ulna bone was marked out in the same way as the gull bone, with the number one section being the most proximal, closest to the humerus and ulna joint. After the process was performed on the ulna and each section was checked to insure that no damage was done to the bone, sectioning of the right humerus began. Because the right humerus bone was already damaged prior to receiving it, the damaged side became the most proximal, which coincidentally is still the

proximal epiphysis portion of the bone. **Figure 13c** shows the right humerus bone before sectioning began. In order to conserve the right humerus only half of the bone was sectioned. This can be seen in **Figure 17** below.



Figure 17: The right humerus of the vulture after sectioning. Where the bone attaches to the body or other wing bones is labeled respectively.

Ch. 3.1.2.4: Common Raven

Finally, the right raven wings were sectioned in the same way that the vulture wings were with **Figure 13d** showing the raven humerus bone before sectioning. Again, since there were limited quantities of each bone, sectioning started with the ulna. After sectioning half of the ulna bone, each sample was checked to ensure that there was no damage done to the specimens.

Ch. 3.1.3: Sample Preparation

After all the samples were sectioned from their respective bones, various preparation techniques were used in order to observe different properties and structures.

Ch. 3.1.3.1: Epoxy Sample Preparation

The first preparation technique was to embed the samples in epoxy, purchased from Buehler. The type of epoxy used was the EpoxiCure® system which is a system of resin and hardener. This system required the mix

quantities to be five parts or grams of the resin and one part or gram of the hardener. This technique allowed for the observation of cross sections of the bone as well as the microstructures that constitute them using various optical microscopes. The samples were placed in a SamplKup[®], purchased from Buehler, with the cross section of the bone touching the bottom and then filled with epoxy solution. This can be seen in **Figure 18** below.

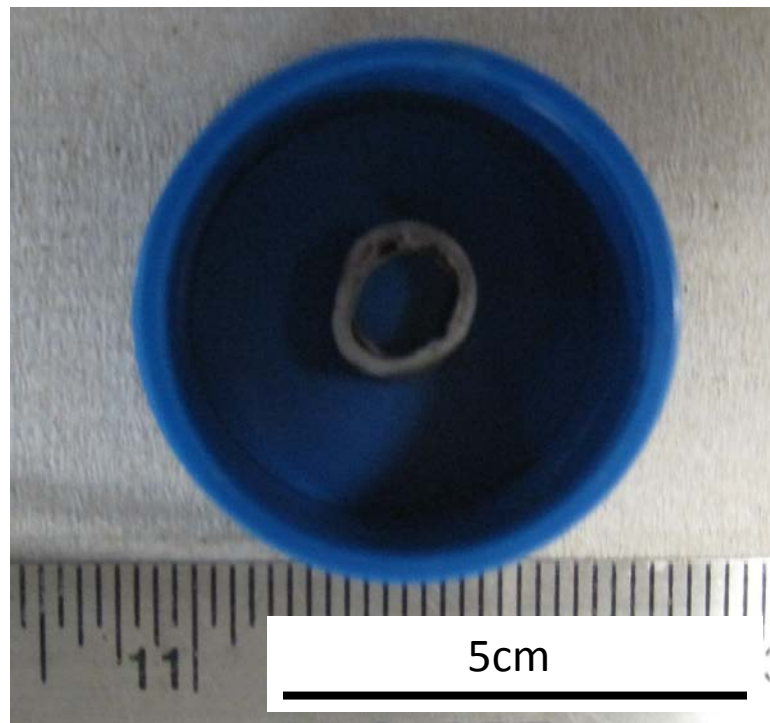


Figure 18: SamplKup[®] with a 5mm section of the vulture humerus prior to filling with epoxy solution. Once filled with epoxy solution, the setup is left alone for 24hrs to allow for the epoxy to dry and harden.

Then by allowing the epoxy solution to harden for twenty four hours, it becomes a puck, which can then be polished using the Leco VP-160 Grinder/Polisher. The puck has two sides and the side with the sample is where the polishing will take place. The grinding pads that were used were the CaribiMet[®] 2 Abrasive Discs, purchased from Buehler, which were made of

Silicon Carbide (SiC) and ranged from grits of P180, most coarse, to P4000, ultra-fine. After using the grinding pads on the epoxy puck, MicroCloth[®] polishing cloth in conjunction with MicroPolish II[®] Alumina powders, both of which were purchased from Buehler, suspended in deionized water of first 0.3 μ m powder, then 0.05 μ m powder were used on the puck to remove any surface scratches that the grinding pads may have left behind as well as give the specimen a clean and polished surface. Once this process is finished, the epoxy puck is stored in a sealed bag so as to protect the now exposed and polished surface of the sample from any unnecessary scratches. Four bone samples that were set in epoxy can be seen in **Figure 19** below.

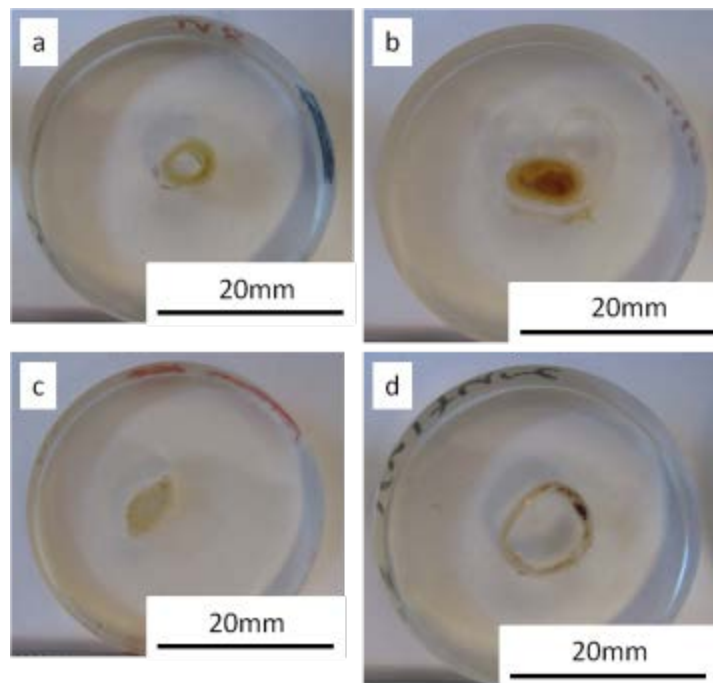


Figure 19: Exposed surface of the humerus bones from different species after grinding and polishing. a) Common Raven, b) California Gull, c) Pekin Duck, d) Turkey Vulture.

By setting the samples in epoxy, micro-hardness indentations and measurements could be performed on the now exposed and polished cross-

sectional surface of the bone. However, before any hardness testing was done, steps were taken to ensure that all the samples began in the same state. Each sample puck was first immersed in a container filled with Hanks Balance Salt Solution (HBSS)TM and allowed to soak for roughly one hour. This allowed the bone samples to rehydrate so that during the testing process, the sample condition would be similar to, if not the same as if the bone was still within the wing of the bird. Once the epoxy pucks finished soaking in the HBSSTM, they were removed, dried, and taken to the Leco Hardness Testing Machine located in the Undergraduate Research Laboratory at UCSD. In addition to enabling micro-hardness testing, setting the samples in epoxy also allowed for imaging using the Axio optical microscope.

Ch. 3.1.3.2: Water and Ash Content

The next preparation technique was used to measure the amount of water and ash content that the bone is composed of. Firstly, three 5mm sections of the humerus bone from one of the species were chosen and placed inside vials that were filled with enough HBSSTM to cover each sample. The caps were placed back on the vials and the samples were allowed to soak in the HBSSTM. Depending on the time the sample was initially placed in the vial, the soak time in HBSSTM varied between twenty-four to forty-eight hours, since it was difficult to remove each sample at exactly twenty-four hours. After the allotted time passed, the samples were taken out of the vials and the excess liquid on each was dried with a dry towel. Next, the samples were

weighed on the Denver Instrument Analytical Balance; these values will be referred to as the initial weight. Once the measurements were taken, the samples were placed on a ceramic tile and loaded into the Thermolyne Muffle Furnace. The furnace was heated to 105°C [41, 42] using the Eurotherm Temperature Controller. The samples were left inside the muffle furnace for 4 hours to dry and then removed and weighed again; these values will be referred to as the dry weight. Calculating the difference between the initial and dry weights of each sample gives the amount of water, or water content, in each. Following this, the samples were again placed on the ceramic tile and loaded back into the muffle furnace. The samples were left inside the muffle furnace for twenty-four hours at 550°C [42-45], using the controller to maintain the temperature in the furnace. After twenty-four hours, the samples were removed and weighed again; these values will be referred to as the ash weight. The difference between the dry and ash weight of each samples gives the amount of protein in each while the ash weight gives the amount of ash, or ash content in each.. This process was repeated for the bones of each species.

Ch. 3.1.3.3: Deproteination

The third preparation technique that was used in this study was deproteination. Deproteination is the process of removing or eliminating protein. There are many methods used for deproteination, but due to what was available in the laboratory the method used in this study was one that utilized

a sodium hypochlorite (NaOCl) solution [40, 46, 47]. This solution is found in regular bleach, Clorox Bleach[®], at a concentration of 5.6%, and in pool bleach, Buckman's Liquid Shock[®], at a concentration of 12.5%. Three samples were again chosen from sectioned humerus of one species. These samples were first weighed and then placed inside vials, which were then filled with regular bleach. After roughly twenty-four hours the samples were removed from the vials and weighed. Once the measurements were taken and recorded, the used bleach was disposed of and the samples placed back into the vials. The vials were then filled with fresh Clorox[®], capped and again left alone for twenty-four hours. This process was repeated until the weight of the samples, after removal from the vials, reached a steady value, which took roughly two weeks to complete. Due to the length of time it took to complete this process, Liquid Shock was used instead because of its higher NaOCl concentration. With this NaOCl solution, less time was required to fully deproteinize the samples and have them reach a steady weight value. However, the disadvantage of using a higher concentration of NaOCl solution is that it may affect the structure. Therefore, care was taken to ensure that the structures that were to be observed were not affected. Deproteination was done mainly to observe which portions of the bone were organic and inorganic. This process was repeated for the bones of each species.

Ch. 3.1.3.4: Scanning Electron Microscope Sample Preparation

Finally, the last preparation technique used in this study was SEM sample preparation. To prepare samples for SEM imaging, one 5mm section that was cut from the humerus bone of each species was taken and fractured. A specimen mount was then obtained and carbon tape was placed over the surface. Then the fractured pieces of the 5mm section for each species were carefully placed on top of the carbon tape so that the fractured surface of each specimen can be seen and imaged by the SEM. After placing the samples onto the specimen mount, they were taken to the SEM. Because SEM images are generated through the detection of electrons that are emitted from the sample, it is important that the sample be conductive and since natural materials are not naturally conductive, they need to be coated with a thin layer of conductive material. Depending on what target was installed on the particular day the SEM sample was coated, gold, chromium, or iridium was used as the conductive material and the Emitech Sputter Coated was used to apply the coating to the samples. Coating the samples mostly prevented localized heating at the site of interest, which could cause structural damage or difficulty in capturing the image due to image drifting on the screen. After coating, the sample was placed inside the SEM for imaging.

Ch. 3.2: Facilities and Equipments

The facilities that were used during this study were the Nano3 facility in the California Institute for Telecommunications and Information Technology

Center (CaliT2), the research laboratories of Professors Marc Meyers and Joanna McKittrick at the University of California San Diego (UCSD), the Undergraduate Research Laboratory at UCSD, and the Pediatrics Diabetes Research Center at UCSD's School of Medicine. The Nano3 facility in CaliT2 housed many of the optical equipments and sample preparation machines that were used in this study while the equipments found in the research laboratories of Professor Marc Meyers and Joanna McKittrick and the Undergraduate Research Laboratory were used mainly for measurement and testing purposes. **Table 1** lists the equipment found in the Nano3 facility.

Table 1: Equipment found at the Nano3 facility in CaliT2

Equipment at the California Institute for Telecommunications and Information Technology Center (CaliT2)
Phillips XL30 ESEM [48]
Axio Fluorescence Microscope [48]
Keyence VHX1000 Microscope [48]
Emitech K575X Sputter Coater [48]

Ryan Anderson, a process engineer at the facility, provided training on the equipments found in Nano3. The Emitech Sputter Coater was use prior to the use of the Phillips Environmental Scanning Electron Microscope (ESEM) machine in order to coat the specimens with a very thin layer of conducting material. This coating prevents to sample from charging when exposed to the electron beam of the Scanning Electron Microscope (SEM). The SEM was used to take high magnification images of the microstructures that make up the whole specimen. Along with taking high magnification images of the

microstructures in the specimen, the SEM machine was also able to perform elemental mapping which show the type of elements and percentages of those elements that the specimen consists of. The Axio Fluorescence Microscope was mainly used as a high magnification optical microscope to gather “macro” images of the specimen (macro when compared to the high magnification images taken with the SEM machine). The Keyence VHX1000 Microscope is capable of capturing high resolutions images as well as stitching together these high resolution images into a three dimensional image. This provides a different look on the structures and sub-structures that make up the specimen. In addition to the three dimensional image generation capabilities, various measurements and angle imaging could be made that would not have been possible otherwise. Next, **Table 2** lists the equipments found in the research laboratories of Professor McKittrick and Meyers’.

Table 2: Equipment found at the research laboratories of Professor McKittrick and Meyers

Equipment in the Research Laboratories of Professor McKittrick and Meyers
Leco VP-160 Variable Speed Grinder/Polisher [49]
Struers Accutom-2 Precision Cut-Off Machine [50]
Ryobi 9” Band Saw [51]
Denver Instrument XA-200 Electric Analytical Balance [52]
Thermo Scientific Thermolyne Heavy Duty Muffle Furnace FA1730 [53] w/ a Lindberg Eurotherm 818P Temperature Controller [54]

Various students in Professor McKittrick’s and Meyers’ lab group provided the necessary training to operate the many types of equipment that are found in the research laboratories. The Leco Grinder/Polisher was used to polish the

surface of samples mounted in epoxy so that they would be ready to take to the SEM or optical microscope for surface imaging. The polisher also aided in providing a smooth surface on which to perform micro-hardness indentations. The Struers Accutom-2 was used to precisely section certain samples so that specific areas or surfaces of the bone could be view and measured. Without the Struers Accutom-2, sectioning portions of the bone would most likely result in cracked, broken, or mangled samples. The Ryobi Band Saw was used to quick section portions of the bone for analysis. These portions did not have delicate interior structures so therefore coarse measurements and cuts were desired for quick sample turn around. The analytical balance was used to accurately measure the mass of a sample. Finally, the muffle furnace was used to heat specimens to a certain degree Celsius in order to remove all the water and/or all the protein content. This allowed for the calculation of the percent water in that particular bone sample as well as the percent mineral. **Table 3** lists the equipment found in the Undergraduate Research Laboratory.

Table 3: Equipment found in the Undergraduate Research Laboratory

Equipment in the Undergraduate Research Laboratory
Leco M-400-H1 Hardness Testing Machine [55]

Yen-Shan Lin, who is a PhD student in Professor Meyers' lab group, provided training on the Hardness tester in the Undergraduate Research Laboratory. The hardness-testing machine was used to determine the toughness of a material after it was mounted in epoxy and polished. Finally, **Table 4** lists the equipment that was used at the Cartilage Tissue Engineering Laboratory.

Table 4: Equipment found at the Cartilage Tissue Engineering Laboratory

Cartilage Tissue Engineering Laboratory
SkyScan In Vivo Microtomograph 1076 [56]

Esther Burak, a researcher at the Cartilage Tissue Engineering Laboratory, gathered the data using the micro-computed tomography machine (μ CT). Michael Porter, a PhD student in Professor McKittrick's and Meyers' group helped in coordinating the time to use the μ CT as well as helped with the 3-D generated images shown later in this study.

Chapter 4: Results and Discussions

The following chapter has been divided into two main components: structural and optical characterization of the bones from each species, and mechanical properties of the bones from each species. Each section contains an analysis and discussion of the results that were found during the study.

Ch. 4.1: Structural Characterization and Optical Observation of the of the Bones in each Species

This section will describe the results of the characterization of the wing bones from various species of birds both optically and structurally. It will become clear that the structural characteristics of the bones are largely dependent on the species of bird as well as how the wings are used to generate lift and thrust. This section will also attempt to explain why these structures occur and why they differ from species to species.

Characterization of the avian wing bones started with the notion that most birds have the capability to fly and thus the structure of their bones must factor greatly into allowing them to do so. Optical characterization of the bones was performed on all species that were used in this study. However, certain steps had to be taken to ensure that all the bones started in the same condition prior to analysis. Some of the bones, those of the gull and duck, were still “fresh”, meaning that the meat and flesh was still attached to the bones. The flesh and meat on these bones had to be removed first before

characterization could begin (for detailed explanation of the cleaning process see chapter 3). The other bones, those of the raven and vulture, were dried out and only leftover bits of flesh could still be seen on the bones. These raven and vulture bones needed to be immersed in HBSS™ in order to have them somewhat close to the condition that they would be in if they were in the body. Once the preparation of the bones was completed, sectioning began for each species (for detailed explanation of the sectioning process see chapter 3). After sectioning, documentation of the bones from each species was done to characterize the different internal structures found within.

Ch. 4.1.1: California Gull

Beginning with the California Gull wing, cutting the left humerus bone lengthwise allowed for the observation of the interior of the bone. After cleaning the interior of the bone, it was observed that there were sections that were hollow, shown in **Figure 14**, as well as sections that had internal supports. These internal supports can be seen in **Figure 20** below.

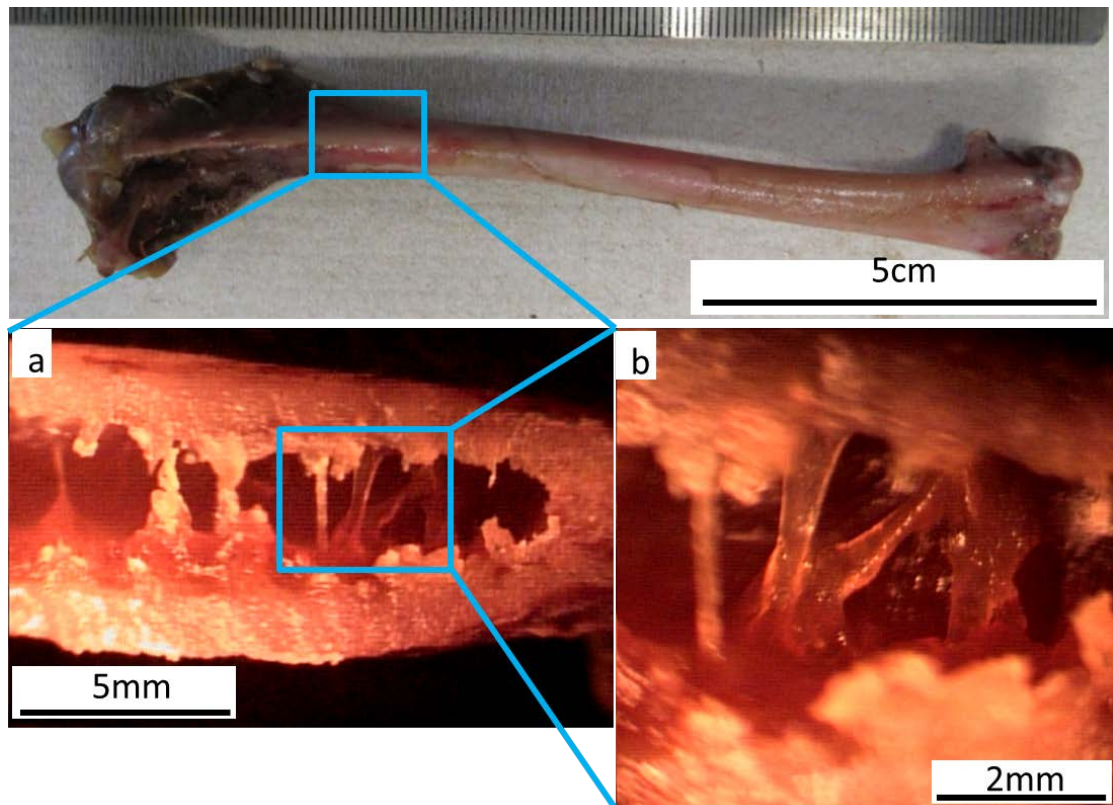


Figure 20: a) A portion of the length-wise cross section of the gull humerus bone. b) Zoomed image of the internal structures of the gull humerus bone.

These internal supports, or struts, are found only in the end portions of the bone as shown in **Figure 21**.



Figure 21: Approximate location of the struts found within the gull humerus bone.

The bone was then taken to the Keyence VHX1000 microscope in the Nano3 facility to get higher magnification images of the struts. These can be seen in **Figure 22**.

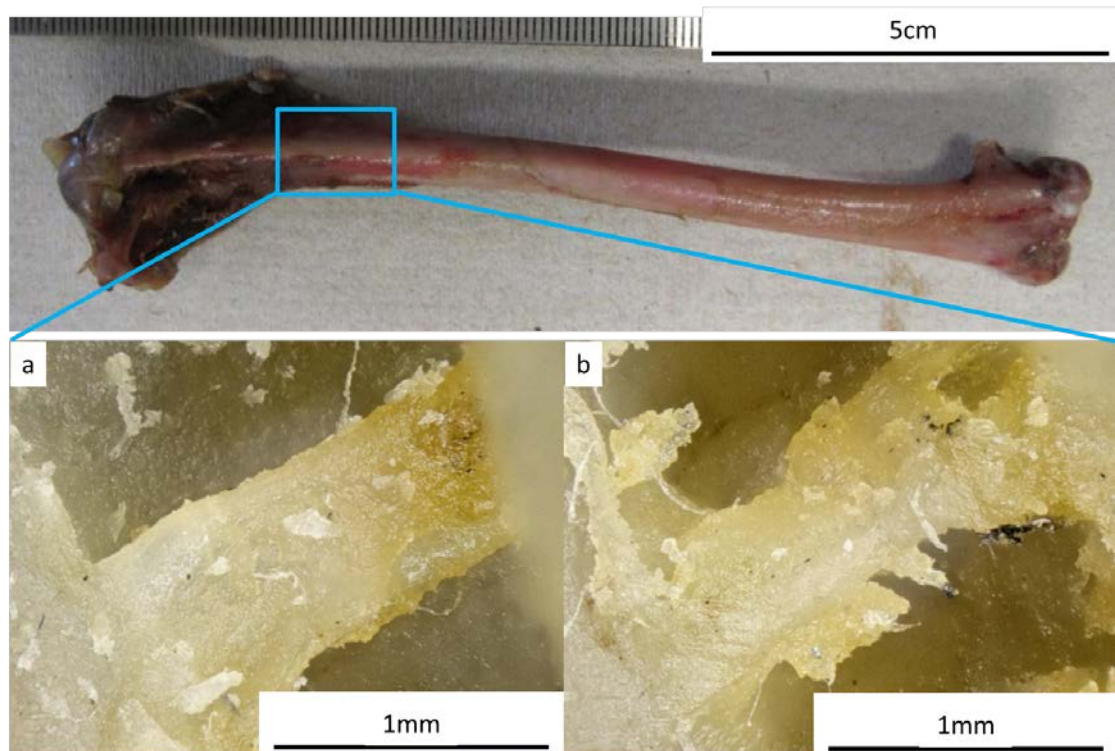


Figure 22: a) and b) show two different struts found within the humerus bone of the gull using the Keyence 3-D optical microscope.

Under a microscope, these two struts were carefully excised using a scalpel. Strut (b) was kept as is and reimaged under the Keyence microscope. This can be seen in **Figure 23**.

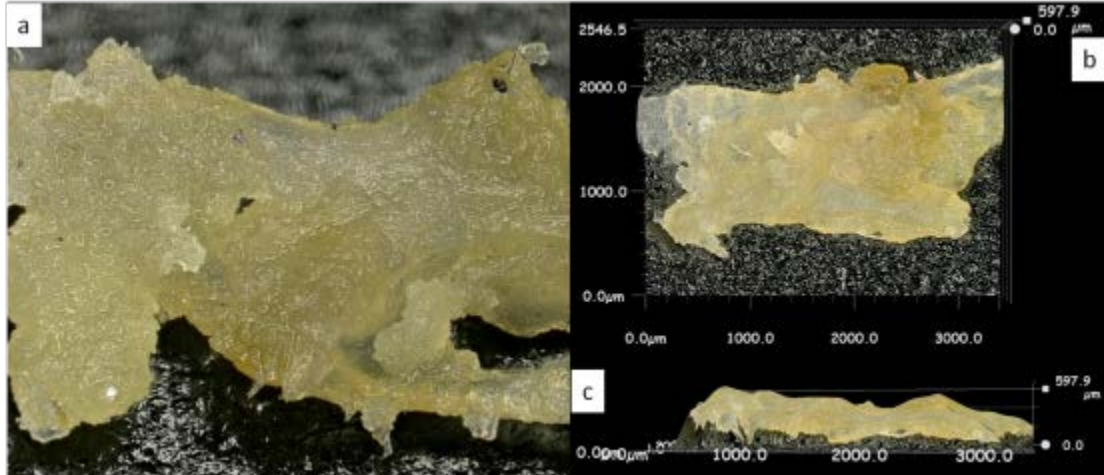


Figure 23: Strut (b) from Figure 22. a) Shows a high resolution image of the excised strut from the humerus bone of the gull using the Keyence 3-D optical microscope. b) Shows a top down view with dimensions. c) Shows a side view with dimensions.

After these images were taken, strut (a) was put through a deproteination process (for detailed explanation of the deproteination process see Chapter 3).

Deproteinization was done on the strut, with an initial weight of 0.0008g, so as to determine its composition, whether it was mainly made of protein or mineral.

After two weeks of deproteination using the Clorox[®] bleach, the amount of protein removed from the strut, roughly 0.0005g, suggested that it was composed mostly of minerals, although the resolution of the instrument at this

level could slightly skew the measurements. Once the strut was fully

deproteinized it was taken to the Phillips XL30 ESEM for observation and

since the strut was extremely small in size, it was first coated to avoid

localized heating. **Figure 24** shows the strut under the SEM after

deproteination.

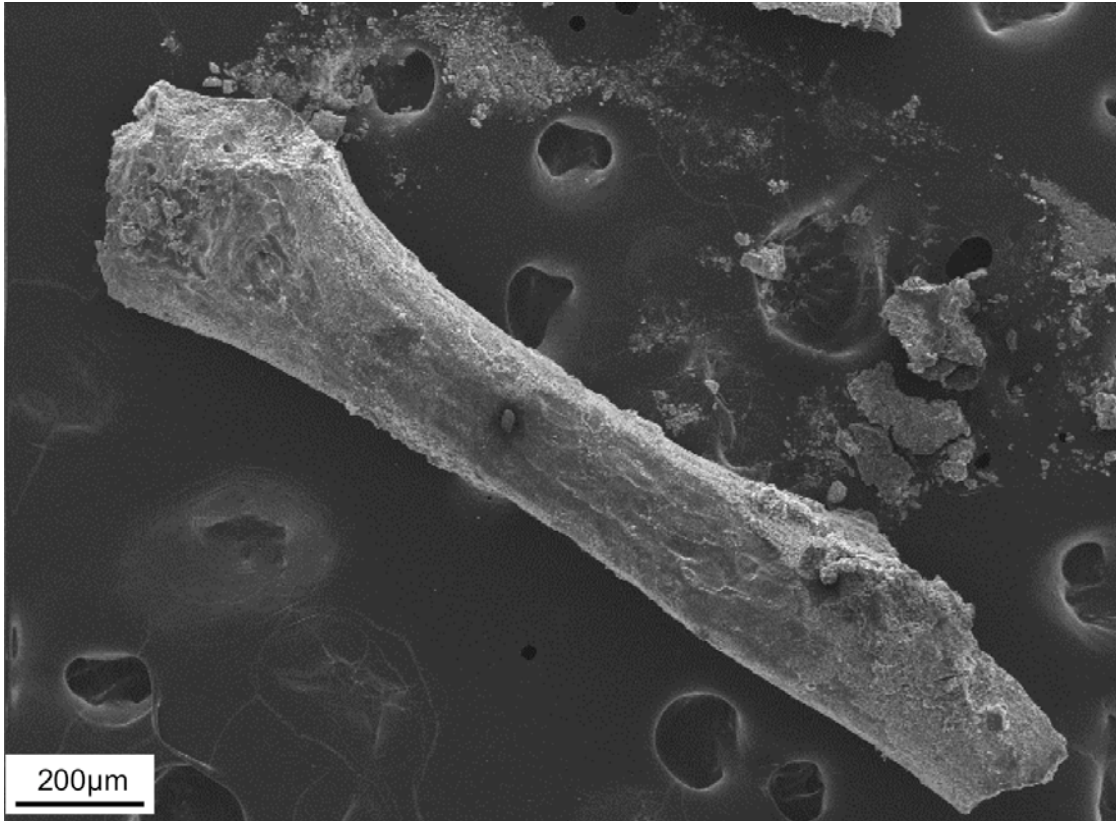


Figure 24: Gull strut (a) from Figure 22 after the deproteination process under the SEM.

The strut was then fractured so as to image the cross section of the strut as well as to determine its structure. Under SEM, it could be seen that the strut consisted of solid bone and that many microstructures could be seen on the fractured surface. These microstructures are most likely channels through which blood flow through. The fractured surface of the strut is seen in **Figure 25** below.

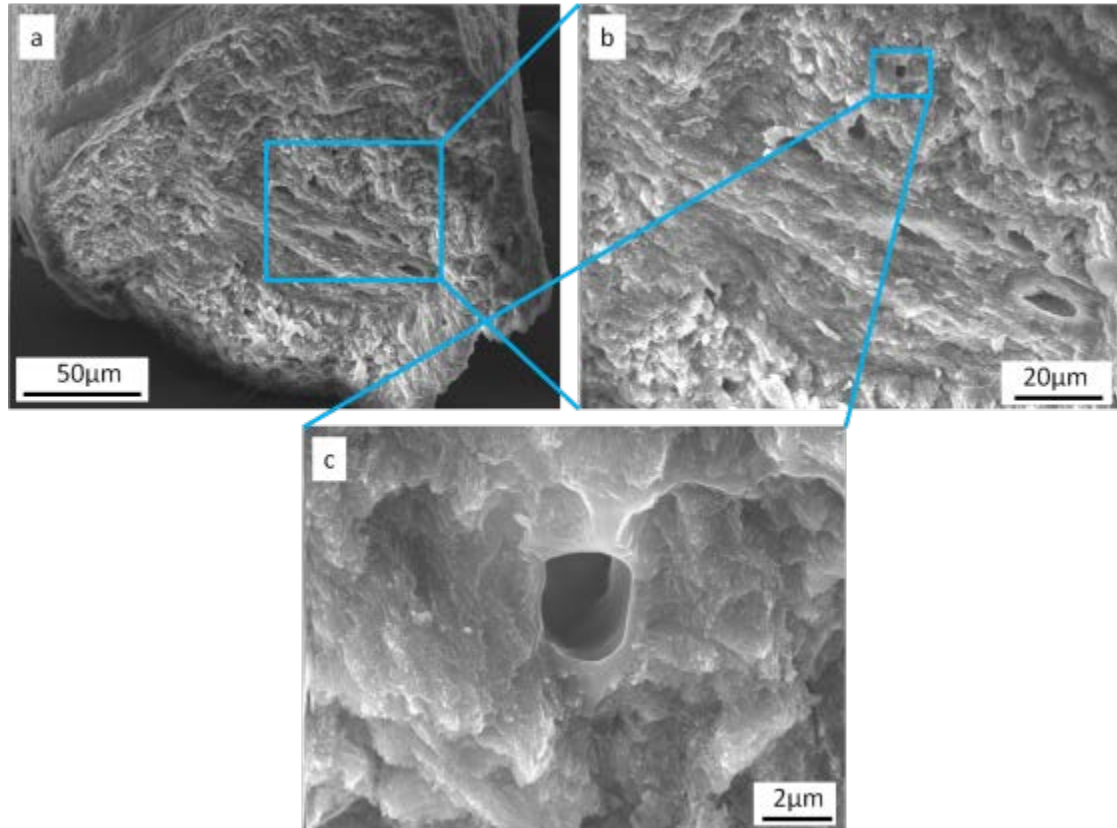


Figure 25: a) Fractured surface of the deproteinized strut from the gull humerus. b) Shows the microstructures within the strut. c) Shows one of the many microstructures within the strut

After having observed the microstructures within the strut, the cross-sectional surface of the humerus bone was then prepared to observe the microstructures within the cortical region of the bone. A 5mm section of bone that was cut from the gull humerus bone was embedded into epoxy and polished until the cross-sectional surface of the bone was exposed from the epoxy and free from scratches (detailed explanation of the epoxy and polishing process see Chapter 3). Once the surface of the sample was exposed and polished, it was taken to the Axio microscope for low magnification imaging. The images taken by the microscope revealed that the microstructures found within the gull humerus bone are similar to those of

bovine bone [16]. Structures such as osteons, vascular channels, lacuna spaces, and Volkmann's canals were all seen in the cross section. These structures are shown in **Figure 26** below while images of the bovine bone are shown in **Figure 27**. The structures found in these two figures are similar and all of them together form the basic building blocks of bone. The difference between the figures is the size of each microstructure, which is most likely dependent on the size of the bone and the age and size of the organism.

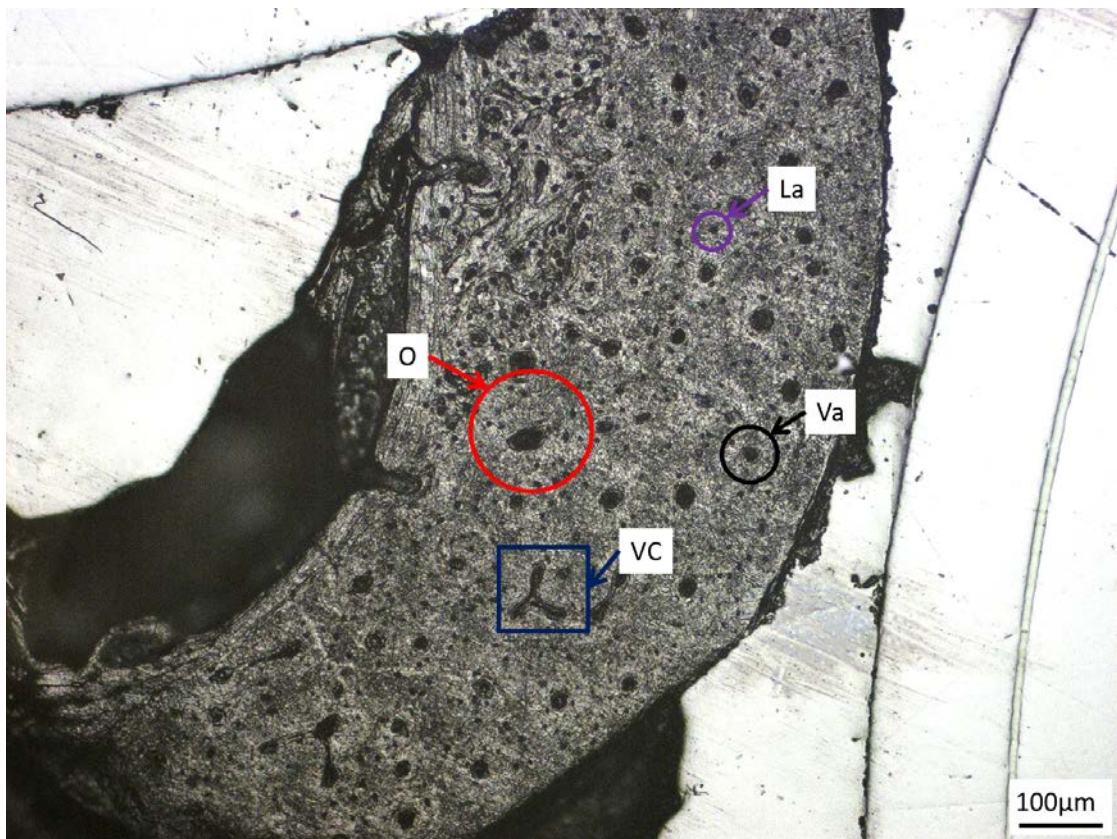


Figure 26: Cross sectional image of a section of the gull right humerus cortical bone showing the microstructures such as osteons (O), vascular channels (Va), lacuna spaces (La), and Volkmann's canals (VC).

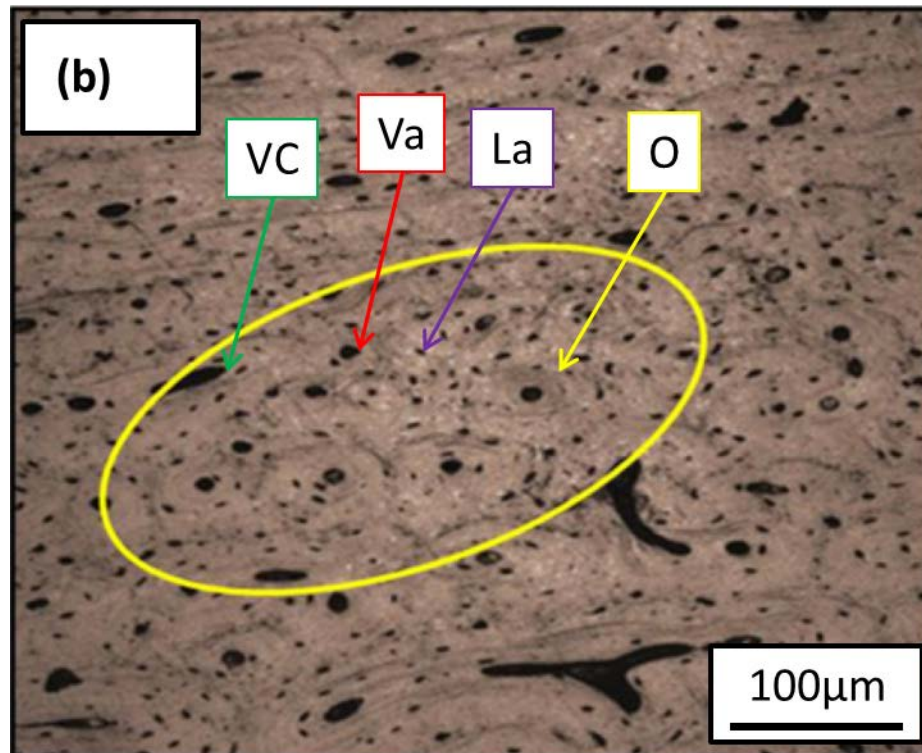


Figure 27: Optical image of untreated bovine cortical bone. Some of the microstructures can be seen in the yellow oval. Osteons (O); Lacuna Spaces (La); Vascular channels (Va); Volkmann's canals (VC) [57].

Three additional 5mm sections of the right gull humerus bone were then deproteinized for two weeks. After the deproteination process, one of the three sections was fractured and taken to SEM to study the fracture surface. **Figure 28** shows the fractured surface of the gull humerus bone. This was done so as to study the mineral phase of the bone without altering its original structure.

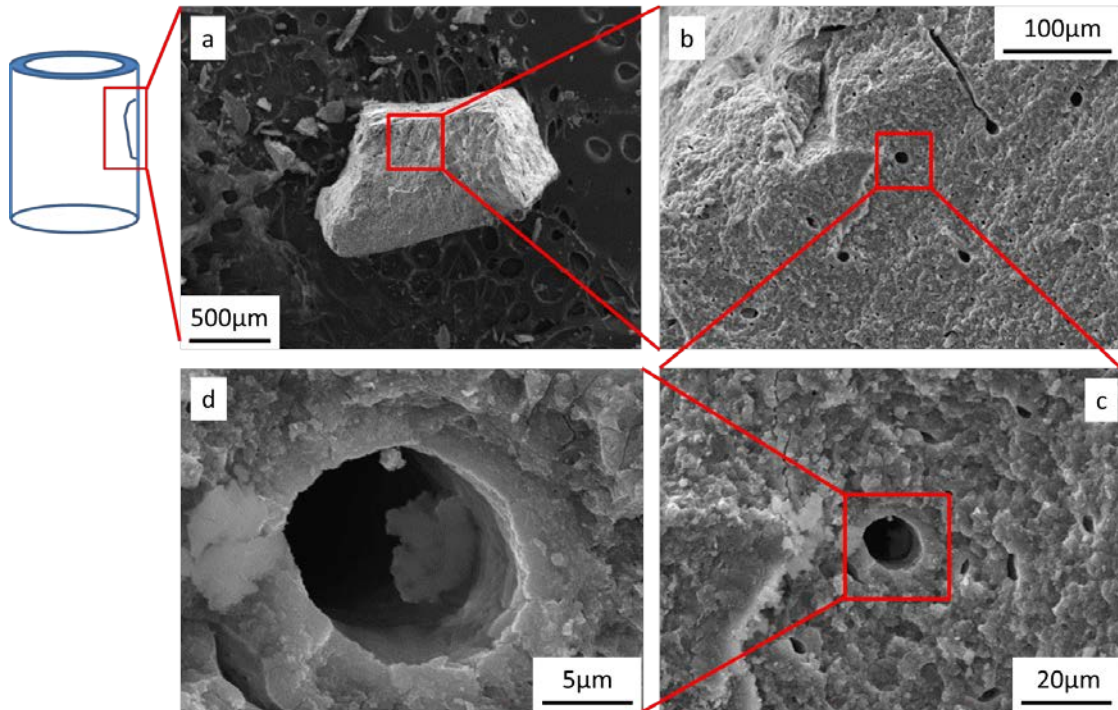


Figure 28: Fractured surface of a deproteinized section of the gull humerus bone. a) through d) shows increasing magnification of the microstructures that were seen in the bone.

Ch. 4.1.2: Pekin Duck

The next species that was studied was the Pekin Duck. 5mm sections of the duck humerus bone were cut and labeled. During the sectioning process, a few differences were immediately noticed between the duck humerus and the gull humerus. The first difference was in the cutting behavior of the two bones. With the gull humerus, the bone needed to be pushed through the Ryobi band saw and this resulted in a slightly jagged edge and plenty of bone dust. While, with the duck humerus, the bone needed no help going through the band saw, similar to a knife slicing through a warm stick of butter. In addition, cutting the duck humerus produced clean edges and little to no bone dust. Another observation was that during the removal of the flesh

from the bone, there were certain places where if the correct amount of force was applied; portions of the bone could be removed by hand. This difference between the cutting behaviors of the two bones as well as the observation seen during the flesh removal process, gives the notion that the gull bone is tougher than the duck bone. The next difference was in the internal structures of the duck bone. With the duck humerus, no internal struts were found in any section of the bone. In place of the struts, trabecular bone was found. This can be seen in **Figure 29** below. The trabecular bone looks slightly red due to the marrow still inside.



Figure 29: Cross sectional view of the duck humerus bone, taken from the most proximal end of the bone not including the proximal epiphysis, showing the trabecular bone with the marrow still inside.

The trabecular portions were found near the ends of the bone, which can be seen in **Figure 30**. The gull on the other hand had struts in these portions. What was similar between the humerus bones of the two species was the fact that the middle section of the bone was completely hollow.

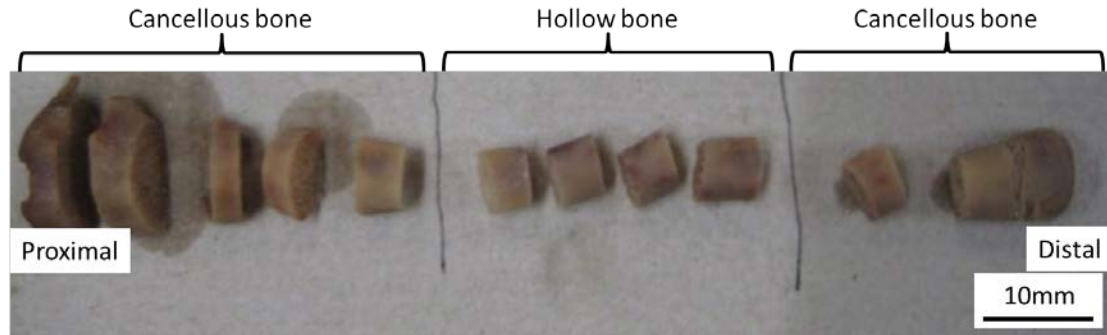


Figure 30: The duck humerus bone after sectioning. The trabecular portions of the bone are found in the ends of the bone while the middle section is hollow.

After the sectioning was finished and the marrow cleaned out of each portion, one of the trabecular sections was taken to the Keyence microscope. One of the functions of the Keyence microscope is that it is capable of taking high magnification / resolution images and then stitching those images together to form a larger image. **Figure 31** utilized this function to create a large high-resolution image of the trabecular portion in the duck humerus bone.



Figure 31: High resolution of the trabecular portion of the duck humerus bone.

Next one of the hollow sections of the duck humerus was taken and embedded into epoxy for optical observation as shown in **Figure 18**. After polishing the sample, the cross section of the humerus bone was imaged

using the Axio microscope. These images showed the microstructures, osteons, lacuna spaces, vascular channels, and Volkmann's canals, that constitute the duck humerus. These structures can be seen in **Figure 32** and are similar to the structures that were seen in the gull humerus bone.

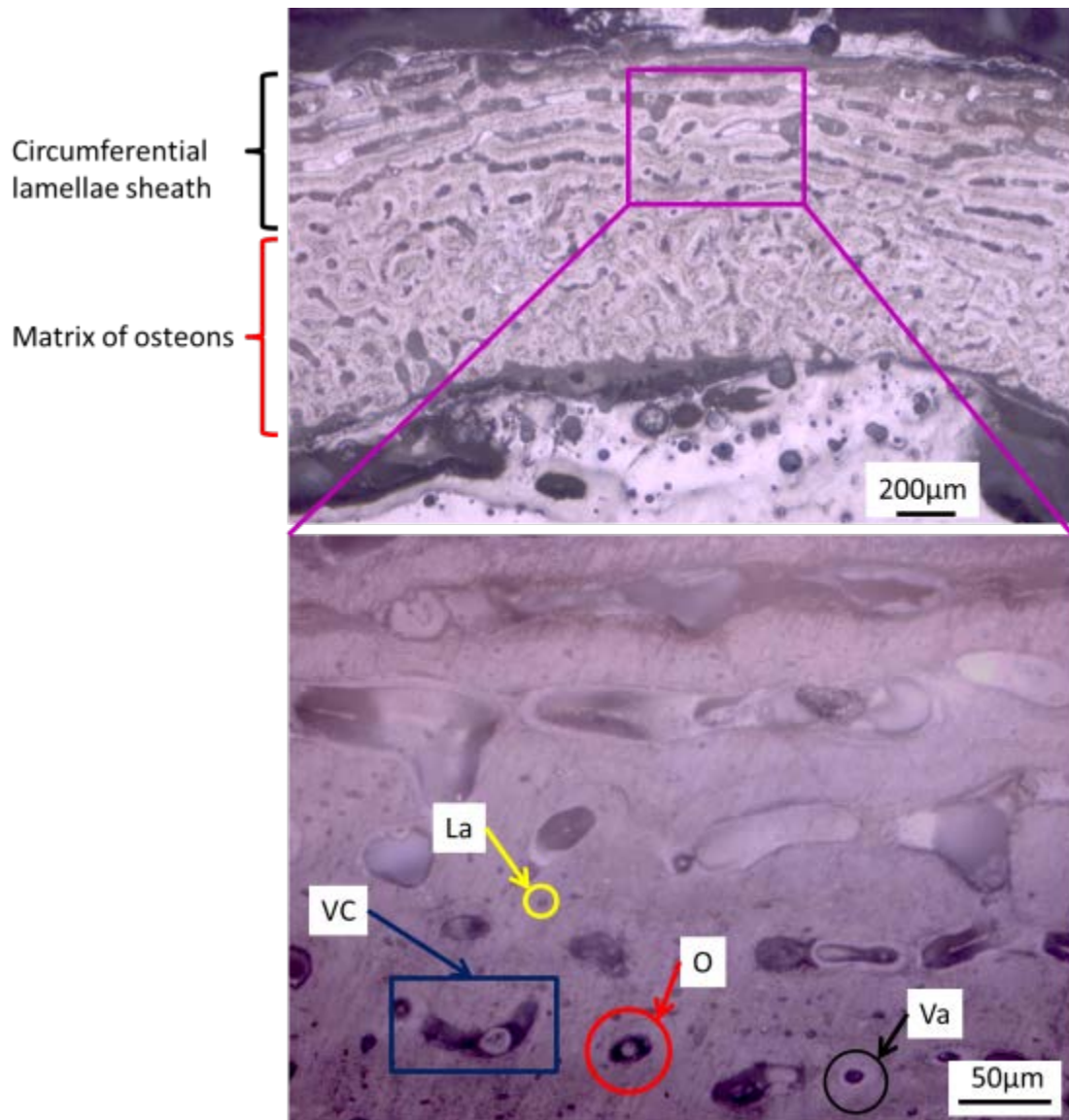


Figure 32: a) Optical image of a cross section of the duck humerus bone. Also shows the different regions within the cross section of the duck humerus bone. b) Higher magnification of the duck humerus bone showing the microstructures. Osteons (O); Vascular channels (Va); Lacuna spaces (La); Volkmann's canal (VC)

However, there is a distinct difference between the gull and duck humerus bone. In the duck humerus bone, there are two vastly different regions of structures, a matrix of osteons and a circumferential lamellae sheath. The circumferential lamellae sheath surrounds the matrix of osteons. These regions, which can be seen in **Figure 32**, were most likely still in the process of growing and reforming, in other words a young bone. This outer sheath that surrounds the osteonal matrix is extremely similar to what is seen in young bovine bone as shown in **Figure 33**. Judging by the similarity between the two bones, the duck and the young bovine, it seems to suggest that the duck bone that was obtained originated from a young duck.



Figure 33: Cross sectional image of young bovine bone showing the outer sheath.

After imaging the cortical section of the duck humerus bone, the focus was then diverted to the trabecular section. Because the trabecular bone is surrounded by the cortical bone, it was sectioned by cutting off the surrounding cortical bone, thus leaving a rectangle of roughly 7 x 5 x 5mm

(length x width x thickness) in size. This rectangle was then put through the deproteination process for a week using the Liquid Shock[®] as the vehicle for the sodium hypochlorite. After a week, the rectangular trabecular bone was taken to the SEM. It was observed that after the deproteination process, the rectangular trabecular bone was extremely brittle and any amount of force resulted in the sample breaking. This deproteinized trabecular bone can be seen in **Figure 34**, which shows some of the structures that are also seen in the cortical bone. Looking closely at the high-resolution image of the trabecular bone in **Figure 35c**, it can be said that these structures are similar to that of the struts found in the gull humerus bone shown in **Figure 25a**.

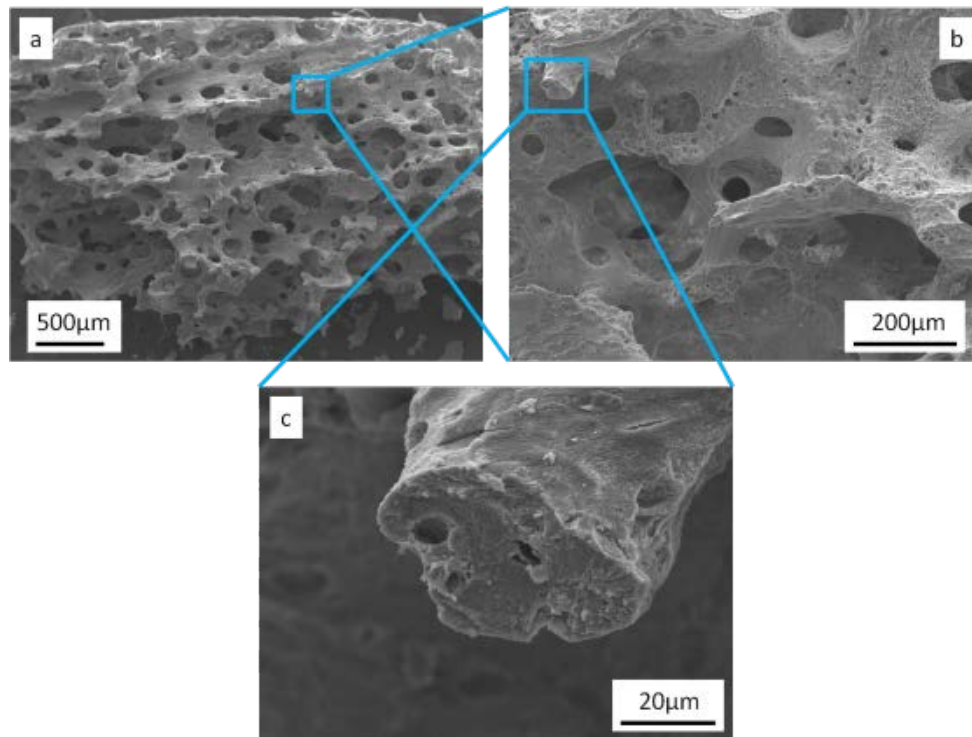


Figure 34: a) SEM image of the deproteinized duck trabecular bone cut out from the duck humerus bone section shown in Figure 30. b) and c) are higher magnification images of the structures within the trabecular portion.

Ch. 4.1.3: Turkey Vulture

As mentioned before only the right wing of the vulture was used for sectioning and studying due to the limited number of wing samples. As explained in Chapter 3, the ulna of the right vulture wing was the first bone to be sectioned, mainly a practice bone, to avoid any possible mistakes or damage to the humerus bone. However, an interesting observation as well as features arose during the sectioning of the ulna bone. While sectioning the ulna bone, it was observed that the inside of the bone was devoid of bone marrow or fluid of any kind, which was completely different when compared to the gull and duck bones. The gull and duck bones contained bone marrow and some type of clear fluid that when cut into, caused the clear fluid to seep out and create a smell that was nearly unbearable to be around. In addition to lacking marrow and any fluid of any kind, it was noticed that the ulna bone had features within that looked like reinforcing structures, or henceforth referred to as ridges. These ridges can be seen in **Figure 35** below.



Figure 35: The reinforcing structures or ridges found in the ulna bone of the vulture.

Further sectioning of the ulna bone revealed that these ridges were only found in end portions of the bone, similar to that of the struts found in the gull humerus bone. The difference between struts and ridges is that struts are like beams that span the inner diameter of the bone reinforcing the structure while ridges are like braces that support the inner diameter. Ridges may also have an appearance similar to that of the rib cage. One of the 5mm sections was taken and cut in the radial direction in order to obtain a clearer view of the struts that span the inner diameter. These struts seen in **Figure 36** are only a representation of the many struts found within the ulna bone since not all the struts form a crisscross pattern.

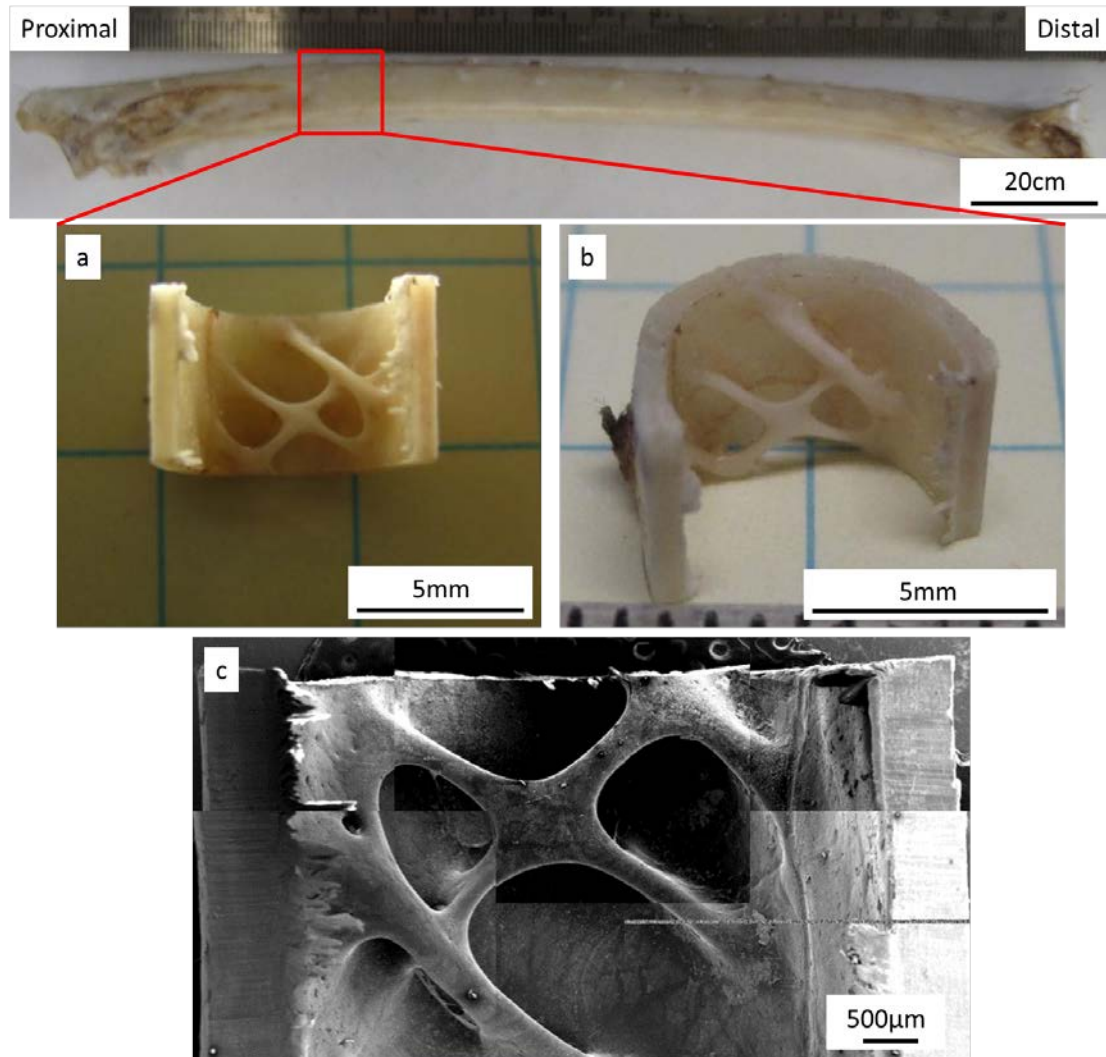


Figure 36: a) Top down view of the struts inside the right ulna bone of the vulture. b) Angled view of the struts. c) Stitched SEM images of the struts.

Following the characterization of the ridges, one of the 5mm sections of the ulna bone was embedded into epoxy with the cross section facing down as shown in **Figure 18**. Once the epoxy resin hardened, the sample was polished until the cross sectional surface of the ulna bone was free of scratches. This epoxy puck was then imaged using the Axio microscope. Again, as with the gull and duck bones, microstructures such as osteons, lacuna spaces,

vascular channels and Volkmann's canals. These structures can be seen in **Figure 37**.

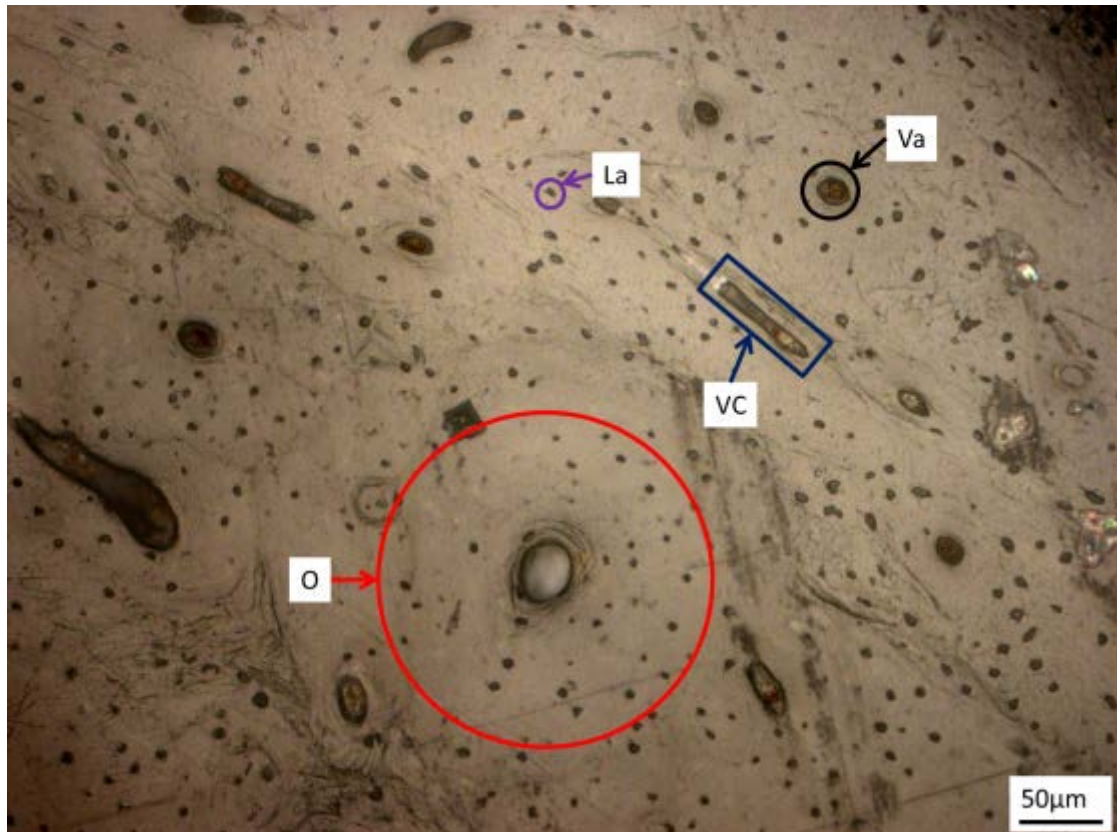


Figure 37: Cross sectional image of the vulture's right ulna bone showing structures similar to that of the gull and duck bones. Osteons (O); Lacuna spaces (La); Vascular channels (Va); Volkmann's canals (VC)

The optical image of vulture ulna bone reveals that the microstructure is more similar to that of the gull than the duck bone. In addition to the high magnification images of ulna bone, several low magnification images were taken and pieced together so that the entire cross section could be observed. This also helped to show the cross sections of the ridges that were found within the ulna bone. The stitched image of the cross section is shown below in **Figure 38**. From this optical image, it can be seen that the ridges are most

likely hollow contributing to the fact that the bones need to be strong yet light weight.



Figure 38: Entire cross section of the vulture's right ulna bone. This optical image also shows the ridges that provide support to the bone.

After the ulna was characterized, sectioning began on the right humerus bone of the vulture wing. As mentioned previously in Chapter 3, only half of the humerus bone was sectioned due to the limited amount of samples on hand. **Figure 17** shows the right humerus bone after sectioning along with

the section numbers for each specimen. It was observed that there were ridges and struts within the humerus bone, which can be seen in **Figure 39**.

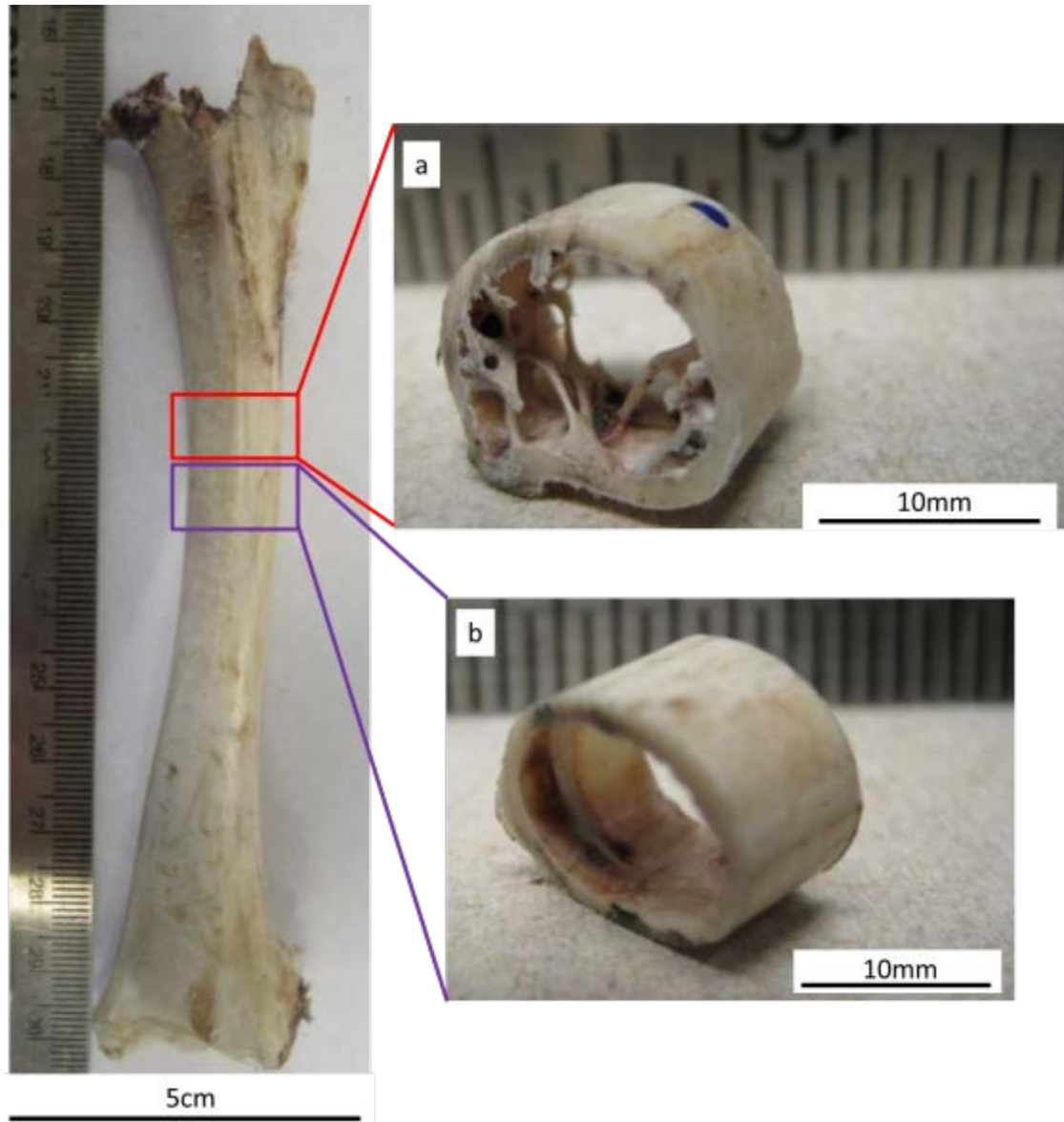


Figure 39: a) Section #4 of the vulture's right humerus bone showing struts within. b) Section #5 of the vulture's right humerus bone showing a ridge along one side of the inner circumference.

The growth of the struts and ridges within the bones of the vulture was extremely intriguing so therefore it was decided that micro-tomography should be done on the left vulture wing. The bones of the left wing were separated

from each other and cleaned (for detailed explanation of the cleaning process see Chapter 3). After the bones were cleaned, the ulna, carpometacarpus, and phalanx were taken to the SkyScan microtomograph machine (μ CT) located in the Cartilage Tissue Engineering Laboratory. Four total scans were done in different locations on the ulna, carpometacarpus, and phalanx bones. The humerus bone was not scanned because the size of the bone was too large to fit into the μ CT machine. These scans can be seen in **Figure 40**, **Figure 42**, **Figure 43**, and **Figure 45**. With the exception of **Figure 42**, which is a 50.8mm scan, each scan was limited to a 25.4mm scan to reduce the time required to complete each scan. In addition, the scale that each scan was done at was determined by the inner diameter of the ridges measured in **Figure 38**. The inner diameters of the ridges were measured to be roughly 354.3 μ m with a standard deviation of 100.2 μ m, so therefore the scale of 36 μ m/pixel was chosen for each scan.



Figure 40: a) Image of the ulna of the vulture's left wing. b) μ CT scan from the middle portion of the ulna. c) A top down view of the ulna. d) A cutaway view revealing the struts within the ulna.

Figure 40, as mentioned before is a 25.4mm scan of the middle section of the ulna bone which shows a couple struts in this region. However, from looking at the preview scan, it was seen that these few struts, along with one or two more, were the only ones in the entire middle section.

In addition to the struts seen inside the bone in **Figure 40**, the small bumps located on the trailing edge of the bone were observed to be hollow on the inside as seen in **Figure 41**. These bumps, or quill knobs, are the attachment points for the feathers as concluded by Kurzanov [58, 59], who also saw these bumps on the ulna bone of the *Avimimus*, a small dinosaur that lived in the late Cretaceous in what is currently Mongolia. These attachment points are seen along the length of the bone and are where the secondary feathers attach to [60].



Figure 41: A cutaway showing that the small bumps, or quill knobs, on the ulna bone are mostly hollow on the inside.

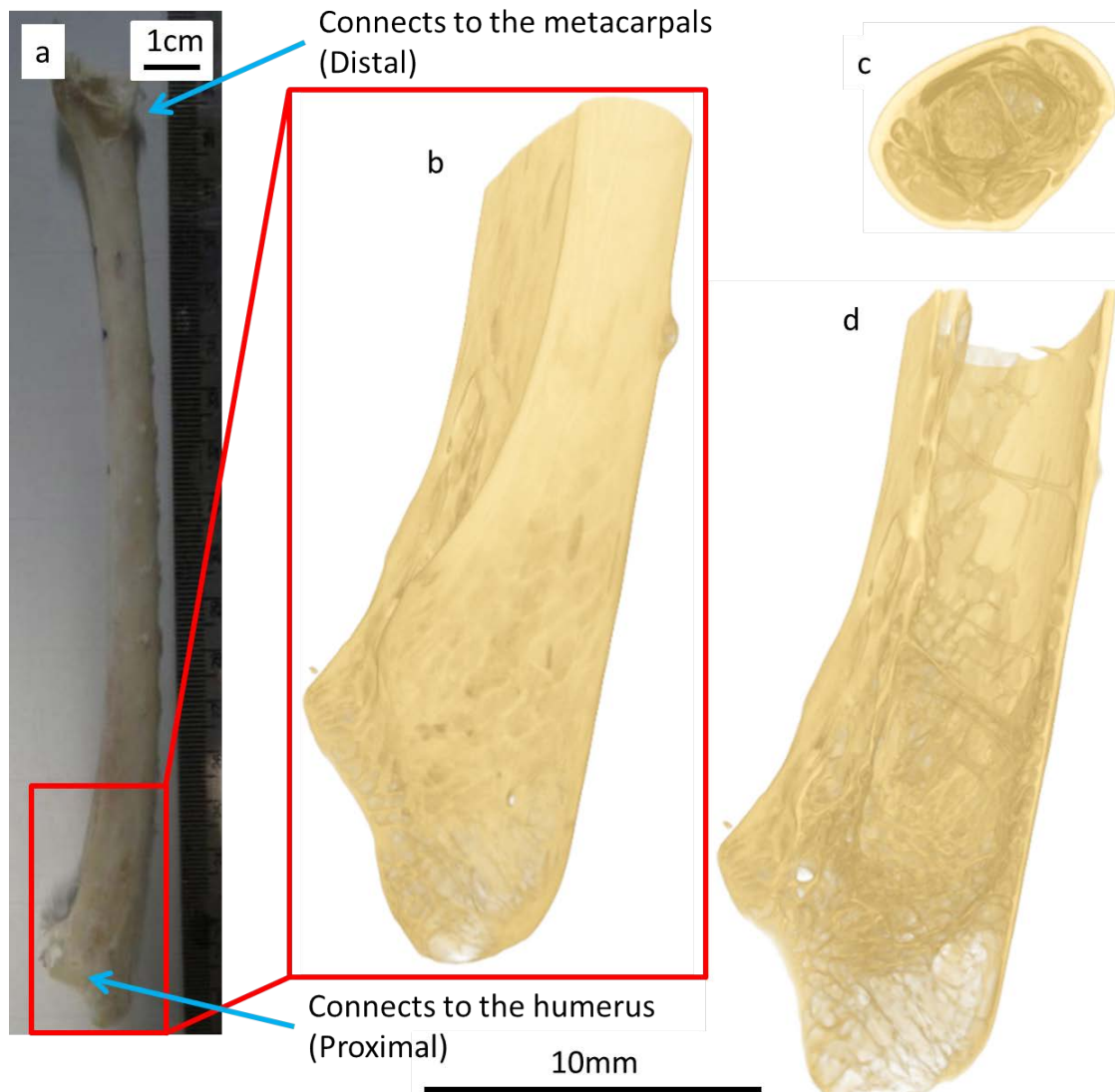


Figure 42: a) Image of the ulna of the vulture's left wing. b) μ CT scan of the end portion of the ulna. c) Top down view of the ulna. d) Cutaway view of the ulna that shows the internal structures found in the bone.

The next scan, **Figure 42** shown above, as previously mentioned, is a 50.8mm scan of the end portion of the ulna bone. Initially this area was not chosen as a scan location but due to the large number of structures that were seen during the preview scan, it was decided that in addition to scanning this area, the length of the scan would be doubled so as to see all the structures within. This figure shows that not only does the ulna bone have ridges in certain areas, but

struts as well. It also shows that the number of struts and ridges within the bone increases as one gets closer and closer to the end of the bone.



Figure 43: a) Image of the carpometacarpus and phalanx of the vulture's left wing. b) μ CT scan of the end portion of the carpometacarpus. c) Top down view d) A cutaway view that reveals the struts and ridges.

The third scan, a 25.4mm section shown in **Figure 43**, was of the end portion of the carpometacarpus bone. Prior to performing the μ CT scans on the vulture wing bones, it was seen in D'Arcy Thompson's book On Growth and Form [61, 62], that the metacarpal bone was "stiffened after the manner of a Warren's truss" [63]. The image that Thompson shows in his book can be seen in **Figure 44**.

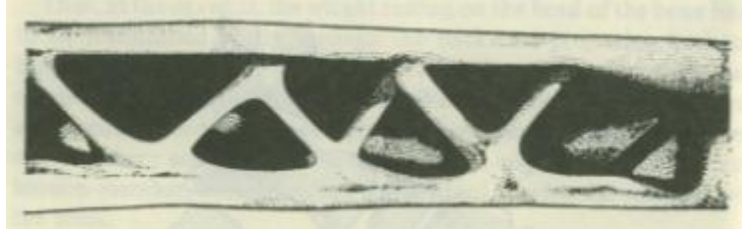


Figure 44: Image of the metacarpal bone from a vulture's wing taken from Thompson's book [62, 63].

This image was the driving force behind the μ CT scan of the carpometacarpus bone. But during the preview scan of the carpometacarpus bone, it was seen that the internal structures were clustered mainly around the end portions of the bone. Therefore, only the end portion of the carpometacarpus bone was scan. However, after performing the scan, it was observed that the carpometacarpus bone was indeed stiffened with the aid of struts and ridges, but not in the manner of a Warren's truss. These struts and ridges appeared to form within the bone on an "as needed" basis and did not always form in the same plane. This suggests that due to the varying changes in the air profile, the wing bones of the bird may have experienced many different forces on its wings causing the bone to reinforce itself to better support the bird in flight.

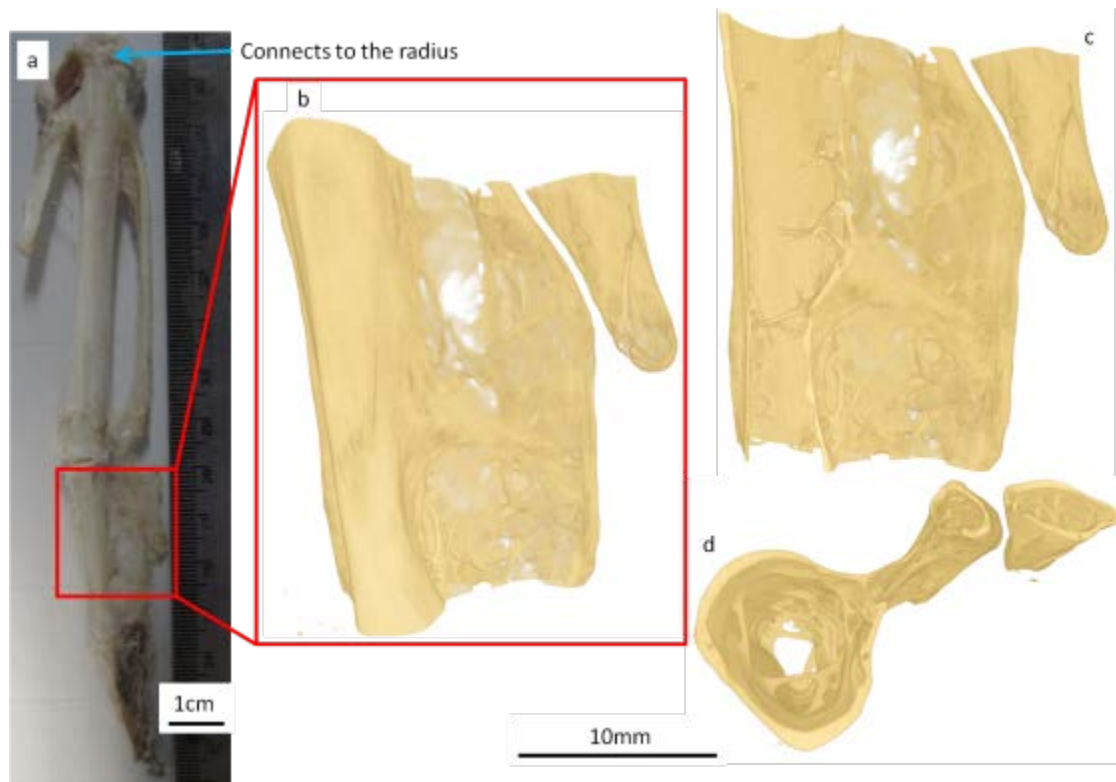


Figure 45: a) Image of the metacarpal and phalanx of the vulture's left wing. b) μ CT scan of a portion of the phalanx. c) A cutaway view revealing the struts and ridges inside. d) Top down view of the phalanx bone.

The last scan, a 25.4mm section shown in **Figure 45**, was of the first and second phalanx bones. In this section, struts seem to be the more dominant structure within the bone.

Ch. 4.1.4: Common Raven

The ulna bone of the raven's right wing was sectioned first. The ulna bone for the raven was similar to the gull and duck bone in that during the sectioning process, clear fluid seeped out and bone marrow was seen within the bone. After removing all the bone marrow from each sample that was sectioned, it was noticed that the bone contained no struts or ridges of any kind. Next, the humerus bone was then sectioned. The marrow inside was

cleaned out and as with the raven ulna bone, it was noticed that there were no struts or ridges within the raven humerus bone. Since no internal supports were found within the raven humerus or ulna bone, one of the samples from the humerus bone was encased in epoxy and polished for optical observation using the Axio microscope as seen in **Figure 46**. This image shows that the raven bone also contains the same or similar microstructures as seen in the gull, duck, and vulture bones.

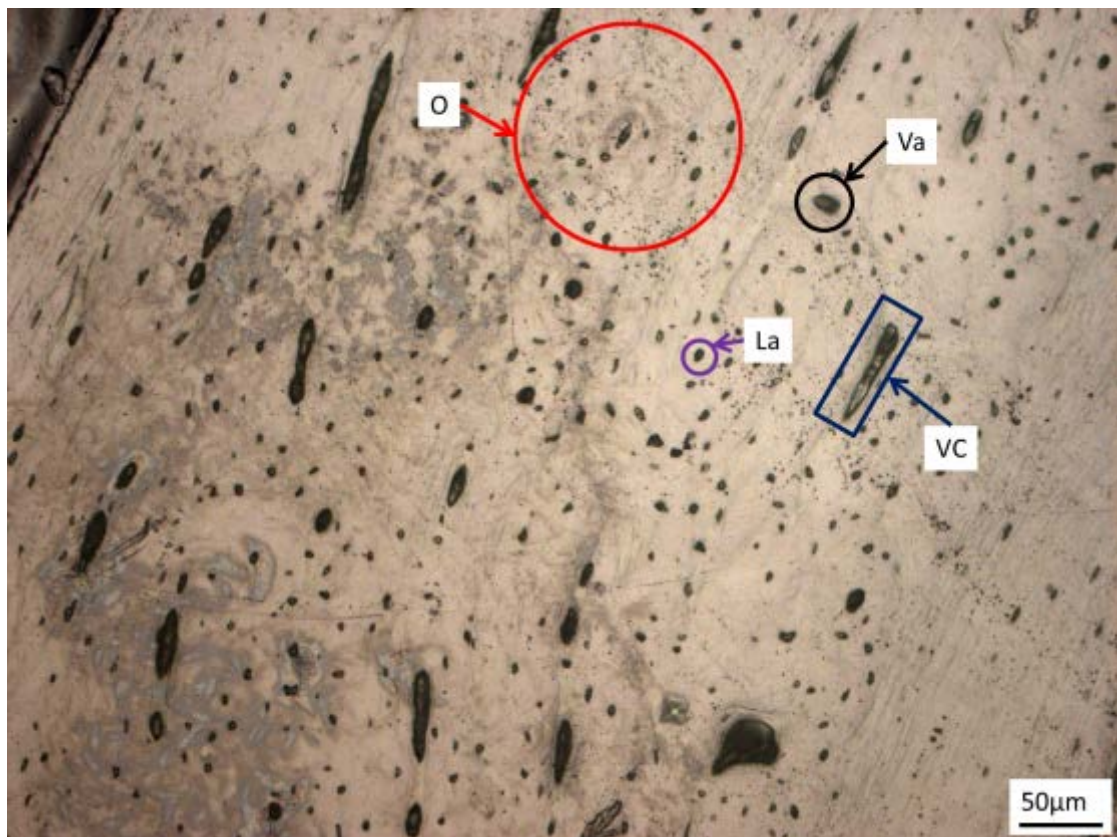


Figure 46: Cross sectional image of the raven's right humerus bone showing similar microstructures to that of the gull, duck, and vulture. Osteons (O); Lacuna spaces (La); Vascular channels (Va); Volkmann's canal (VC)

Ch. 4.1.5: Summary

From all the optical characterizations done on the bones of each species, it can be seen that the interior morphology of the bone varies. **Table 5** summarizes each avian species used in this study as well as what sort of internal structures that are found within the bones of each species. In addition, **Figure 47** shows the location of the internal structures for the bones of each species while **Figure 48**, is the collection of images that show the microstructures seen on the cross sectional surfaces of the bone from each species.

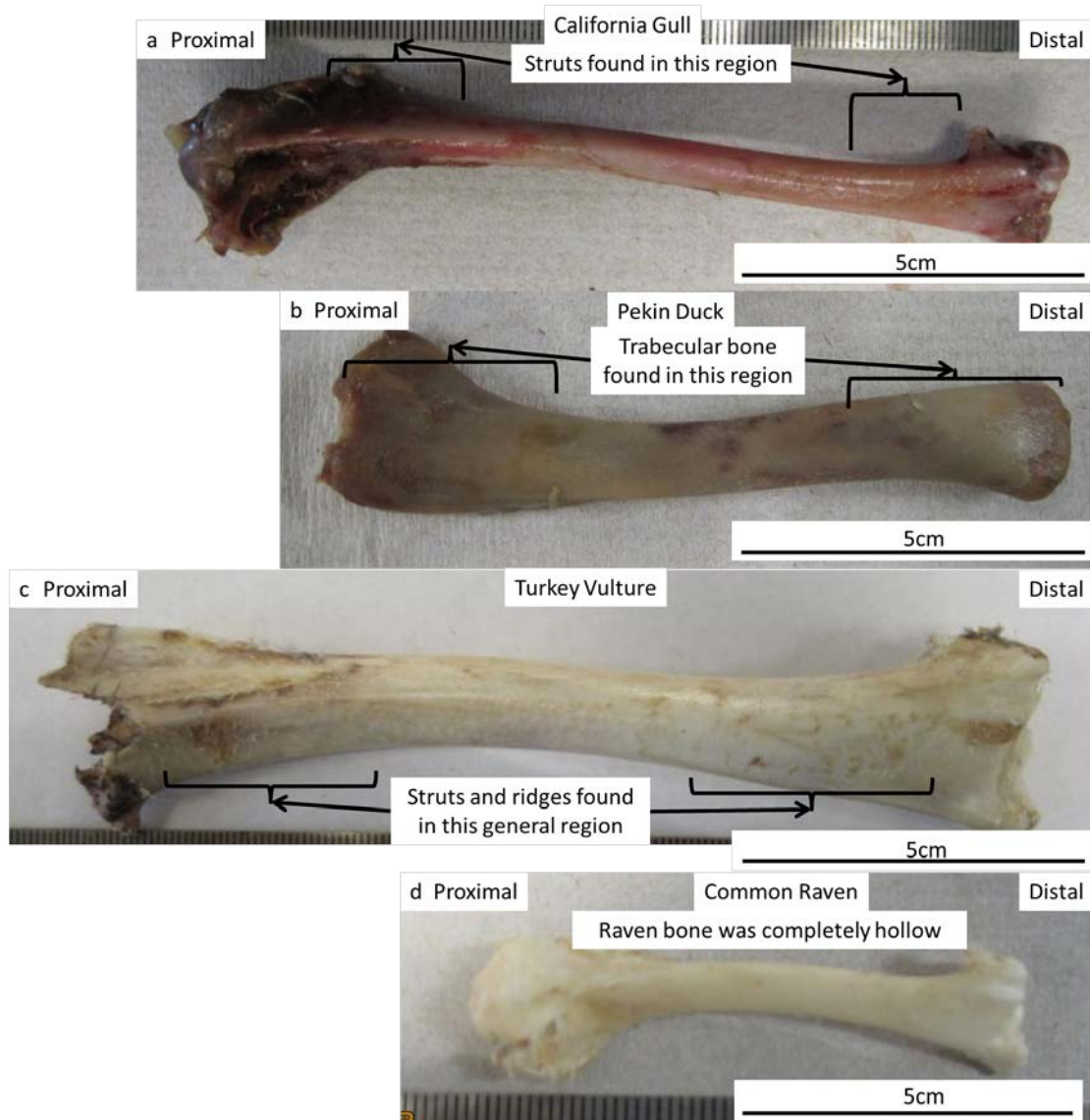


Figure 47: General location of where the internal structures were found in each bone with the exception of the raven bone. a) gull humerus, b) duck humerus, c) vulture humerus, d) raven humerus.

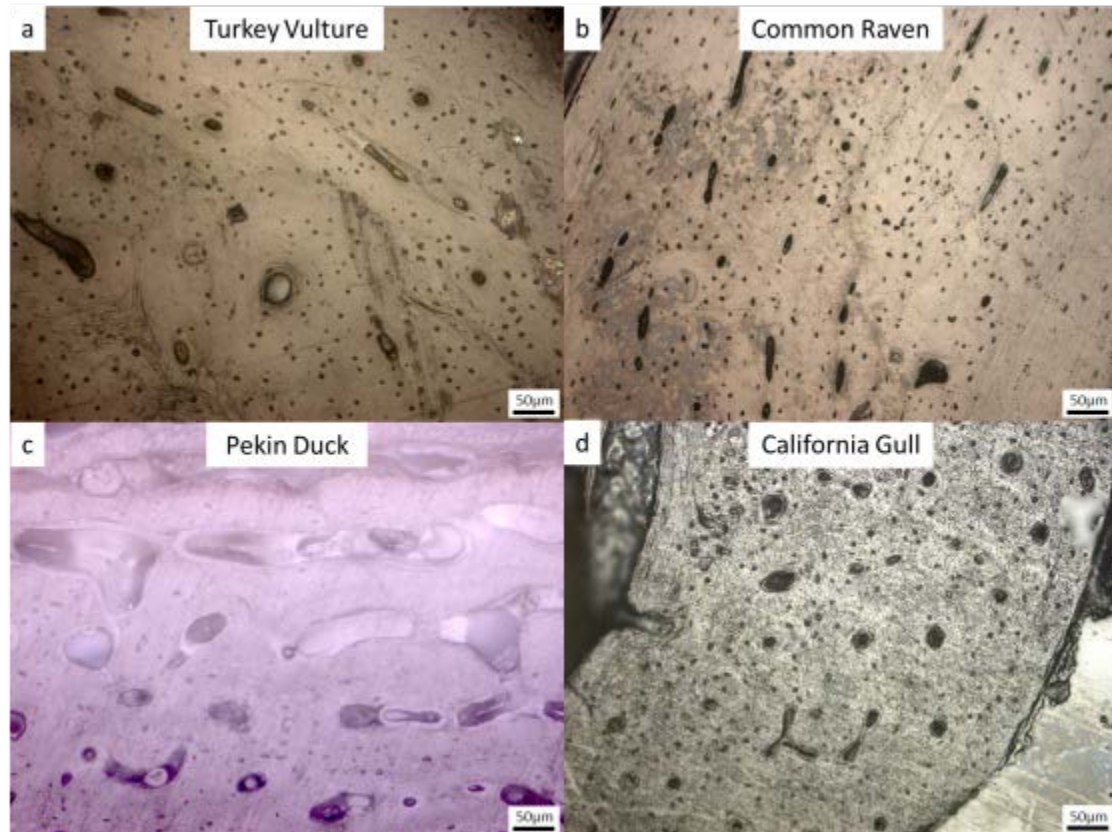


Figure 48: Microstructure comparison for each bone. a) vulture, b) raven, c) duck, d) gull.

Table 5: Table of avian species used in this study. The flying styles, wingspan, interior structures, and the structure locations within the bone are also listed.

Species	Flying Style	Wingspan (average)	Interior Structures	Structure Location
California Gull	Soaring	1.3m	Struts	Ends of the bone
Pekin Duck	Land (non-flying)	0.9m	Trabecular Bone	Ends of the bone
Turkey Vulture	Soaring	1.74m	Struts + Ridges	Ends of the bone
Common Raven	Flying	1.17m	None	N/A

A possible explanation for the variation in the interior morphology or structures of the bone is that each of the birds studied here has different methods of

achieving and sustaining flight. The California Gull, with its average wingspan of 1.30m [64], achieves and maintains flight by flapping its wings in an up and down motion as well as soaring through the air. When the gull is flapping its wings the down motion, or down stroke, provides the majority of the thrust needed for the bird to increase or maintain its speed, while the up motion, or upstroke, generates the rest. On top of the flapping motion, the gull also has the ability to search for, find, and use thermals, which are upward movements of air, to soar through the air. By utilizing these thermals, gulls are able to glide through the air without expending much energy [65]. Due to their ability to soar in the air, the gull is referred to as a soaring or gliding bird in this study. The Turkey Vulture, with its average wingspan of 1.74m [66], is similar to the gull in that it also soars or glides through the air using thermals. However, the vulture differs from the gull in that it rides the thermals to gain altitude. Once they reach the top of the thermal, they glide slowly downward and will catch another thermal when the need to gain altitude arises again. Generally the only time that a vulture will flap its wings is during takeoff and landings. In addition to gliding or soaring on the thermals, the vulture also soars at low altitude utilizing a teetering motion instead of thermals to soar through the air [66, 67]. As with the gull, the vulture is referred to as a soaring or gliding bird in this study due to their ability to soar and glide through the air. Thus by gliding and soaring through these thermals, the gull and vulture are more or less holding their wings in a stationary position, with small angle adjustments to correct for the non-uniformity of rising hot air, while the rising air imparts a

force to the underside of the wing creating lift. Because the wing bones of the gull and vulture need to withstand the natural downward force due to the bird's weight and gravity as well as the lift forces created by the rising thermals, it appears that struts and ridges, the ridges only seen in the vulture, have developed within the bone to support and reinforce them with minimal amount of additional weight. These struts and ridges do not seem to be forming in the same direction, plane or location within the bone when comparing the left wing to the right wing of the gull and vulture. In addition, the amount of struts and ridges found within the bone seem to differ when comparing the left and the right wings. Therefore, since all bones within the body are in a constant state of growing and re-growing by rebuilding the matrix of osteons [68, 69], it seems to be that the struts and ridges found within the bone of these two species only form on an "as needed" basis, strengthening the bone in place where a higher than normal stress is exerted on the bone by the pressure differences caused by the fluctuation of the air flow over and under the wings of the bird.

On the other hand, the raven, with an average wingspan of 1.17m assuming that the bones in this study originated from a Common Raven [70], is constantly flapping its wings to increase or maintain its speed and or altitude. The raven will occasionally soar through the air but not for very long. In addition, the raven is also known to be very agile in the air, performing aerial stunts such as barrel rolls and dives [70]. Because the ravens flap their

wings more often than not, it is referred to as a flying bird in this study. Since the raven is constantly flapping, the lift and thrust forces that are generated are divided between the up stroke and down strokes of the wing. Therefore, the wings are not subjected to the constant lift forces seen during the soaring motion of the gull or vulture. Also, because the raven is generally lighter in weight, the downward force acting on the wings due to the weight and gravity is less when compared to that of the gull and vulture. The lighter load on raven's wings coupled with its physical size may possibly explain the absence of struts and ridges inside the bone.

Finally, the Pekin Duck, with an average wingspan of 0.90m assuming that the wingspan of the Pekin Duck is similar to that of its parent breed the Mallard [71, 72], is generally too heavy to fly. However, if the duck is light enough, they are capable of flying for an extremely short period of time [71]. Since this species of duck is mostly overweight and stays either on land or water, it is referred to as a land bird in this study. In addition, since the ducks used in this study were purchased from a local grocery store, it could be that the wings of the duck were also clipped and thus completely preventing it from flying. Therefore, either because the wings of the duck are tucked close to the body most of the time or because the wings were clipped, they are neither exercised nor exposed daily to the lift, thrust, and gravity forces that are experienced during flight. Thus, instead of developing struts and ridges within the bones and thereby strengthening the bones for flight, the wing bones of

the duck developed trabecular bone. Trabecular bone is normally less dense and weaker when compared to compact bone but has a higher surface area.

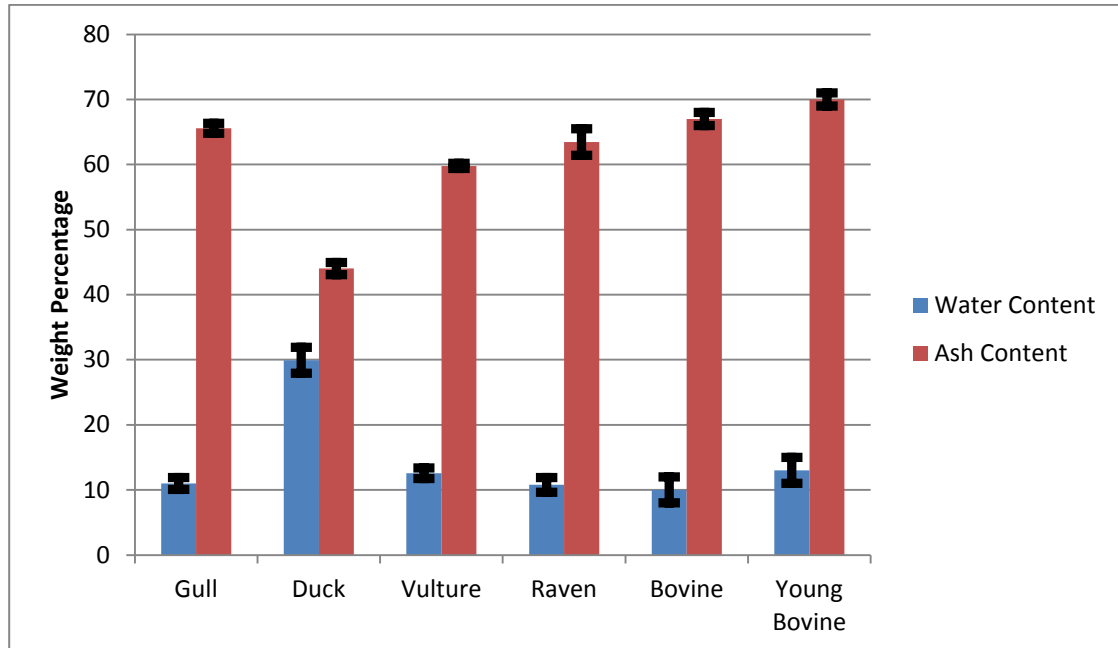


Figure 49: Chart showing the water and ash content of all the avian species studied and compared with that of the bovine and young bovine [57].

In addition to observing the different structures found within the bones of each species, water and ash content analysis was done on multiple samples from the humerus bones of each species and compared to values from bovine and young bovine femurs [57]. From the chart, shown in **Figure 49**, it can be seen that the duck has the lowest ash content percentage as compared to the others. This could be due to the fact that the species of duck that was used in this study most likely seldom or never used its wings for flight. Because of this, a slight drop in bone mass or mineral content may be expected. In addition, the Pekin Duck, which can be found in grocery stores, is normally killed at an extremely young age, around three months old, while the

average life expectancy of a fully mature adult duck is around nine to twelve years. This could also help to explain the lower mineral content seen in the duck bone. Looking at the ash content percentages of the gull, vulture, and raven humerus bones, it can be seen that they have nearly the same ash content values as the bovine and young bovine femur bones. This suggests that the wing bones of these birds need to be as stiff as a bovine femur bone to resist all the different forces that act upon the wing during flight.

Ch. 4.2: Mechanical Properties of the Bones from each Species

This section will describe the results of the mechanical characterization of the wing bones from various species of birds. It will become clear that the structural characteristics of the bone either help strengthen it or reinforce it to withstand the rigors of flight.

Mechanical characterization of the avian wing bones started with generating mechanical tests that would not damage or destroy the bones. This was important since the wing bones were difficult to obtain and the struts and ridges within the bone were even more difficult to find without the aid of a μ CT scanner. Therefore, the chosen mechanical test to be done on the wing bones was micro-hardness indentation testing. This test was chosen mainly because the indentations made on the surface of the samples were extremely small and with a little polishing, the indentations could be quickly removed to return sample surface to pre-indentation conditions. Micro-hardness indentation testing was performed on the wing bones of all the species that were used in

this study and therefore steps had to be taken to ensure that all the bone samples started in the same condition prior to testing. Since not all the 5mm samples that were sectioned for optical characterization were used, these remaining samples were taken and cleaned of any marrow or fluid (if any) that still remained. After cleaning, the samples were embedded into epoxy, as seen in **Figure 18**, and allowed to cure for twenty-four hours. The next day, after the twenty four hours have passed, the epoxy pucks were removed from the SamplKup® and the side with the sample was grinded and polished. **Figure 19** shows a few bone samples after the grinding and polishing process (for a more detailed explanation on the grinding, polishing, and sample preparation for hardness testing please refer to Chapter 3). Once the preparation for the samples was completed, hardness testing was done using the Leco Hardness Testing Machine. The machine is fitted with a Vickers Hardness tip, a diagram of the Vickers tip is shown in **Figure 50**, and before each bone sample was indented, a rectangular block of aluminum 6061-T651 was used to confirm that the tip was neither damaged nor was the machine miscalculating the hardness value, which outputs the results as Vickers Hardness. The equation used by the machine to calculate the Vickers Hardness is shown in **Equation 1** below.

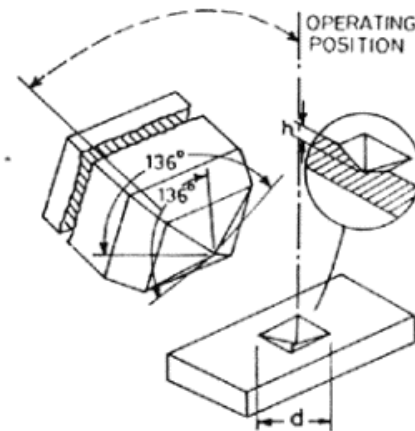


Figure 50: Diagram of the Vickers Pyramid Diamond Indenter [73]

$$HV = \frac{2F \sin \frac{136^\circ}{2}}{d^2} \text{ or } HV = 1.854 \frac{F}{d^2}$$

Equation 1: Vicker's Pyramid Diamond hardness number (HV) equation. F is the applied load in kgf while d is the average length of the diagonal left by the indenter in mm [73].

The load for the micro-hardness indentation on the aluminum block was selected to be 10.19kgf (100.00N) since this load imparted a reasonable sized indentation. The reported hardness value of the aluminum block was then compared with known values, obtained from the ASM Aerospace Specification Metal Inc. website [74], to confirm that the machine was working properly. After it was confirmed that the micro-hardness tester was operating properly, each bone sample was loaded into the machine. The load on the machine was then changed to 1.02kgf (10.00N), since 10.19kgf would create too large of an indentation on the surface of the tested samples. Roughly ten to twelve indentations were performed on each bone sample surface with indentations done on various areas of the sample surface. Once the testing was finished the average micro-hardness value was calculated. The California Gull, with an

average Vickers Hardness value of 66.57HV (653.05MPa) and a standard deviation of 5.18HV (50.80Pa), the Pekin Duck, with an average Vickers Hardness value of 41.17HV (403.85MPa) and a standard deviation of 4.44HV (43.56MPa), the Turkey Vulture, with an average Vickers Hardness value of 53.55HV (525.33MPa) and a standard deviation of 5.17HV (50.72MPa), and the Common Raven, with an average Vickers Hardness value of 59.84HV (587.03MPa) and a standard deviation of 5.48HV (53.78MPa), are show in the following chart, **Figure 51**. This chart also compares these hardness values to those of the bovine and young bovine femur bones [57].

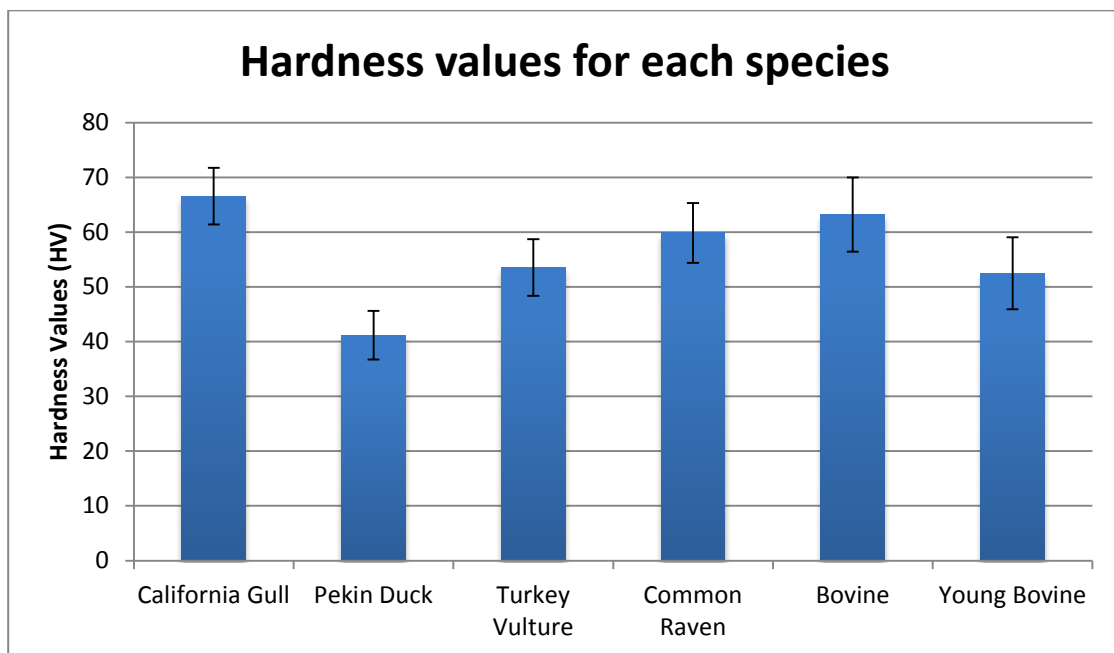


Figure 51: Average micro-hardness value of the humerus bones from each species and compared to the micro-hardness values of the long bones in the bovine and young bovine [57].

From this chart, it can be seen that the hardness values of the humerus bones for the California Gull and Common Raven are similar to that of the long bones for the bovine. Since hardness testing gives insight on how resistant a

material is deformation, it can be correlated that the wing bones of the California Gull and the Common Raven are nearly equal in resistance to that of the bovine long bone. An explanation for this is that the various loads experienced by the wing bones of the bird are similar to the loads that the long bones of the bovine experience. In the bovine and young bovine, the loads that the long bones experience day-to-day are due to gravity, weight, and the reaction forces applied by the ground. On the other hand, the long bones of a bird experience gravity, weight, lift, thrust, and drag forces during day-to-day flight. These various forces acting on the wing bones can be seen in **Figure 52** below. The added forces that the wing bones experience could help to explain the similarities in hardness values seen in the gull, raven, and bovine.

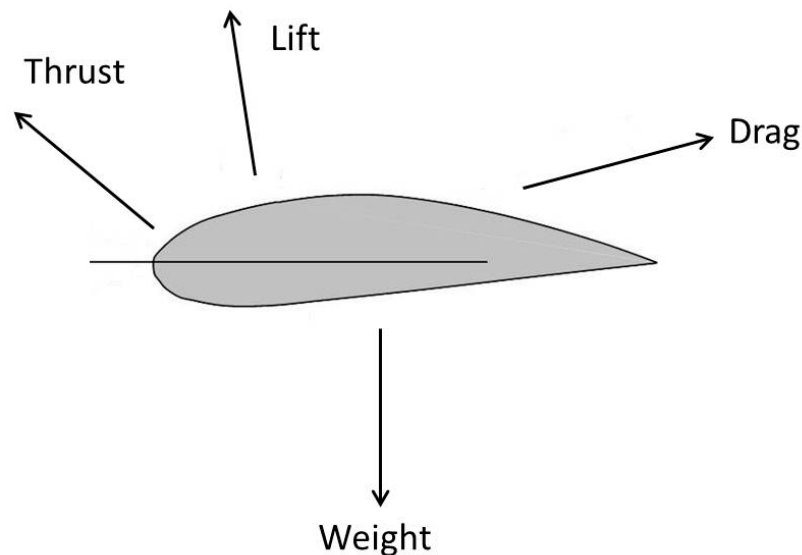


Figure 52: Diagram of the forces that act upon the wing of a bird.

The Turkey Vulture on the other hand, has a hardness value similar to that of the young bovine. Normally, it would be expected that the Turkey Vulture would have similar hardness values to that of the California Gull, but it could

be possible that the bones of the Turkey Vulture used in this study were from a relatively young vulture. However, comparing the size of the California Gull and the Turkey Vulture to the size of the Common Raven, it would seem that the hardness value for the gull and vulture should be higher than that of the raven since the raven is considerably smaller than the other two. An explanation of the similar hardness values for these three species could be that the gull and vulture wing bones developed interior struts and ridges, thereby helping to reinforce the bones to withstand the forces it experiences during flight. In addition to allowing the bones to be slightly less resistant to deformation than the raven's bones, the struts and ridges may also allow the bones of the gull and vulture to have the slightest bit of flexibility. This tiny bit of flexibility in the bones is possibly needed due to the fact that the birds are constantly changing the angle of their wings to adjust to the varying changes in the air profile of the thermals that they ride. Finally, from the chart, it can be seen that the Pekin Duck has the lowest hardness value when compared to the three other bird species and the bovine. The best possible explanation for this is that the life style of the duck is far different than that of the three other species of birds as well as the bovine. As explained earlier, this particular species of duck, if light enough, is capable of flying for an extremely short period of time, but is generally overweight and therefore has difficulty flying. Because the wings generally are neither being used for flight, compared to the wing bones of the other species of birds studied here, nor for any load bearing activities, compared to long bones of the bovine, the deformation resistance of

the duck's bones is not required to be higher than that of the other species show in the chart. Also, the age of the duck needs to be kept in mind since the hardness of the bone does still depend on the age of the animal.

Chapter 5: Summary and Conclusions

A structural and mechanical study of the structural characteristics of the wing bones from four different species of birds is presented. These four species were the *Larus californicus*, *Anas peking*, *Cathartes aura*, and the *Corvus corax*. The wing bones of these birds represent a biological material that has structural hierarchies that range from the micro to the macro-scale. The goal of this study is to contribute to the expanding knowledge base of biological systems and how the structure of these wing bones can be modeled in hopes of inspiring new designs, redesigns, and/or novel techniques. From these examinations, the following conclusions can be made.

The process of sectioning and cleaning the wing bones from each species is discussed at great lengths. By utilizing a combination of different tools, it was observed that the wing bones contained varying internal structures near the end portions of the bone, such as the struts and ridges seen in **Figure 20** and **Figure 35**, which help strengthen it. Another type of internal structure also observed near the end portions of the wing bones was a matrix of trabecular bone. In addition, a similarity between the wing bones of all four species of birds was the fact that the middle section of the each of the wing bones contained little to no internal structures, either struts, ridges, or trabecular bone. Out of the four species of birds that were used in this study, the struts were found in two of them, the California Gull and the Turkey

Vulture, while the ridges were only found in the Turkey Vulture. As for the remaining two species of birds, the Pekin Duck was observed to have trabecular bone, seen in **Figure 31**, within its wing bones while the wing bones of the Common Raven were completely hollow, save for the bone marrow within.

By looking into the habits of each species of bird used in this study, it was determined that these internal structures play an influential role in supporting the bones during flight. As previously mentioned, each of the birds used in this study has a vastly different flying style, which affects how their wing bones are shaped and strengthened. The California Gull, which is a soaring bird, achieves and maintains flight by flapping its wings. In addition, the gull is able to use thermals to not only soar or glide through the air while using less energy, but glide from thermal to thermal to prolong their flight duration without having to flap their wings. The Turkey Vulture is also a soaring type bird, in that it soars or glides through the air using the thermals. But the vulture differs slightly from the gull because it rides the thermals to gain altitude. As soon as it reaches the top of the thermal, it glides slowly downward and will catch another thermal to gain altitude when it needs to. The only time the vulture will flap its wings is during takeoff and landing. This type of flying style, soaring or gliding through the air on thermals, generally requires the bird to hold its wings in a stationary position while the rising air applies a force to the underside of the wing. The wings of these two species of bird need

to be strong and tough enough to withstand gravity, lift, and thrust forces so therefore it seems that the wing bones of these birds have developed these internal structures to help reinforce the bone in certain places without adding much weight. But, these struts and ridges do not look to be forming in the same direction and location when looking at the left and right wing from each species. Therefore, since all bones within a body are in a constant state of growing and re-growing, it seems that the struts and ridges found within the wing bones of these two species are forming on an “as needed” basis strengthening the bone in places where a higher than normal stress is exerted on it. In addition, because the gull and vulture soar on the thermals, their wings are constantly making small angle adjustments to correct for the minute changes in the profile of the thermals. These small changes in the wing angle may also help to explain the non-uniformity of the struts and ridges seen within the bone when comparing the left to the right wing of each species. As for the Common Raven, which is a flying bird, needs to frequently flap its wings in order to increase or maintain its altitude and/or speed. It will occasionally soar through the air but not for very long. This type of flying style, repeated flapping of the wings, subjects the wings to a varying range of lift and thrust forces due to the down and up stroke of the wings as well as the differences in the air pressure with each flap. However, these forces that act on the wing are divided between the down and up strokes of the wing. This coupled with the fact that the raven is generally lighter in weight when compared to either the gull or the vulture possibly explains the lack of struts and ridges within the

bone. Finally, because the Pekin Duck, which is a land bird, is either too heavy or had its wings clipped rarely uses its wings for flight. Since the duck's wings are not exercised, their bones did not develop internal structures like struts and ridges. The duck's bones did however develop trabecular bone which is less dense than the compact bone.

In addition to studying the flying habits and bone structure of each species of bird, mechanical testing was done on samples of bone that were sectioned from the wings. Due to the fact that there were limited numbers of bones from each species, micro-hardness testing was done on the cross sectional surface of the sectioned bone samples. This allowed for analysis of the bones without destroying them. From **Figure 51**, it can be seen that the hardness values for the California Gull and Common Raven have similar hardness values to that of the bovine. This is most likely due to the fact that there are more forces acting on the wing bones of the gull and raven as compared to the number of forces acting on the long bones of the bovine. Both have gravity, weight, and reaction forces acting on them, but the wing bones of the bird also have lift, thrust, and drag forces acting on them as well as shown in **Figure 52**. As for the Turkey Vulture, the hardness value is similar to that of the young bovine which could possibly be due to the age of the bird when it died. However, one would expect that because of the size of the gull and vulture, the hardness value for both these birds should be higher than that of the raven. But the development of struts and ridges within the wing

bones of the gull and vulture may have allowed the wing bones of these two species to slightly less resistant to deformation. This lower resistance to deformation may also be necessary to allow the birds to constantly adjust their wings to the changing air profile of the thermals. As for the duck, because it does not use its wings often, they are not subjected to the forces seen during flight which could mean that the wing bones of the duck are not required to be as resist to deformation as the other species in this study.

In conclusion, understanding the evolution of avian wing bones and how the flying habits of each species affects the structural development of the wing bones should aid in improving current designs as well as the design of new and novel structures. Our current technologies are capable of incorporating the designs created by nature and by modeling future structures after these designs, not only will we be able to create stronger yet lighter structures but may also help the environment as well.

Chapter 6: Future Work

Further mechanical testing should be done on the wing bones of these species after more samples have been obtained. These tests such as a 3 point bend test should focus on bending the bone to observe how much force is required to stress the bone before it begins fracturing. However, existing 3 point bend fixtures found in the lab are not suitable enough to test the flexibility of these bones since the bone will be unconstrained in certain places thereby allowing it to slide, roll, or slip before the testing is completed. Therefore, a new 3 point bend fixture should be created that will constrain the bone, thus preventing it from moving in the x and y direction. This new fixture should be able to hold the bone so that different orientations of the bone can be tested. This new fixture should also allow the bone some space to flex in the z direction; otherwise additional unwanted forces will be applied to the ends of the bone. The bone should be placed in the fixture so that the applied load from the 3 point bend tester is in the same direction as the lift and thrust forces that the wing is exposed to during flight. After these tests have been performed and the data analyzed, the following 3-D prototype models should be made.

1. 3-D prototype model of an existing tubular man-made structure.
2. 3-D prototype model of an existing tubular man-made structure with areas of lower strength.

3. The same model from variation number 2 should be used but the areas with lower strength should now include internal struts and ridges to help reinforce the structure.
4. The same model from variation number 1 but with the addition of internal struts and ridges

During the testing of these prototype models, the same force and direction of the applied force should be kept constant throughout. Ideally, the amount of material used to create the prototypes of variation number 1, 2, and 3 should be roughly the same since the idea is to test the strength of the struts and ridges without having weight create additional variables in the testing. The prototype models for variation 4 should be higher in weight when compared to the remaining three since variation 4 would be testing the idea that the struts and ridges would strengthen the bone while only slightly increasing the weight. The results from the testing of these variations of the same tubular man-made structure would help quantify the theory that the struts and ridges help reinforce and/or strengthen the structure with minimal amount of material. Additional testing should be done after the results from these experiments have been analyzed to see if this theory applies to different shapes and structures.

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