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A Histological Approach to Taphonomy: The Freeze-Thaw Cycle and Water Immersion

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A Histological Approach to Taphonomy: The Freeze-Thaw Cycle
and Water Immersion

by

William A. Souchick

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment of the
requirement for the
Degree of Master of Arts
Department of Anthropology

Western Michigan University
Kalamazoo, Michigan
April 2001

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ACKNOWLEDGMENTS

I would first like to thank my committee chair, Dr. Tal Simmons, for all of her advice, insight, and most of all, patience. I would also like to thank Dr. Robert Anemone and Dr. Laura Junker for their comments and help as well. Thank you all for helping me bring this thing to fruition at last.

I would like to thank Dr. JinPing Yang of the University of Texas Medical Branch. Dr Yang was solely responsible for the polymer embedding and the final sectioning of all of the samples. Without his help, this study could not have been completed.

I would also like to thank Dr. Robert Eversole of the Biological Imaging Center for his assistance with the microscopy and specimen image capturing.

Appreciation is also extended to Dr. Stephen Nawrocki for his helpful comments and suggestions on an early draft of this study.

Lastly, I would like to thank my wife, Erin. Without her unwavering support and love, this project would never have been possible.

William A. Souchick

A HISTOLOGICAL APPROACH TO TAPHONOMY: THE FREEZE-THAW CYCLE AND WATER IMMERSION

William A. Souchick, M.A.

Western Michigan University, 2001

Previous taphonomic studies have failed to fully study how freeze-thaw cycles, fresh water immersion and salt water immersion affect bone microstructure. Knowledge of how these environs modify bone can help narrow post mortem interval estimations or allow medico legal investigators to differentiate between perimortem damage and damage caused by the surroundings. To date, previous studies of water immersion and freeze-thaw cycles have failed either to relate their findings to forensic taphonomy or to address how bone microstructure was modified. This study was conducted to determine how bone was modified by freeze-thaw cycles and water immersion and to apply the findings to forensic taphonomy.

A set of humeri and ribs of freshly killed pigs was subjected to six freeze-thaw cycles. Another set was subjected to immersion for eight weeks in an aquarium filled with fresh water, while the last set was immersed for eight weeks in an aquarium filled with salt water. The samples from all treatments were cross-sectioned, embedded, and sectioned. Microscopic study of the samples yielded no results, which correlated to treatment. When compared against a set of control samples, those subjected to treatment did not appear to differ morphologically.

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CHAPTER I

INTRODUCTION

This study was conducted in order to examine the differences between bony materials exposed to different environmental conditions. This was done in order to determine if a microscopic evaluation of such bones could help medico-legal investigators identify the conditions to which they were exposed. After careful consideration of existing literature, three areas were identified that needed further study. These three areas were: bony material exposed to freeze-thaw cycles, fresh-water immersion and salt-water immersion.

By studying animal remains subjected to treatments meant to replicate various environmental conditions for specific durations of time, it may be possible to determine, or narrow, the postmortem interval for remains discovered in these contexts. Additionally, these studies may help eliminate confusion between perimortem and postmortem damages by demonstrating the effects these environments have upon these remains.

Animal bone has been used in bone modification studies because it is easier to obtain than human bone samples, and there have been no noticeable differences between studies conducted on various land mammal bone and human bones (Gifford, 1981; Shipman et al., 1984; Johnson, 1985). This study used the bone of adult pigs (*Sus scrofa*) to study the effects of various forensic taphonomic processes upon bone

modification, as the microstructure of pig's bone is very similar to that of humans (Nawrocki, personal communication 1999).

This study may allow forensic investigators to recognize bodies exposed to freezing and thawing, fresh or salt-water immersion, and possibly to narrow postmortem interval estimations through the histological study of bone. Since it is known that freezing confounds normal postmortem interval estimations, (Iskan and Loth, 1997; Zugibe and Costello, 1993) a closer look is warranted. Additionally, the ways in which bone is modified by immersion in fresh or salt-water has been poorly understood, thus warranting a more thorough study of these taphonomic processes.

CHAPTER II

LITERATURE REVIEW

Historical Review of Taphonomy

Forensic taphonomy is that part of taphonomy that attempts to apply the study of the "laws of embedding" (Efremov, 1940:93) to medico-legal investigations. Taphonomy, as a discipline, has taken several forms over the last 80 years. The earliest workers, mostly Germans, strove to understand the processes by which vertebrate remains become embedded within their natural surroundings (Efremov, 1940); the bulk of these studies were conducted between 1912 and 1935 (Haglund and Sorg, 1997).

Efremov (1940) took this early German work and built upon it, eventually coining the word taphonomy. Efremov used the word taphonomy to describe a discipline that studied the "transition (in all its details) of animal remains from the biosphere into the lithosphere..." (Efremov, 1940:85). Even though Efremov coined the word taphonomy, and made many substantial early contributions to the study of taphonomic processes, his influence outside of Russia was meager until the 1960s (Gifford, 1981).

Later taphonomic studies continued to emphasize the recreation of paleoecological contexts (Behrensmeyer and Hill, 1980; Shipman, 1981), the processes that produce weathering (Behrensmeyer, 1978), the process of

disarticulation of vertebrate skeletons (Hill, 1979), the ability to distinguish carnivore scavenging from evidence of hominid bone modification (Shipman and Phillips, 1976; Shipman and Phillips-Conroy, 1977), and the study of early hominids through the tools they made or the material artifacts they left (Behrensmeyer, 1975; Brain, 1981). While these taphonomic studies did not attempt to relate findings to the forensic sciences, as they were primarily paleontological in scope and purpose, many principles were discovered that set the stage for use in forensic science research in the mid 1980's.

Forensic taphonomy as a term wasn't coined until 1986 (Micozzi, 1997), though workers in the forensic sciences have been using the principles of forensic taphonomy for much longer. Early studies of entomological succession on decomposing animal remains set the stage for later work on human remains.

An early study by Reed (1958) examined the decomposition of dog carcasses and proposed a system of four stages of decomposition: fresh, bloated, decay, and dry. A later study by Payne (1965) described six stages of decay for carcasses exposed to arthropods and five stages for carcasses protected from arthropods. He effectively showed that insect scavengers contributed immensely to the process of decomposition, and without them, carcasses decayed much slower. Payne et al. (1968) continued along this line of inquiry and conducted a study focusing on insect scavenger effects on buried carcasses and found that insects were also present and aided in decomposition. Furthermore, these early studies noted that insect activity was dependent upon temperature, but none tried to further analyze this phenomenon until Johnson's (1975) study. He studied seasonal variations in insect populations on

carcasses and found that the colder temperatures of winter not only slowed insect activity, but bacterial and fungal activity as well. As a result of these slowed processes, the carcasses "went through a different decomposition process which skipped the Decay stage" (Johnson, 1975:84), or aerobic protein decomposition. The results of these early studies were later applied to human cadaver studies.

Rodriguez and Bass (1983) studied insect activity and its relationship to human cadavers, reporting results similar to those cited by Reed (1958), Payne et al. (1968) and Payne and King (1970, 1972). They established that insect activity and the extent of tissue decomposition could be used to determine the postmortem interval for humans, thus giving forensic investigators and forensic anthropologists a potentially useful tool. Furthermore, these studies helped investigators recognize normal patterns of tissue decomposition. Additional results reported by Rodriguez and Bass (1985), and by Bass (1997), further linked time since death with the degree and type of insect activity. Research conducted in arid climates (Galloway et al., 1989) also demonstrated that insect activity hastened decomposition time. The aforementioned studies dealt largely with the application of forensic taphonomy to the study of the decay of soft tissues, and in these cases little was done to apply these results to bony tissues.

Bone modification studies by paleontologists have long been concerned with distinguishing bones modified by hominids from those modified by natural environmental agents (Marshall, 1989), and how the bony tissue came to become embedded within its surroundings (Efremov, 1940). Forensic taphonomists have been able to take principles gleaned from these inquiries and to further apply them to

medico-legal inquires.

Forensic anthropologists have long used taphonomy to answer questions about human remains. The aforementioned work by Bass bears this out. Earlier examples can be found in the work of Stewart (1979) in which he describes the ways in which a bone may become stained by soil. This in essence was an early application of taphonomy to a forensic investigation. Furthermore, he described how a bullet might damage a cranium. This again can be seen as a part of forensic taphonomy, since it is important to be able to distinguish a gunshot wound from an artifact caused by burial or some other natural process.

The ability to discriminate between the marks left by natural processes and the marks left in the act of a crime is one of the most important aspects of forensic taphonomy. These marks are, more often than not, borne out upon the skeletal remains. Sauer (1984) illustrates this succinctly with a discussion regarding the differences between injuries incurred near or at the time of death (perimortem) and those caused after death (postmortem) by other taphonomic events.

The recent volume edited by Haglund and Sorg (1997) gives examples of continuing research into how remains are affected by taphonomic processes. This volume served to unite many different aspects of taphonomy as they are applied to the medico-legal community and to demonstrate how different disciplines can contribute to forensic taphonomy.

Forensic taphonomy is a discipline that continues to grow from addition of new practitioners and from the effects of continued interaction from other disciplines. A review of the existing literature has shown that there are still areas of taphonomy

that require additional study. Little work has been done to explore how bone microstructure is altered, or modified, by the effects of freezing and thawing, fresh-water immersion, and salt-water immersion.

Cold Weather Bone Modification

Bodies exposed to freezing temperatures can remain well preserved for long periods of time due to freeze-drying, or sublimation (Artamonov, 1965; Smith and Zimmerman, 1975; Vreeland, 1979). With regard to faunal activity, Reed (1958), Payne (1965), and Johnson (1975) recorded that arthropod, bacterial and fungal activity was decreased on cooler days and that decomposition proceeds more slowly on these days. A later study by McBride (1994) examined the effects of cold weather on insect succession and found that it was sufficiently different as to be distinguishable from succession patterns in warmer climates. In essence, the frequency, appearance and occurrence of certain insects changed over the course of a winter season in Michigan.

Micozzi (1986) was one of the first researchers to conduct studies on the effects of cold on soft tissue decomposition; he found that the decomposition of frozen and then thawed carcasses proceeded differently from non-frozen carcasses. After being frozen, carcasses decomposed at an increased rate after thawing (Micozzi, 1986, 1991, 1997; Perper, 1993; Ubelaker, 1997). Furthermore, Micozzi (1986, 1991, and 1997) described frozen-thawed carcasses as decomposing from the outside-in and fresh animals as decomposing inside out. This distinction was due to the differences in bacteria present in these two situations. In freshly killed animals decomposition occurred largely as a result of decay from within due to the presence

of naturally occurring enteric bacteria. When the animals were frozen, the decrease in temperature effectively killed any enteric bacteria; therefore, bacteria acting upon the carcasses came from the external environment. Furthermore, freezing and thawing damaged the skin and permitted easier entry for foreign organisms and scavenger insects and it also promoted faster skeletal disarticulation. Although it has been well documented that repeated freeze-thaw cycles could have the potential to fracture bone (Miller, 1975; Micozzi, 1997; Ubelaker, 1997), this phenomenon has been poorly documented and the concept has not been applied to forensic taphonomy in a controlled regime.

Repeated freezing and thawing causes cell organelles to be disrupted (Lillie, 1965) due to ice crystal formation (Zugibe and Costello, 1993). Although not yet studied, it is not unreasonable to believe that this may also happen to the cells of bones. It has been reported that bone tissues are composed of various percentages of water - anywhere between 8% (Klepinger, 1984) and about 20% (Evans, 1973) - that, upon freezing, would expand by a factor of about 9% (Wood and Johnson, 1978). This process could cause splits to form in bone. Miller (1975) found exactly this when experimentally frozen, defleshed bone was allowed to thaw. Parallel and longitudinal cracks formed that ranged from surface cracks, penetrating the bone up to a millimeter in depth, to cracks penetrating through to the marrow cavity (Figure 1). The action of freezing may have caused micro cracks to form within the cortical bone (Figure 2). These cracks originated at the cement lines around osteons and propagated throughout the bone causing a visible fracture to appear (Evans, 1973). In other studies, Sadek-Kooros (1972, 1975) subjected bone to various freezing and

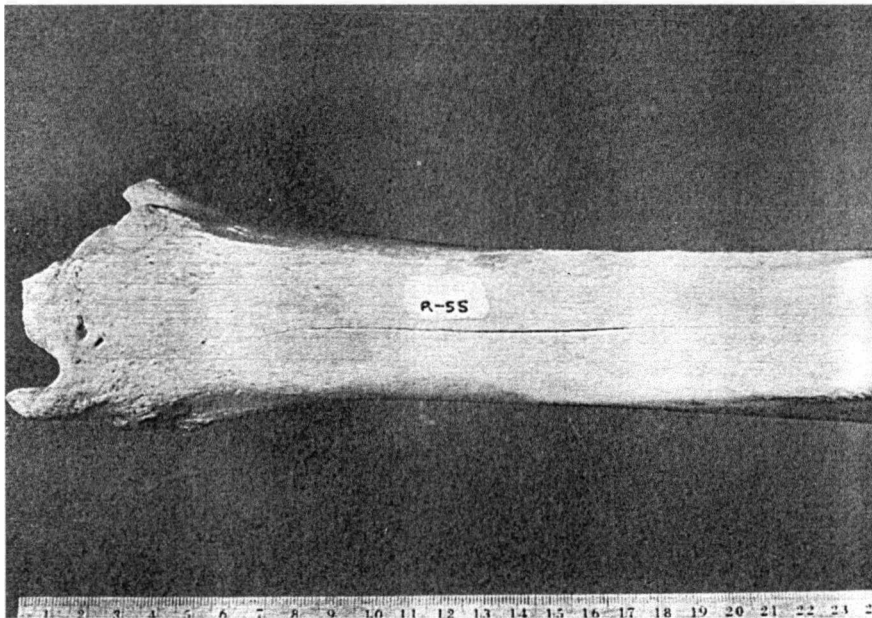


Figure 1. Longitudinal Crack in Cow Bone Caused By Freezing and Thawing.
(from Miller 1975:xxxvii)

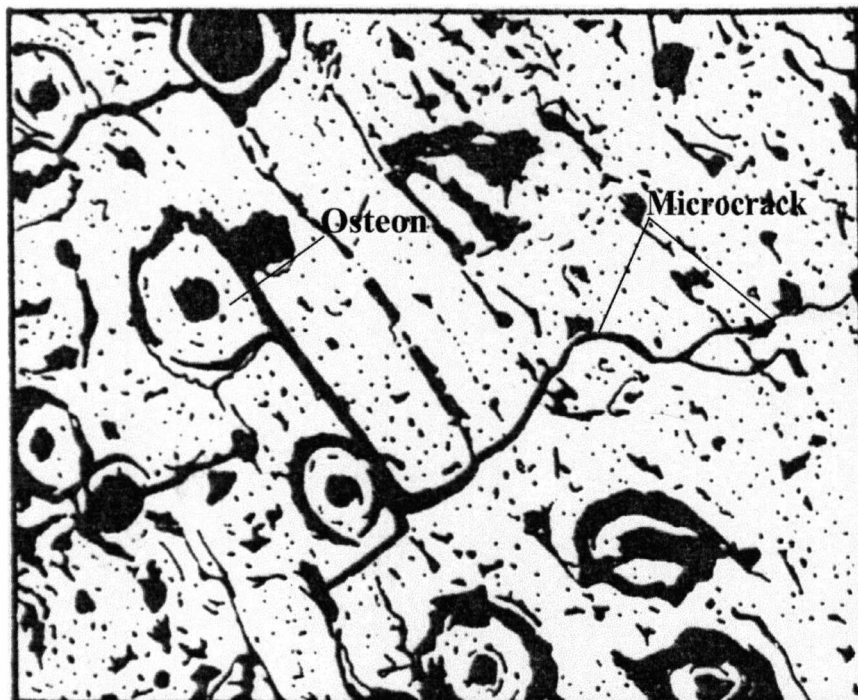


Figure 2. Bone Microstructure After Microcracking. (from Johnson 1985:433).

thawing times in order to replicate spiral fractures seen in cases of supposedly hominid caused bone modification. The results did not mention any naturally occurring fractures, but this may have been because the studies by Miller (1975) and Sadek-Kooros (1972, 1975) had different purposes. In both studies, the emphasis was neither histological nor related to forensic taphonomy.

Fresh-Water Immersion

A large body of literature exists examining the transport of skeletal remains in rivers (Koch, 1989). Early fluvial transport studies examined the movements of skeletal remains in artificial fluvial environments (Boaz and Behrensmeyer, 1976). These were conducted with an emphasis on relating the results to understanding fossil assemblages in paleontology. Additional experiments were conducted to reconstruct paleoecological contexts (Hanson, 1980), but these lacked a consideration for soft tissue decomposition. Overall, there has been a continuing lack of knowledge about the histological modification of bone immersed in both salt and fresh-water (Haglund, 1993).

Payne and King (1972) studied insect succession and decomposition of pig carcasses in fresh-water. They found that the carcasses went through six stages of deterioration: (1) submerged fresh, where the carcass was under water; (2) early floating, where decompositional gases caused the carcasses to float; (3) floating decay, where the carcasses continued to float and to be decomposed by insects; (4) bloated deterioration, where most of the exposed tissues were eaten away by insect activity; (5) floated remains, where the remains were quite deteriorated; and finally, (6) sunken remains, where the bones and bits of remaining skin sink to the bottom.

Another review of fresh-water decomposition (O'Brien, 1997) has reported that fresh-water has a preservative effect upon internal organs due to its promotion of the formation of an adipocere "shell" (Cotton et al., 1987). Adipocere is a grayish white material formed from the saponification of the organism's lipids (Gill-King, 1997). Formation of adipocere in fresh-water immersed remains occurred best in water 21° to 45° Celsius (O'Brien, 1997). Additional insults to the body occurred from fish and crustaceans, which fed upon the soft tissues (Mottonen and Nuutila, 1970; Haglund 1993). These organisms were able to skeletonize a body within 2 weeks (Mootonen and Nuutila, 1970). The earliest areas that were attacked were the lips, nose and eyelids (Mootonen and Nuutila, 1970; Haglund, 1993). Bodies tended to disarticulate in predictable ways if floating in open water, with the degree of flexibility of the joint as a major influencing factor (Haglund, 1993). For instance, the highly flexible radio-carpal, tibial-tarsal, elbow and knee joints disarticulated first, with the less flexible vertebral column persisting the longest. While it was well known how bodies decomposed in fresh-water, little was known about the modification of bone in these contexts.

The largest body of literature concerning fresh-water bone modification concentrates on damage caused by collisions with rocks, floating debris and the lake bottom. Since large lakes are characterized as having counterclockwise patterns of surface movement causing currents known as upwellings and downwellings to occur, it can be expected that objects (i.e. bodies) in lake water are in almost continual movement (Boyce et al., 1989). Erosion of exposed skeletal elements can occur due to the current dragging them across the lake bed or from collisions with rocks

(O'Brien, 1997). Erosion can also occur due to impacts with abrasive matter laden winter ice (Boyce et al., 1989), or skeletal elements can become embedded within ice and be dragged along rocks or other obstacles causing damage to the bone (Irving et al., 1989). While macroscopic damage to bone in fresh-water has been noted, as evidenced above, damage to microscopic, histological structures have not, and there has been no application to forensic taphonomy.

Marine-Water Immersion

The study of bone modification in marine contexts suffers from a dearth of literature similar to that of fresh-water bone modification. While taphonomic studies have been conducted to assess fossil preservation in marine contexts (Parsons and Brett, 1991), little forensic taphonomy research has been done (Sorg et al. 1997). Sorg et al., (1997) believe this is due to two factors: human remains in marine death assemblages are rare; and recovery of these remains is rarer still. There are numerous factors governing the postmortem fate of a human body including temperature, salinity of the water, scavenger activity, and presence or absence of body coverings. Since accounting for this variability is difficult, establishing the postmortem interval is problematic, but a study of the type of bone modification seen in marine contexts is still warranted.

Arnaud et al. (1978) described the effects seawater had upon human bone immersed in the Mediterranean Sea for approximately one thousand years. They described the bones as being well preserved, but with a covering of slime, which had penetrated into the medullary cavity. Upon investigation, this slime was found to be composed of grains of sand and silt. Histological analysis showed that the

microscopic structures were recognizable and that there were discolored areas in the cortex near the periosteal and endosteal surfaces. Marine scavengers account for much of the bone modification seen in these contexts.

Fish are responsible for most of the soft tissue loss of a body, but affect bony tissues little (Mottonen and Nuutila 1970; Sorg et al., 1997) except for instances when large predatory fish like tiger sharks are able to scavenge whole body parts, flesh, and bone from decaying bodies (Rathbun and Rathbun, 1997). Aquatic arthropod scavengers, like their terrestrial counterparts, can be responsible for great amounts of flesh decomposition. It is also thought that many species of mollusks (e.g., clams, mussels, oysters, and snails) and echinoderms (e.g., sea lilies, sea stars, brittle stars, sea urchins, and sea cucumbers) could be important decomposers, but their role is poorly studied (Sorg et al., 1997). Barnacle remains attached to bone can be important indicators of postmortem interval, but there are many confounding variables to this line of study. Overall, the biggest impact marine scavengers have upon bone modification comes in the form of bioerosion. Bioerosion can come in the form of grazing by mollusks and boring by bryozoans. These have not been well studied so their effects on bone tissue are not well known. Although it is recognized that a body can be skeletonized in marine contexts in as little as one month (Boyle et al., 1997), or in some cases, as little as two weeks (Mottonen and Nuutila, 1970), little actualistic research into bone modification on either a gross or histological level has been done.

CHAPTER III

MATERIALS AND METHODS

Each experimental group in this study was composed of both ribs and humeri. These specific bones were used to show possible differences in modification of bones with differing porosity (Hanson and Buikstra, 1987) and size, since smaller bones are known to preserve poorly (Shipman, 1981; Galloway et al., 1997). Specimens were defleshed with the aid of a knife. It was not essential that every bit of flesh be removed from the specimen, as techniques to deflesh bone with dilute acids or boiling in a soap solution might have damaged the specimens histologically, and thus introduced confounding variables into the analysis.

The first group contained five rib and five humerus control specimens. No treatment was performed upon them. The second group contained four fleshed and four defleshed humeri, and four fleshed and four defleshed ribs for a total of eight ribs and eight humeri exposed to six complete freeze and thaw cycles. The third and fourth groups consisted of specimens exposed to eight weeks of immersion in fresh and salt-water, respectively. Both of these groups were comprised of two fleshed humeri and ribs and two defleshed humeri and ribs, for a total of eight specimens in each treatment.

The humeri and ribs of freshly killed pigs were obtained from a local butcher. Care was taken not to store the specimens in a freezer and to use them promptly.

Each specimen was labeled individually; the freeze-thaw specimens had labels placed on the trays they sat in and the water-immersed specimens were tagged with waterproof plastic labels.

Those used in the freezing and thawing treatment were set on a tray and then placed in a freezer kept at about -1° to -2° Celsius. They were allowed to freeze for a period of 5 days to assure complete freezing. The specimens were then removed and allowed to thaw at room temperature (about 20° Celsius) for 48 hours. This was completed a total of six times. The specimens were then prepared and evaluated for the presence or absence of long fractures and for the number of microcracks.

For freshwater immersion, the specimens were placed in a twenty-gallon aquarium filled with water from Clear Lake, in St. Joseph County, Michigan. Five-gallon jugs were filled with water from the lake and a small bit of silt from the lake bed. To replicate the currents present in a lake, a fish aquarium filter, with the filter element removed, was allowed to run, causing water circulation, and an air pump was used to oxygenate the water. The water temperature in lakes is highly variable, depending on the season, the depth of the lake, the water volume, and density (Cole 1994). To simplify matters, temperature was maintained between 19.4 and 21.8 Celsius, a temperature range for Lake Ontario in April (O'Brien, 1997). This temperature was maintained with the use of an aquarium heater and checked twice daily with a thermometer to assure that the range was maintained. A small desk lamp simulated sunlight with a 75-watt Grow Light bulb installed in it. This was done to assure that natural algae growth would occur, in order to simulate normal lake conditions where algae might modify the bone in some fashion. After eight weeks,

the specimens were removed and evaluated for presence and number of tunnels from microorganisms and algae observed, presence or absence of adipocere, and presence or absence of discoloration.

To replicate marine conditions, a twenty-gallon fish aquarium was filled with water that was treated with Instant Ocean brand sea salt. Once again, to circulate the water, a fish aquarium filter, with the filter element removed, was run continuously, and an air pump oxygenated the water. The temperature was kept at about 6 Celsius, the average temperature of 75% of the water in the oceans, and slightly alkaline (>7 pH) (Taber and Dubach, 1972) with the aid of a thermometer and a pool testing kit, respectively. A lamp with a 75-watt Grow Light bulb was used to stimulate any alga growth. After eight weeks, the specimens were removed and evaluated for evidence of bone modification due to tunneling, presence or absence of adipocere, presence or absence of slime (Arnaud et al., 1978), and presence or absence of discoloration.

Specimen preparation proceeded according to an outline described by Stout (1989) and Yang (personal communication 2000). For fleshed bone, the author with the aid of a kitchen knife, taking care not to damage the bone, removed the flesh manually. Then, a ½-inch cross-section of bone was removed from all specimens using a standard hacksaw. In order to stabilize the specimens during cutting, they were secured firmly in a small vise. Both fleshed and defleshed bone samples were then degreased by immersion in acetone filled, individual glass containers (Stout and Paine, 1992) for a period of 48 hours (Yang, personal communication 2000). After the prescribed time, the acetone was removed and then Dr. JinPing Yang of the University of Texas Medical Branch embedded and thin sectioned the specimens.

The specimens were again placed in a glass container and covered with a methyl methacrylate monomer. They were then placed in a vacuum dessicator so that they would be infiltrated by the monomer. At this point, air is driven out of the bones and replaced by the monomer, so care must be taken to keep the specimens covered. After a period of 48-72 hours the specimens were completely embedded in the monomer (Yang, personal communication 2000). In order to complete the procedure, the excess monomer was poured off and partially polymerized methacrylate added in its place. The specimen jars were then placed in an oven kept between 40°-45° Celsius for 48-72 hours to complete polymerization (Yang, personal communication 2000). The blocks were then sectioned down to between 50 μ and 150 μ and sandwiched between two glass slides.

The slides of the specimens were examined by the author using a Nikon Microphot FXA light microscope equipped with a Javelin Chromachip II ccd image capture camera. The slides were examined visually under 40x power and 100x power magnification. The images were also captured with a commercially available program, Meta Morph. These captured images were also examined with the unaided eye for any sign of morphological damage as well.

CHAPTER IV

RESULTS

The author evaluated all of the specimen slides qualitatively. Initially, it was hoped that this study would yield results that would be able to be quantitatively evaluated, but due to varying quality of specimen preparation and lack of any identifiable damages to the samples, a only qualitative analysis was used. Any specimens that showed some indication of having been damaged were double checked by a member of the author's thesis committee (Dr. Tal Simmons).

The specimens subjected to the freeze-thaw cycles did not show any signs of having been damaged by the treatment under 40x magnification. All of the specimens were morphologically very similar to those included in the control sample (Figure 3). Additionally, there was no physical difference between those that were fleshed and those specimens that were defleshed. When magnification was increased to 100x, results were identical (Figure 4).

Similarly, those specimens subjected to fresh-water and salt-water immersion did not show any sign of damage (Figures 5 and 6). Again, there was no difference in the specimens that were fleshed and those that were defleshed. Comparing fresh-water immersed specimens with those that were immersed in salt-water yielded no differences in histological appearance either. Under 40x magnification and 100x magnification, both groups appeared similar to each other and to the control group as

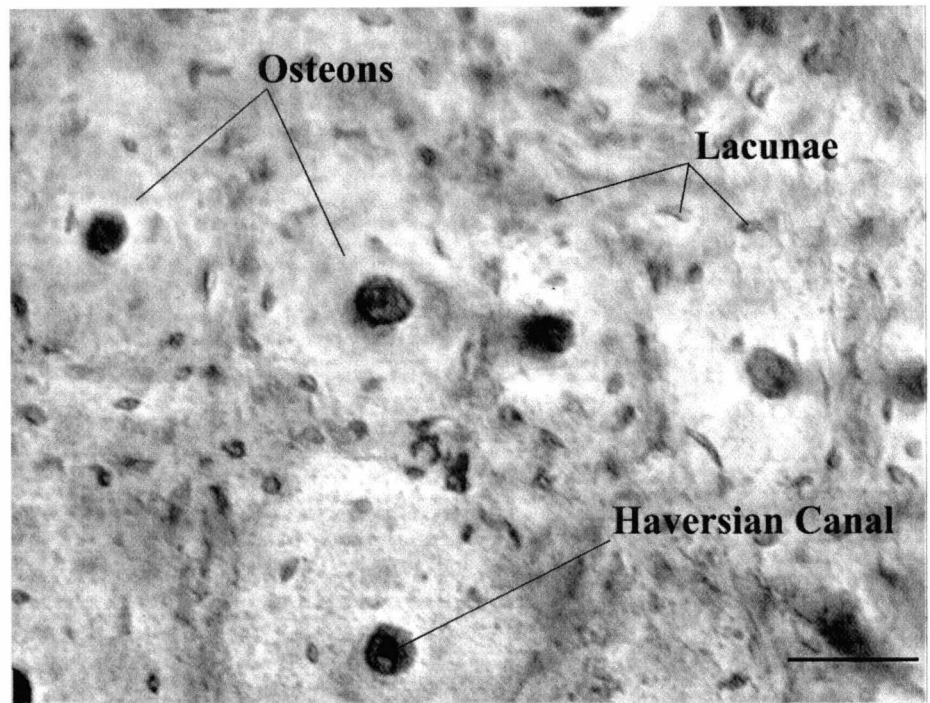


Figure 3. Representative Image of a Control Specimen. (Bar= 100 μ m).

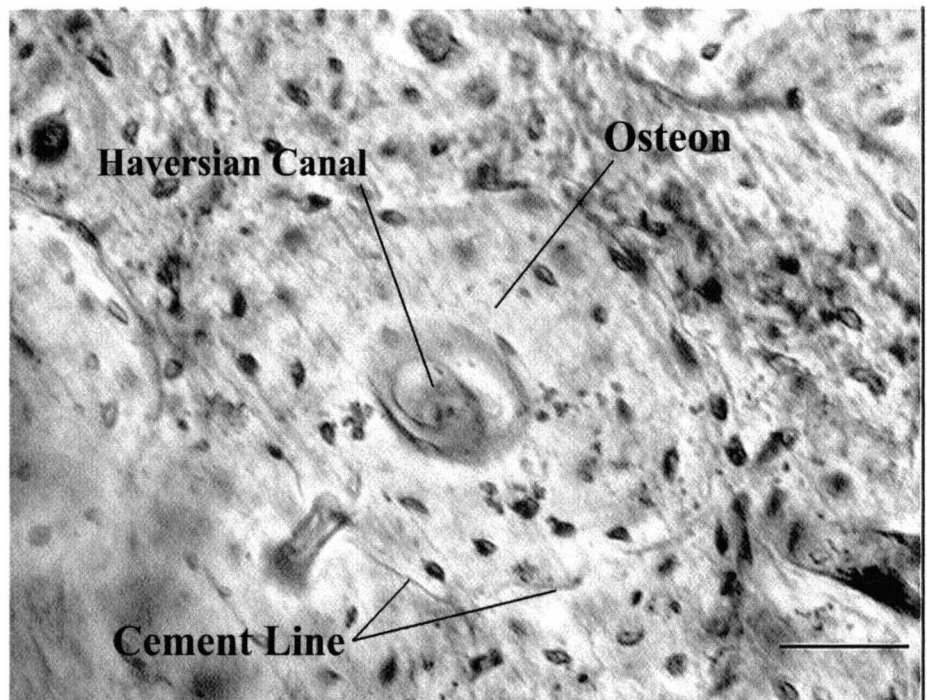


Figure 4. Representative Image of a Freeze-Thaw Specimen. (Bar= 100 μ m).

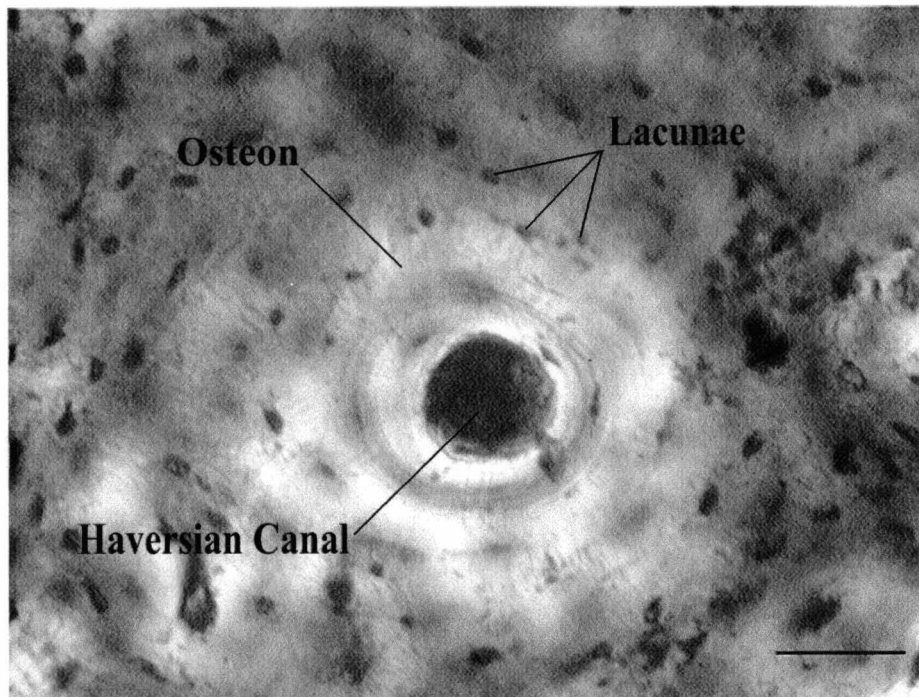


Figure 5. Representative Image of a Fresh-Water Immersed Specimen. (Bar= 100 μ m).

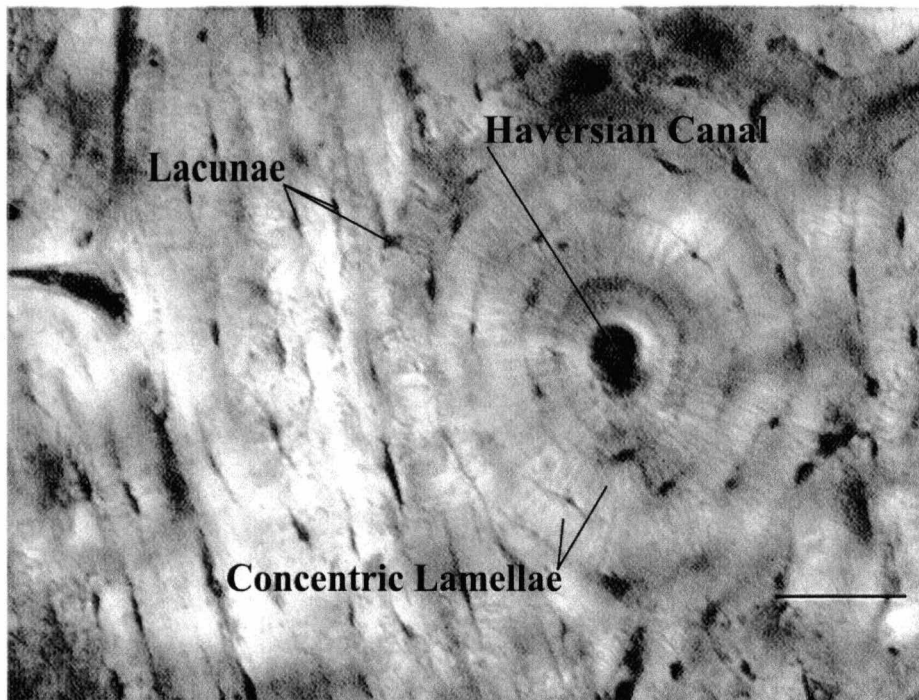


Figure 6. Representative Image of a Salt-Water Immersed Specimen. (Bar= 100 μ m).

well. The only area in which these three treatments differed was on external appearance.

The surface of the fleshed freeze-thaw specimens appeared greasy and shiny as well. They were obviously decomposing as there was decompositional odor noticeable. Otherwise, the specimens did not exhibit any expected surface cracking nor did they exhibit microscopic damage.

The fresh-water immersed specimens had a great deal of adipocere present. The fleshed rib specimens were almost entirely adipocere in composition. Where some of the specimens had lain against the bottom, there was evidence of algae growth. Furthermore, there was a very strong decompositional odor present.

Similarly, the salt-water immersed specimens exhibited a great deal of odor. Upon removal, however, no adipocere was noted upon the specimens. Overall, the salt-water immersed specimens appeared better preserved than the fresh-water immersed ones. Whether this was from the salinity or the cooler temperatures maintained (6° Celsius as opposed to 19° to 21° Celsius for fresh-water immersion) is not known, but both factors probably contributed.

CHAPTER V

DISCUSSION AND CONCLUSIONS

This study was unable to qualitatively demonstrate any difference between bones exposed to the aforementioned conditions and those bones that were fresh. Actualistic studies such as this are fraught with many difficulties, which may account for negative results. The act of replicating natural conditions is difficult at best. Exposing the freeze-thaw group to a series of six complete cycles is an example. No literature exists to suggest that remains deposited in the winter months would be exposed to six freeze-thaw cycles (a total of six weeks in this experiment); this parameter just happened to fit in roughly with the amount of time that the water-immersion groups immersed (eight weeks). Additionally, the material used in the freeze-thaw group was that of very fresh remains. The natural elasticity of the fresh bone tissue may have negated the effects of freezing, giving the appearance that freeze-thaw cycles have no effect upon bony tissue. As a counterpoint to this, the study by Miller (1975) used fresh cattle long bones that were frozen and when allowed to thaw, cracks formed. Some differences exist between the study by Miller (1975) and this one. First, Miller froze the bony material in a commercial freezer at a temperature of -20° Celsius, while this study subjected the fleshed and defleshed specimens to a temperature of -2° Celsius. Second, the bones used in Miller's study were allowed to freeze for three weeks, while the specimens in this study were

allowed to freeze for only five days. The longer period of freezing may have acted to sublimate the moisture in the bone, in effect drying it out. Indeed, Miller (1975) observed that the cracks he produced were related more with drying than the action of thawing. A further study may yield different results if dry remains were used initially or if the remains were subjected to the lower temperatures and longer duration in Miller's study in order to sublimate the moisture within the bone.

The second and third parts of this study, fresh and salt-water immersion, may have suffered from the greatest difficulties. The specimens in the fresh-water portion were immersed in water kept at a certain temperature. This temperature (between 19.4° and 21.8° Celsius) was similar to that of Lake Ontario in April (O'Brien, 1997) and was chosen in order to demonstrate how bone may become modified in a lake in a Northern climate. Any lake's average temperature could have been used though, and a change in the temperature at which the specimens are maintained may affect how the bone is modified. Additionally, the use of an air stone to aerate the water may be problematic. A body of water has differing oxygen content at differing depths, temperatures (Dorit et al., 1991), and current flows. Introduction of a constant oxygen supply can be seen as unnatural, but conversely, in order to replicate a body of water, oxygen must be introduced as it is in nature from aquatic plants and the perfusion of oxygen from the air (Dorit et al., 1991). What effects the amount of oxygen in the water may have had upon the results of this study are unknown and will not be known unless a study is conducted that specifically manipulates it. Finally, there was no interaction between the bony material and any aquatic life that may have been in the water. There was some evidence of algae growth, but this study was

unable to take into account the effects small crustaceans and fish may have on a set of remains.

The salt-water immersion portion of this study suffers from similar problems. The temperature of 6° Celsius was chosen to represent the average temperature of 75% of the water in the ocean (Taber and Dubach, 1972). This is another example of the difficulties with actualistic study. The temperature chosen doesn't represent the temperature of any particular body of water, but merely the average of 75% of the water. Therefore, any results concluded from this study may have been difficult to apply to waters that may be significantly warmer or colder (*i.e.* water in the Caribbean versus water from the Arctic Ocean). Finally, there was lack of interaction between the remains and any sort of flora or fauna. Unlike the remains subjected to fresh-water immersion, there was no evidence of algae growth on this set of specimens.

Overall, this study was unable to demonstrate any difference between the microstructures of bone subjected to freezing and thawing, fresh-water immersion or salt-water immersion and the microstructures of bone subjected to no treatment. This study was able to show that there were observable external differences between the specimens in this study. The salt-water immersed specimens appeared the best preserved of all, then the freeze-thaw specimens; the fresh-water immersed specimens appeared the most decomposed.

Future studies should further focus upon freeze-thaw cycles and their relation to bone microstructure. An important aspect of this research would be to incorporate the work of Miller (1975) more fully into the research design. Specifically, any

specimens used should be frozen at a lower temperature for a much longer period of time. A much larger sample size must also be used in order for any results gained to be statistically significant. Finally, it may be useful to determine if there is a threshold effect to damage caused by freezing and thawing. From this study it has been determined that periods of freezing as little as five days had no effect upon bone microstructure. There was no apparent cumulative effect from freezing the specimens five days at a time for six weeks, a total of thirty days, or just over three weeks. It may be instructive to determine just what length of time is needed before bone microstructure is altered by freezing.

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