The Effects of Particulate-Laden Water on Skeletal Trauma

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Abstract:
Bone weathering has been well studied and documented regarding skeletonized remains in terrestrial environments. However, little work has been published on the process of abrasion to bones that are submerged in dynamic water. Skeletal material under water is subjected to a different set of stresses than bones left on a ground surface and research to fully document these postmortem changes is required. Increasing instances of maritime disasters, such as the sinking of the Italian cruise ship *Concordia* and the loss of migrant boats in the Mediterranean and Timor Sea, warrant further investigation of the processes of degradation of human remains found in marine environments as a result of both water flow and abrasion by suspended particulate matter. Studying the physical changes that occur over time to bones that may have been injured in a traumatic event such as a shipwreck can provide useful information to investigators. Pig (*Sus scrofa*) ribs inflicted with sharp force trauma wounds were subjected to an environment simulating an underwater decompositional site using a high particulate water wash. Samples were allowed to abrade for set intervals of time and examined using micrometer caliper and stereomicroscopy. This study characterizes the specific changes that occur when injured bones are left submerged in water for extended periods of time. The information gathered from this research can be applied in several areas, including estimation of time since deposition, trauma site identification, and associating remains with a particular event.

Introduction

Forensic examination of bone is not always a straightforward enterprise. A complex interplay of factors contributes to the information able to be obtained from a set of skeletonized remains that is largely influenced by the environment in which the remains were found. Weathering effects on bone have been well studied and documented with respect to skeletonized remains in terrestrial settings (Behrensmeyer 1978, Hockett 1996, DeBattista 2013, Nicholson 1996). The six stages of bone weathering first described by Behrensmeyer (1978) and supplemented by numerous others (Andrews and Whybrow 2005, Todisco and Monchot 2008, Andrews and Cook 1985, Morris 2013) have provided examiners with a valuable timeframe for aging samples discovered in extramural locations. However, little work has been published to expand upon the process of degradation that occurs in bones that are submerged in tidal water for extended periods of time. Previous studies include those of Shipman and Rose (1988) and Fernández-Jalvo and Andrews (2003), though none have specifically focused on abrasion of bone as a result of the combination of agents characteristic of a marine environment such as salinity, wave action, unique biota, particular sediment, etc. It is important to note that bones under water are subjected to a different set of stresses than are bones left exposed to the elements and further research is required to fully understand these postmortem changes.

Coastal areas seem to attract a significant amount of death, whether it be homicides, suicidal drownings, boating accidents, burials at sea, transport from rivers, or disposal sites from land homicides (Pokines and Symes 2013). Natural disasters such as Hurricane Katrina in the United States or the Boxing Day 2004 Tsunami account for a significant number of deaths as well. This, paired with a higher population density and comparatively low vegetation cover, leads to a relatively high frequency of bodies discovered and therefore, a reliable methodology for investigating such recoveries is required (Pokines and Symes 2013). Recent events such as the sinking of the Italian cruise ship *Concordia*, as well as other maritime disasters warrant further understanding of marine taphonomy; in a situation like the *Concordia* accident, it is important for investigators to be able to associate sets of remains with a particular time frame. Especially in situations where criminal charges are being brought, an accurate body count is necessary to determine the severity of the charges and knowing when a set of remains entered the water can help determine whether it was deposited there as a result of the incident being investigated or due to an unrelated event. An article released by the British Broadcasting Corporation (BBC) details Maltese Prime Minister Joseph Muscat's statements that the Mediterranean Sea is "becoming a cemetery" due to the growing number of sinking vessels each year (BBC 2013). Studying the physical changes that occur over time to bones that may have been injured in a traumatic event such as a shipwreck may provide useful information to investigators. The information gathered from this research can be applied in several areas, including estimation of time since deposition, trauma site identification, and associating remains with a particular event.

Materials & Methods

Animal Model

Domestic pig (*Sus scrofa*) ribs purchased from Costco® or ShopRite® in West Haven, Connecticut were used to model human bone. The use of animal analogs, specifically pigs, is a well-accepted practice in scientific studies, as there are numerous issues with the availability and ethics of using human tissues in scientific inquiries of this nature (Aerssens et al 1998, Crowder et al 2011, Haskell 2000, Davis and Goff 2000). Adult S. *scrofa* tissues have been found to be acceptably similar to that of human so as to justify their use in a study meant to be applied to the human species (Aerssens et al 1998). This reason, along with the readily available supply of such tissue, forms the basis of reasoning behind its use in this study. Because the bones were already being sold for human consumption, no approval from the Institutional Animal Care and Use Committee (IACUC) was necessary.

Particulate

The sediment types used for this study were both chosen because of their presence in a marine environment. Sand from a sand pool filter was collected and used throughout the study, as this particular sediment type is found in the environment of interest and is readily available.

Diatomaceous earth is composed of crushed fossilized remains of diatom skeletons, minute organisms found in coastal areas (Antonides 1998). This particulate was also used separately from the sand as an alternative method of abrasion. The abrasive nature of diatomaceous earth has led to its use as a natural household polishing agent (Smith 2007). Laboratory grade diatomaceous earth – such as the material used for this study – appears as a fine white powder of low density.

Sample Preparation

For each trial, whole racks of *S. scrofa* rib bones were acquired and imaged using a Nikon D5100® camera and Leica S6D® stereomicroscope under 10X magnification with a Leica EC3® mounted camera. The samples were defleshed manually with a filleting knife and forceps. These whole bones were then sectioned using a Dremel® rotary tool into three or four roughly inch-long sections, depending on the length of the whole bone. Chop wounds were inflicted manually using a Showtime Six Star Stainless Cleaver® of 0.75mm thickness on the superior and inferior aspects of each rib section (Cut A or B) except for eight segments that were reserved for uninjured controls. Although the manual method of inflicting trauma is less standardized, this study is not comparing data from the cuts relative to each other, but rather comparing rates of change of each cut individually. Therefore, the cuts on each bone did not need to be identical. A small but recognizable hole was made on the bottom left corner of each segment using the engraving attachment of the Dremel® to help orient and properly identify the A and B sides of the segments.

Each of the segments were imaged macroscopically after injury using a Nikon D5100® camera with AF-S Nikkor 13-55mm® lens at 65cm height. The cuts were imaged under a Leica S6D® stereomicroscope under 10X magnification using a Leica EC3® mounted camera and Leica EZ Application Suite® for a more detailed view of the trauma. A General® UltraTech® 6" digital fractional caliper was used to take three replicate sets of measurements of segment width, segment thickness, and cortical thickness at both ends of the segment, as well as length, width, and depth of each cut. This instrument was repeatedly calibrated during the course of its use in order to ensure accurate measurements.

After the bone segments were subjected to experimental conditions and sampled on the appropriate days, the same measurements of segment width, segment thickness, cortical thickness, cut length, width, depth, spurs and surface pitting were taken in replicates of three and these data were imported to a Microsoft Excel® spreadsheet. Mass and volume measurements were not taken because natural bone moisture present in the samples would cause these measurements to be an inaccurate representation of the actual mass and volume of the bone. The segments were also photographed macroscopically and under the stereomicroscope again using the same initial instruments.

Apparatuses

Once the bone segments were prepared as per above, each was placed in one of two apparatuses: either the tumbler or the shaker. These models were used to simulate two different types of high energy water environments; bidirectional wave action was generated by the tumbler, where the shaker simulated unidirectional wave action. Trials in both of the apparatuses were conducted simultaneously.

Tumbler

The tumbler consisted of a rectangular PVC frame supported by a wooden base, to which four 500-mL plastic graduated cylinders were affixed (Figure 1). This frame was then rotated at a steady speed by a 1/70 Horsepower Bodine® fractional horsepower gearmotor provided by the Mechanical Engineering Department at the University of New Haven. A set of two holes was drilled into each of the graduated cylinders so that ¾" wood screws could be screwed into the cross-sectional ends of each bone segment, securing it in place with the "A" cut side facing the top of the cylinder.

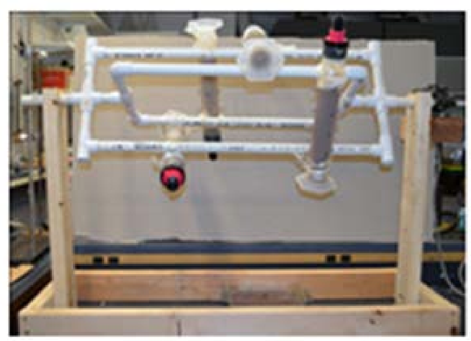

Figure 1: The tumbler apparatus taken with Nikon D5100 camera by A. Appleton

Four bone segments – three experimental (cut) bones and one uncut control bone – were placed in each cylinder (Figure 2). This arrangement was chosen for ease of access during sampling periods; the segments were removed from the top row first, with each sampling progressing toward the bottom of the cylinder. Depending on which type of particulate the trial was focused on, either sand or diatomaceous earth was added to the 50mL or 200mL marks, respectively. This difference was to account for the degree to which each water/particulate solution became saturated. More diatomaceous earth was needed due to the fineness of the grains, which allowed it to form a colloid-like mixture with the water. The particulate was followed by sea water taken from the Long Island Sound in West Haven, Connecticut filled up to the 500mL mark and the cylinders were then sealed with plumbing caps and affixed to the rectangular frame with rubber bands and plastic wrap.

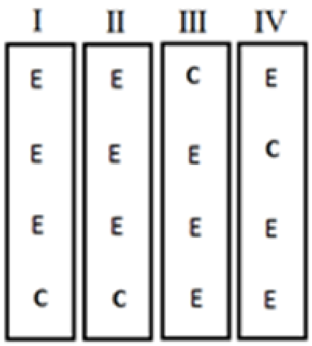

Figure 2: The organization of bone segments in tumbler apparatus; "E" denotes an experimental segment (cut), "C" denotes a control segment (uncut).

After loading, the tumbler began rotations at a speed of approximately 9.5rpm, controlled by a dimmer switch installed on the motor. These rotations simulated bidirectional wave action by washing the particulate and water back and forth over the bone segments with each rotation.

Shaker

The shaker model consisted of a heavy-duty plastic tube 3" in diameter affixed to a Plexiglas platform with zip ties (Figure 3). The bones, particulate, and water were placed inside the tube via rectangular holes cut into the top and the whole platform was placed on an orbital shaker, which induced unidirectional wave motion.

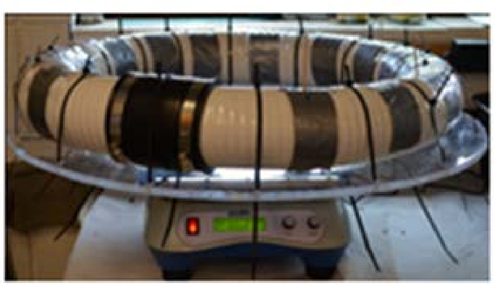

Figure 3: The shaker apparatus taken with Nikon D5100 camera by A. Appleton

For each trial, 3500mL of seawater and 400mL of either sand or diatomaceous earth – depending on the focus of the trial – were added to the tube through the rectangular windows. Small holes were drilled through the medullary canal of each bone sample with a 7/64" drill bit so Small holes were drilled through the medullary canal of each bone sample with a 7/64" drill bit so that a short wooden dowel could be inserted. The bone segment was pushed to Small holes were drilled through the medullary canal of each bone sample with a 7/64" drill bit so that a short wooden dowel could be inserted. The bone segment was pushed to one end of the dowel so that it sat in the particulate-laden water, while the free end of the dowel was passed through a small hole in a plastic cylindrical dome secured over the rectangular hole in the tube. Stationary control bones were placed on a bed of either diatomaceous earth or sand in a glass pan to be sampled with the experimental bones in the apparatuses. The arrangement of experimental and control bones within the shaker is shown in Figure 4. The rotations were carefully controlled by manipulating the speed knob on the orbital shaker and were set at 60rpm for trials using diatomaceous earth and 75rpm for trials using sand. This difference in speed is to account for the extra energy required to suspend the heavier sand in the water, as opposed to the lighter diatomaceous earth.

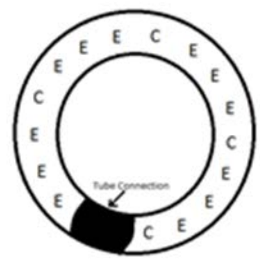

Figure 4: The organization of bone segments in shaker apparatus; "E" denotes an experimental segment (cut), "C" denotes a control segment (uncut).

Conditions

In order to simulate an intertidal marine environment, the water used in this study was taken directly from the Long Island Sound from the beach at the mouth of Oyster River in West Haven, Connecticut. The salinity of these samples was measured with a generic portable refractometer, the majority of this water having a salinity of 30 parts per thousand (‰), which is within the range of normal salinity for such an area (Stewart 2008, Marion et al. 2011). Any water samples that deviated from 30‰ salinity were adjusted by adding appropriate amounts of Instant Ocean Aquarium Salt® to preserve consistency. The water temperature reflected the ambient temperature of the laboratory in which the apparatuses were kept, which was approximately 25°C. This was tested using a glass bulb thermometer.

Sampling Schedule

In order to evaluate the effects of abrasive conditions to bone over time, a consistent schedule was developed in order to assess the changes at each time point. Six samples were taken at 2, 3, 6, 10, 15, and 20 experimental days of exposure in each apparatus. These samples consisted of two experimental (cut) bones and one control (uncut) bone from each apparatus, in addition to a stationary control bone from a glass pan filled with water and whichever sediment was being used for the trial. Due to lack of space, no uncut control bones were available for Sample #2 and #4 (Days 3 and 10).

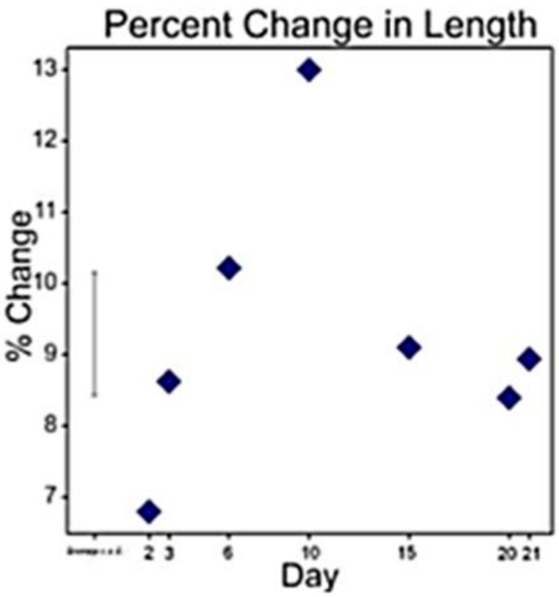

Figure 5: Percent change in length of cuts by day. $F_{(1,451)}$ = 4.36, $p<0.001$

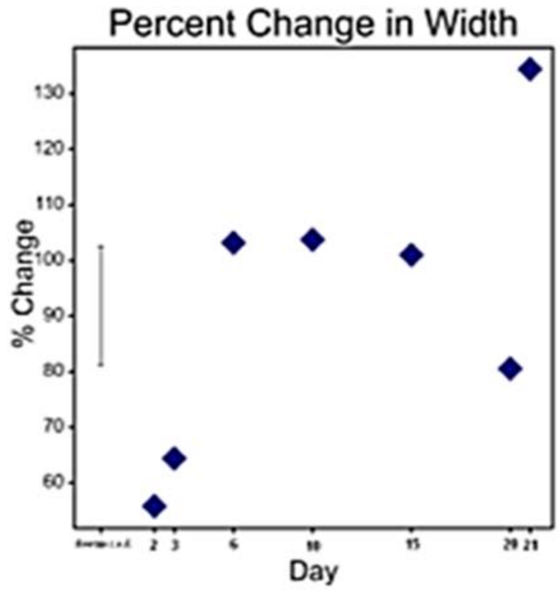

Figure 6: Percent change in the length of cuts by day. $F_{(1+51)}$=3.25, p=0.004

Database Creation

The numerical data collected from the initial measurements and final measurements after sampling was put into a Microsoft Excel® file, which was then uploaded to a VSN International GenStat® database. Columns in the Excel® spreadsheet included the trial number, sample number, days and hours spent in the apparatus, and a calculation of the approximate number of rotations in the apparatus that each bone segment experienced, which was calculated using the rotation speed of each model. The salinity of the water in which each bone segment was kept was also noted. Initial and final measurements of segment width and thickness, cortical thickness, length, width, and depth of each cut, presence of spurs and surface pitting ranking were all included. Calculations of the change in each measurement (Δ = Final – Initial) and the percent change (Δ/Initial x 100%) were performed for each sample on measurements of segment width and thickness, cortical thickness, and length, width, and depth of each cut. In order to statistically assess the presence or absence of spurs in the cuts, a positive observation (spurs were present) received a designation of "1", where a negative observation was designated "2." Thus, when a change in spurs calculation was performed (Final – Initial), a value of 0 denoted no change in the presence of spurs, where +1 reflected a loss of spurs (yes spurs → no spurs) and -1 reflected a gain of spurs (no spurs → yes spurs) after exposure to the experimental conditions. All of these values were then uploaded into the GenStat® database for statistical testing.

Statistics

Analysis of variance tests were performed using the values imported to the VSN International GenStat® Version 16database from the Excel® file in order to determine if exposure to the experimental conditions created measureable morphological changes to each bone segment. Linear regression models were used to determine the presence of any trends or relationships among the data.

Sample Storage and Disposition

When storing the bone segments became necessary – such as before the start of a new trial – the samples were wrapped in paper towel labeled with the segment's ID number, wet with deionized water, and stored in plastic bags in a laboratory refrigerator at 4°C. After the samples were placed in the apparatuses and subsequently processed, the bone segments were placed on labeled paper towels and allowed to dry overnight before being wrapped in the paper towel, placed in a plastic bag which was labeled with the trial number and trial dates, and stored in a laboratory freezer at -20°C.

Results & Discussion

Statistical testing (ANOVA and linear regression) was performed and the results revealed notable changes in the morphology of the cuts. These tests showed a significant difference in the length and width of the cuts between the different exposure days, particularly in the middle days of the trial (Figure 5 & 6). There was a significant difference in the mean percent change in the length of the cuts between all days except between Days 3, 5, 20, and 21. This indicates that Also, there was a significant difference in the mean percent change in the width of the cuts between all days except between Day 2 and Day 3, and between Day 6 and Day 10 and Day 15. This indicates that the experimental treatment did have an effect on the size of the sharp force trauma what was distinguishable based on the length of time the bones were left in the marine environment. Thus, the abrasive effects of the particulates used were powerful enough to remove bone tissue and alter the morphology of the cuts.

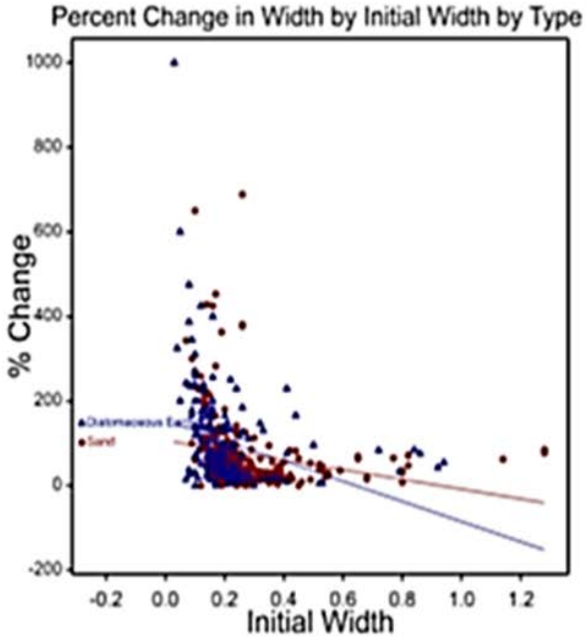

Figure 7: Linear regression showing a negative relationship between the overall percent change in width of the cuts and the initial width of the cuts before experimental treatment. R^2=0.085, $F_{(3,448)}$=15.04, p <0.001; $t_{(448)}$=-2.73, s.e.=17.2, p = 0.007

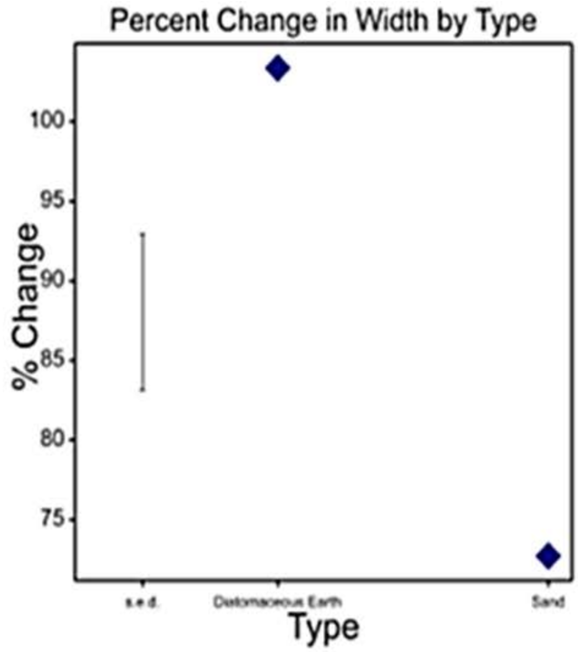

Figure 8: Percent change in width of cuts by type of particulate used in experimental treatment. $F_{(1,451)}$ = 9.75, p = 0.002

Overall, there was a significant negative relationship between the initial width of each cut and the percent change in that width after experimental treatment (Figure 7). If the cuts were on the wider end of the width range, these cuts experienced less of a change in width after experimental treatment than did cuts that were on the thinner end of the width range. This could be due to particulate becoming trapped inside the cuts rather than scraping across the surface, which would decrease the amount of bone tissue lost.

There was also a significant difference in how each substrate affected this relationship. The cuts abraded by diatomaceous earth experienced a greater mean percent change in width after exposure (Figure 8). Upon microscopic observation of each particulate, this result seems reasonable. The surface of granules of diatomaceous earth is sharp and coarse, where the surface of sand grains is slightly rounder. Diatomaceous earth is occasionally used as a polishing agent, which speaks to its abrasive capabilities (Smith 2007). Although sand is also abrasive, its rounded edges could inhibit the removal of bone tissue.

The deterioration of sharp force trauma artefact in bone is an important facet of interpreting the circumstances surrounding the death of a set of remains. This study has shown the time-dependency of such deterioration as seen in the significant differences between the number of days of experimental exposure and the magnitude of morphological change induced in the sample cuts (Figure 5 & 6). Also, the initial size of the trauma appears to have an effect on the percent change after exposure, with narrower initial cuts experiencing a greater magnitude of morphological change than samples with wider initial cuts (Figure 7). Changes in the size of each cut can likely be attributed to tissue loss due to abrasion by either sand or diatomaceous earth. These two particulates appear to have different abrasive strengths, as there was greater tissue loss in the samples exposed to diatomaceous earth than in those abraded by sand (Figure 8). This information serves as a platform from which further research on pinpointing the time of deposition of a set of remains in a marine environment can be initiated and expands the knowledge base of the forensic field.

Conclusion

Exposure to a marine environment, which includes suspended particulate and high-energy wave action, can significantly alter the morphology of sharp force trauma artefact in bone. The implications of such results provide valuable information in determining the length of time that skeletal material has been subjected to such an environment.

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Biography

Avery Appleton is currently a senior at the University of New Haven. Next year, Avery will be graduating with two Bachelor of Science degrees, one in Forensic Science and the other in Premedical Biology, with a minor in Chemistry. Avery is a member of six campus organizations, the most relevant of which being the Coalition to Combat Trafficking in Persons (President). Avery is also a member of the UNH Honors Program and works as a Biochemistry Teaching Assistant.