Effects of Different Types of Water on the Degradation Rate of Human DNA in Bone and Tissue

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Abstract

Forensic investigators heavily rely on Deoxyribonucleic Acid (DNA) analysis in the identification of human remains. In the past, using DNA analysis for identification of human remains that have been recovered from bodies of water has been an issue. This research addresses that issue and analyzes different aqueous environments (salt water, swamp water, and fresh water) and their degradative effects on DNA from human bone and tissue. The purpose of this research was to evaluate the quantity and quality of DNA after a 72 hour period of water immersion to determine whether or not a viable DNA profile would be able to be obtained. In conjunction with the research previously done, a statistical timeline of the rate of DNA decomposition associated with the different water environments can be made that will further aid investigators with the identification process. Also, investigators may be able to utilize this timeline to estimate how long remains have been submerged within a body of water by comparing the quantity and quality of DNA from the recovered remains to the statistical gage formatted by this research. In this study, human bone and tissue samples were incubated for 72 hours in salt water, brackish water, and fresh water. These samples were then removed and DNA was extracted from the bone and tissue of each sample. The extracted DNA was quantified, amplified, and analyzed. The rate of decomposition and the level of degradation was compared to a control sample that was not subjected to water, but allowed to decompose for 72 hours, and a time zero control that was not subjected to any decomposition at all. There was a substantial amount of DNA degradation and loss in both bone and tissue samples that were immersed in water for 72 hours. The bone samples showed on average an 18,900-fold reduction of detectable DNA. Bone samples that were immersed in salt water showed such extensive degradation and loss that the amount of viable DNA was so low that it was unable to be detected resulting in a 54,000-fold reduction of DNA. Tissue samples also showed a significant loss of DNA as well, with an average of a 40-fold reduction of DNA. Tissue samples that were subjected to salt water immersion showed the most DNA loss; 89-fold reduction. These findings show that aqueous environments have substantial degradative effects on DNA from human remains (tissue and bone). The resultants support and confirm the research previously completed, and reinforce the hypothesis that there is considerable DNA loss and degradation after 24 hours of immersion.

Introduction

There are many different avenues that investigators can utilize to identify human remains. In some situations investigators are unable to rely on certain identification tactics due to such extensive damage to the remains, when only a small portion of remains is found such as a foot or a piece of a torso, or even the sheer lack of dental records available. In these cases investigators use DNA analysis to help potentially identify the remains. Especially in cases where large amounts of trauma would be experienced such as a plane crash, investigators heavily rely on DNA identification to determine the identity of the remains that were recovered. On March 8th, 2014 Malaysia Airlines Flight 370 went missing. It has since been theorized that the plane had crashed somewhere in the ocean but the remains of the plane and the victims have yet to be found. When the wreckage is discovered, especially considering the intensity of the crash, the bodies of the victims will be highly decomposed and battered. It will be very difficult to identify the remains of the victims by pure visual identification. Investigators will rely on different methods of identification, such as DNA analysis to try to identify the remains of the victims. Other incidents with mass victims, such as the Tsunami in Indonesia on December 26th 2004, and Hurricane Katrina in August of 2005 required the timely identification of the remains. DNA identification of victims was utilized. Many of these victims had been exposed to water environments for extended periods of time. The exposure to long periods of immersion made DNA analysis difficult.

In order to make a genetic profile that is utilized for DNA comparison, DNA is extracted from a biological sample. After DNA is extracted, a forensic DNA analyst will perform an amplification process on the DNA called Polymerase Chain Reaction, also commonly known as PCR. PCR essentially acts as a highly efficient copy machine for DNA to make multiple copies of DNA so that it can undergo further testing and analysis.

If there is not enough DNA in a sample or if the DNA is too highly decomposed then PCR cannot be performed and a genetic profile is unable to be yielded. When bodies are immersed in water the amount of DNA in skeletal and soft tissues, such as skin, that is available for PCR is decreased overtime due to many different factors. "DNA degradation results from strand breakage, chemical modifications, and microbial attack. These degradative processes reduce the yield of high molecular mass DNA molecules and increase the chance of subsequent PCR failure" [2]. Of the many factors that lead to DNA degradation, one of the biggest factors in aqueous environments is damage due to hydrolysis, or the breakage of chemical bonds through the addition of water [2]. DNA has a high affinity to water and even after death DNA in dead tissues will continue to attract water molecules. When deceased bodies are submerged in large amounts of water for long periods of time, there is a high chance of damage due to hydrolysis.

After being submerged for some time soft tissue will begin to detach from the bone and can drift off if there are currents or flowing water. Also, the remains are subjected to organisms living in the water environment that will feed on the decomposing remains resulting in a lowered DNA yield. The longer that remains are subjected to these conditions the less amount of quality DNA will be available to produce a genetic profile of the remains for identification purposes.

Experimental Samples

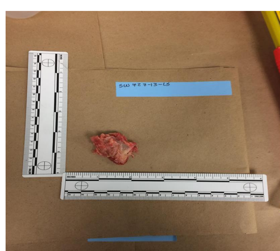

Figure 1. Rib sample before it was placed in the swamp water environment for 72 hours.

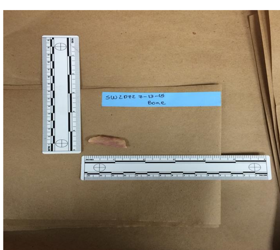

Figure 2. Defleshed bone sample after it was immersed in swamp water for 72 hours.

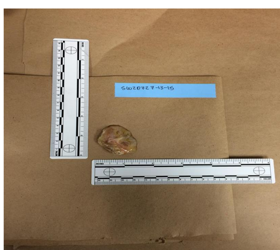

Figure 3. Rib sample after being immersed in swamp water for 72 hours.

Water Immersion Set Up

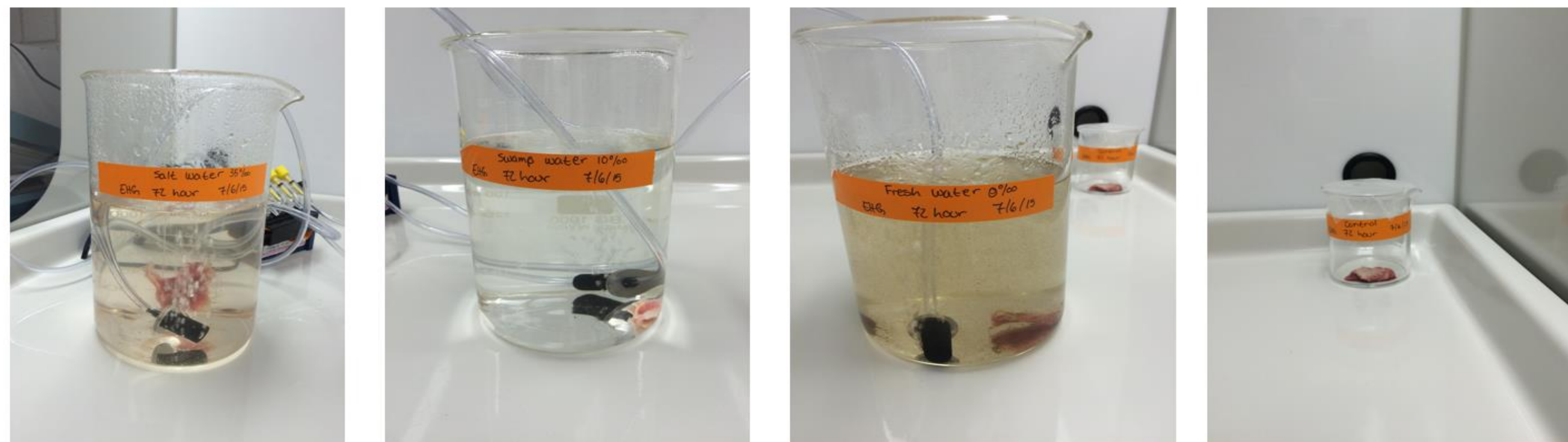

Figure 4. The experimental set up for the 72 hour water immersion experiments. The first three beakers contain a rib sample, water (salt water, swamp water, and fresh water), and an air stone which generates air flow. The last beaker contains the control sample which was not subjected to water immersion.

Data and Results

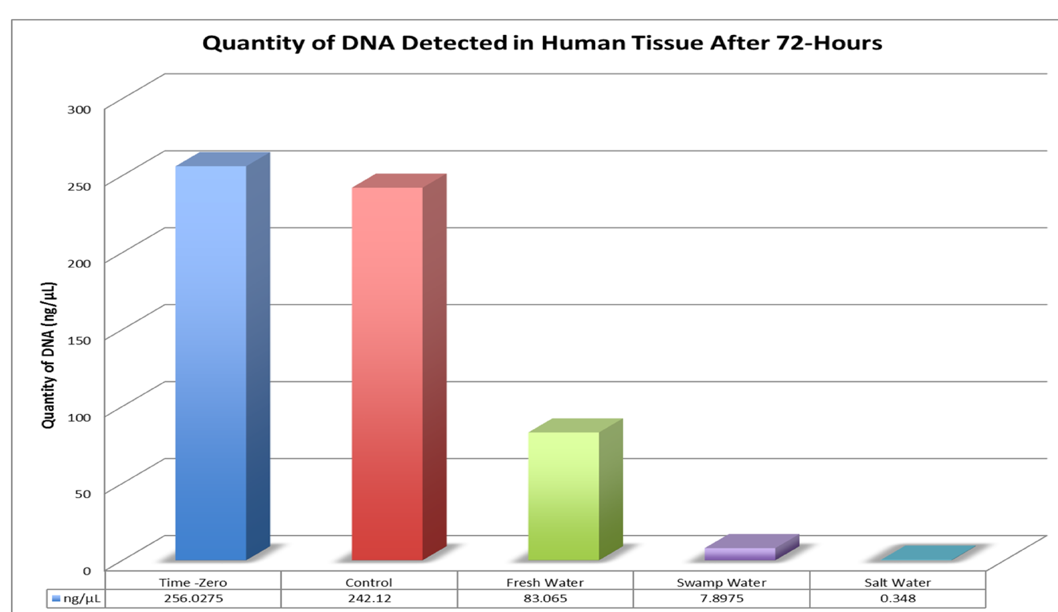

Figure 5. The average DNA quantification results from human tissue samples represented in ng/µL. Fresh water, swamp water, and salt water all showed a dramatic loss in DNA quantity over a 72 hour period of time. This shows that the aqueous environments had a substantial effect on the DNA degradation in this specific time period.

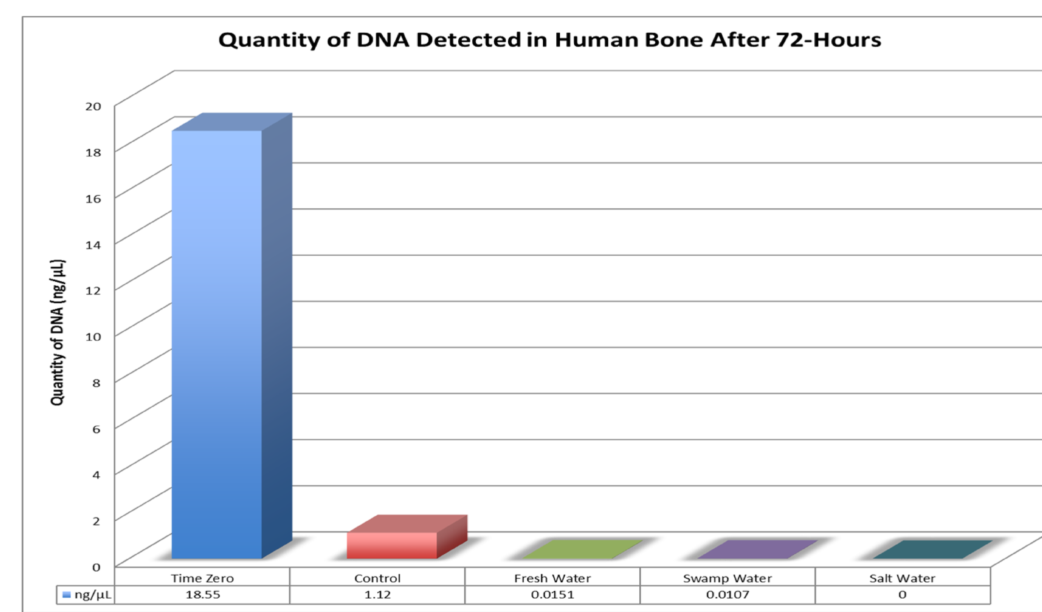

Figure 6. The average DNA quantification results from human bone represented in ng/µL. Fresh water, swamp water, and salt water all showed a dramatic loss in DNA quantity over a 72 hour period of time. This shows that the aqueous environments had a substantial effect on the DNA degradation in this specific time period.

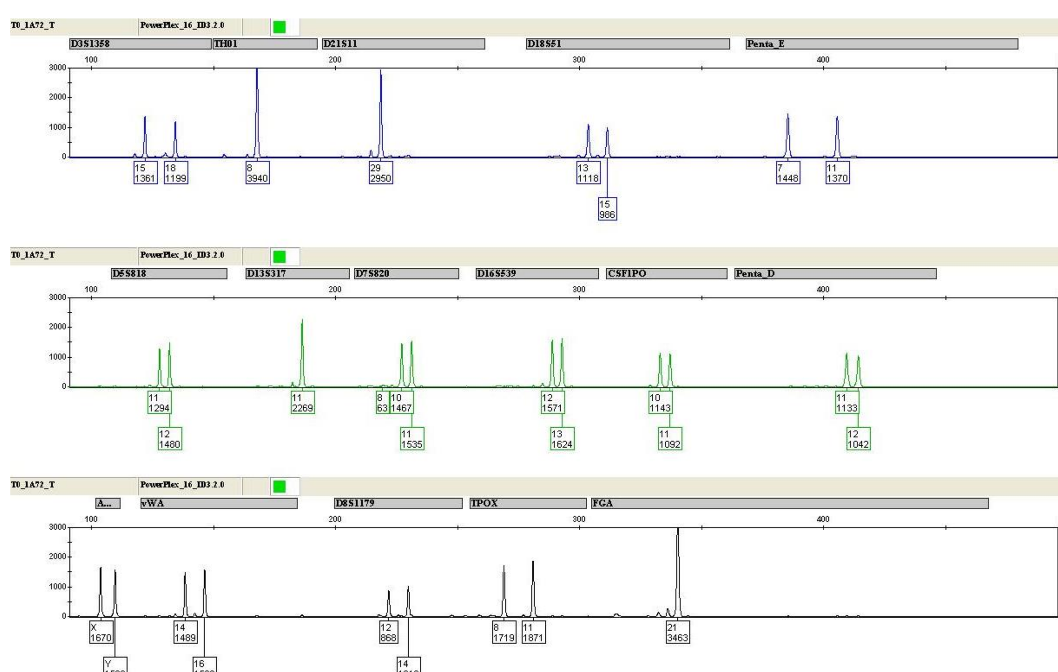

Figure 7. Electropherogram of time zero tissue sample. This shows a genetic profile of human tissue that has not been subjected to decomposition.

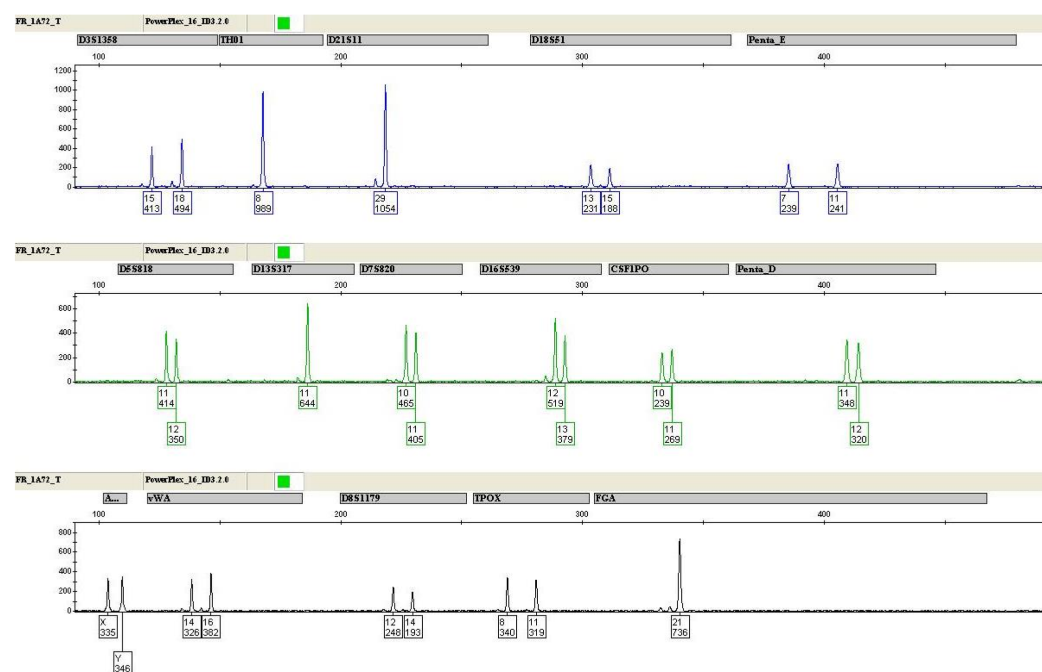

Figure 8. Electropherogram of tissues that has been incubated in fresh water for 72 hours. This shows slight DNA loss in the tissue as represented by the lowered peak heights. Even though DNA loss is present, after 72 hours a DNA profile is still obtainable.

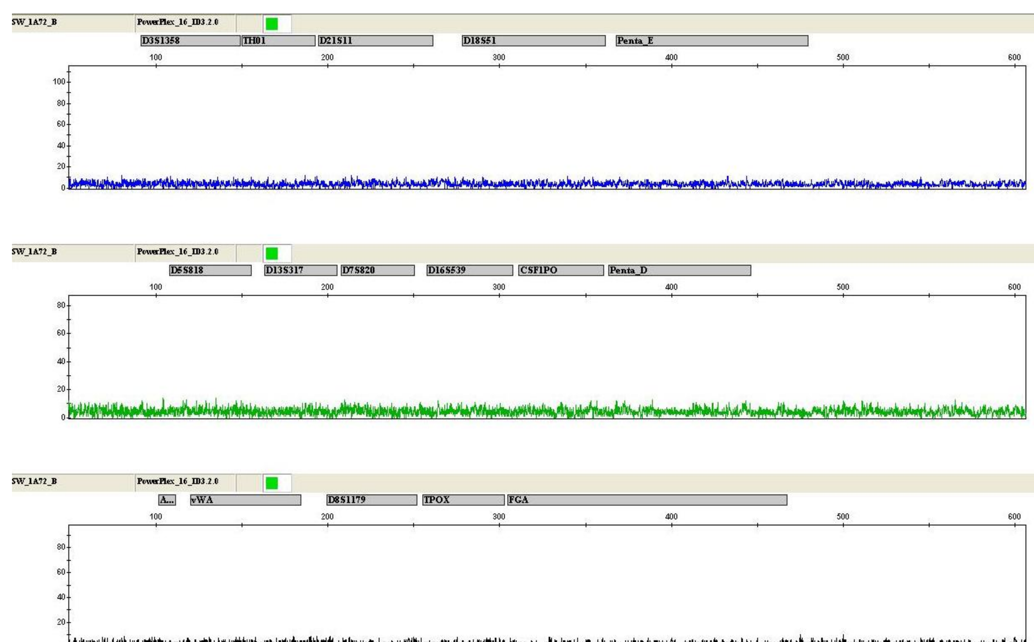

Figure 9. Electropherogram of bone that has been incubated in saltwater for 72 hours. This shows that the DNA loss was so substantial that a DNA profile was unable to be obtained (i.e. no allele calls).

Materials and Methods

Human bone and tissue samples were collected from the Yale School of Medicine Department of Pathology (New Haven, CT) and stored at -20°C until needed. Experimental water was collected from the New Haven Sound and a local freshwater lake. The salinities of the water types were measured using a portable refractometer. Salinity was adjusted by either diluting with deionized water or by adding salt (Instant Ocean® Sea salt). The salinities used were 0 parts per thousand (ppt)(freshwater), 10ppt (salt fen), and 35ppt (saltwater). 1200-1400mL of water was added to three 2000mL beakers and they were labeled accordingly. Each beaker was then aerated by using a pump-system connected to an air stone. 1-2 inch sections of human rib were weighed and photographed. One rib sample was then placed in each beaker and one rib sample was placed in a 250mL beaker containing no water, to act as a control. The samples were incubated in water for 72 hours with water changes every 24 hours. Once the 72-hour period was completed the rib samples were removed, photographed, and weighed. The samples were then defleshed and tissue samples collected.

The bone was pulverized using a SPEX SamplePrep 6770 Freezer/Mill®. 0.5M EDTA at a pH of 8.0 was added to 0.3g – 0.7g of bone powder and was then lysed for 16 hours at room temperature. The bone powder was decalcified by following the procedures stated by the "Connecticut Department of Emergency Services and Public Protection in the Division of Scientific Services for the Forensic Laboratory" [4]. After decalcification DNA was extracted from the bone powder using the "Isolation of Total DNA from Bones and Teeth" protocol from the Qiagen QIAamp® DNA Investigator Handbook [5]. DNA was then extracted from the tissue using the "isolation of total DNA from tissues" protocol from the Qiagen QIAamp® DNA Investigator Handbook [6].

DNA from the bone powder and tissue was quantified using the Quantifiler™ Human DNA Quantification Kit from Applied BioSystems. DNA was amplified using the Promega PowerPlex® 16 HS Kit and the Applied BioSystems® GeneAmp PCR System 9700 thermal cycler. Amplified samples were prepared for injection by using 9.5 µL of Hi-Di™ formamide and 0.5 µL of internal lane standard (ILS600). Separation and detection of the amplified fragments was performed on an Applied BioSystems® Prism 3130*xl* Genetic Analyzer. The data produced from the BioSystems® Prism 3130xl Genetic Analyzer was analyzed using the Applied BioSystems® Genemapper ID v.3.2.1 software.

Discussion

Data were collected for all conditions and values were averaged from six duplicate experiments. DNA loss was observed in the bone samples immersed in all three water environments. The average starting quantity of DNA in the bone (at time zero) was approximately 18.55 ng/µL. The average quantity of DNA that was detected for bone samples that were incubated in freshwater for 72-hours was approximately 0.0151 ng/µL. This was a significant loss of DNA; ~1,200 fold. The average quantity of DNA that was detected for bone samples that were incubated in swamp water for 72-hours was approximately 0.0107 ng/µL; ~1,700 fold. The quantity of DNA from samples immersed in saltwater for 72 hours were so low that it was undetectable by the instrumentation used; ~54,000 fold. The time control bone sample (incubated dry) exhibited some DNA loss, but it was not as significant as the values of the bone samples that were placed in water; ~1.12 ng/µL of DNA (~36 fold).

The tissue from the rib samples closely resembled the findings from that of the bones. The control tissue sample (dry) yielded on average ~242.12 ng/µL of DNA. On average, ~83.1 ng/µL of DNA was detected for tissue samples that were incubated in freshwater for 72-hours; ~3 fold. The average amount of DNA was detected for tissue samples that were incubated in swamp water for 72-hours was 7.90 ng/µL; ~30 fold. The average amount of DNA was detected for tissue samples that were incubated in saltwater for 72-hours was 0.348 ng/µL; ~735 fold.

A substantial quantitative loss of DNA was observed in all samples that were exposed to water immersion for 72 hours. DNA extracted from the bone showed much more extensive DNA loss than that of tissue samples. Since there was less DNA in the bone samples to begin with it resulted in a proportionally larger DNA loss. Of the three of aqueous environments saltwater exhibited the most DNA loss. This was consistent in both the bone samples and the tissue samples. The loss in saltwater bone was so much that even after 72 hours the quantity of DNA was so low that it was undetectable by the instrumentation that was utilized. From these results it is conclusive that water immersion for 72 hours does in fact have a significant effect on the quantity of DNA from human remains.

Conclusions

Water immersion has a substantial effect on the DNA from human remains. 72 hours of water immersion stands out as a critical point in the timeline of DNA loss, especially for bone that was immersed in saltwater. In the research previously done by Shanae Armstrong [1], it was found that there was a critical loss of DNA in between the time periods of 48 hours and 1 week. The results of these 72-hour experiments were consistent with this previous data. At this time period, it was found that there is not enough DNA in bone from human remains immersed in saltwater for a viable genetic profile to be done. Thus, other forms of identification would have to be sought. When compared to the control and time zero samples it is indicative that there is much more substantial DNA loss and decomposition due water immersion, proving that it does in fact have an effect on the human DNA.

References

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