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 DNA analysis in the identification process 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 (saltwater, salt fen water, and freshwater) 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 recovered after a 72 hour period of water immersion. 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 utilize this timeline to try 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. In this study, human bone and tissue samples were incubated for 72 hours in saltwater, salt fen water, and freshwater. These samples were then taken out and DNA was extracted from the bone and tissue of each sample, then it was quantified, amplified, and analyzed. It was found that there was a substantial amount of DNA degradation and loss in both bone and tissue samples that were immersed in water for 72 hours. Tissue samples subjected to freshwater immersion showed the most degradative effects and resulted in the least amount of allele recovery, despite experiencing the least amount of DNA loss. These findings show that aqueous environments have substantial effects on DNA from human remains (tissue and bone).

When human remains are recovered, it is crucial for investigators to identify to whom the remains belong. In cases such as mass disasters or incidents where extensive bodily damage is sustained, methods of identification, such as facial recognition or fingerprint comparison may not be possible. In cases like these, the investigators rely heavily on other forms of identification such as deoxyribonucleic acid (DNA) analysis. DNA analysis can not only act as the primary source of identification but many investigators may also use DNA as a way to confirm other identification methods such as facial reconstruction [1]. Though DNA analysis can play a crucial role in the identification process, extensive DNA degradation may prove to be problematic, possibly making the process completely untenable.

The human body begins to degrade the moment that someone dies, especially on a cellular level. Degradation and the rate at which it happens is influenced by many different factors. The environment that the body is in is one of these. “The following factors affect the progress of decomposition in water and can be easily altered by disposal method: clothing, perimortem trauma, access to the water surface, energy of water movement, biodiversity, floor substrate and geology, body weight, water and air temperature, moisture, pH, partial pressure of O_2 and other gases, and the local chemical environment” [2]. When remains are exposed to aqueous environments for prolonged periods of time the body experiences disarticulation and the detachment of soft tissue. This results in the further exposure of underlying bones and even the loss of full limbs [3]. The body will first lose the bones of the hands and wrists, then the bones in the feet and ankles [3]. This is especially due to the effect of water currents on flexible joints. Joints that are more flexible are more prone to disarticulation than others [3]. The next part of the body that is affected is the mandible and cranium followed by lower legs, forearms, and upper arms [3]. It is not uncommon for remains that are recovered from bodies of water to be skeletonized or have significant portions missing. With that being said, it is also not uncommon to find small parts of a body, such as a part of a foot or a hand, far from the location of the human remains due to water currents and animal interaction [3]. When these smaller pieces are found the investigators must also identify them as well, and in most cases this is done via DNA analysis.

Along with the overall body, DNA becomes degraded as well, especially in aqueous environments. Following cell death, the cellular capabilities that protect DNA from strand breakage are no longer functioning [1]. DNA becomes much more prone to degradation from strand breakage, microbial attack, as well as chemical modifications [1]. DNA is highly reactive with water, and can easily be affected and damaged by hydrolysis. Even after death, DNA will attract water molecules which will interact with the DNA and result in damage via “*deamination* (loss of an amine group) of bases, as well as *depurination* (loss of adenine and guanine) and *depyrimidination* (loss of thymine and cytosine)” [1]. These chemical

modifications, along with damage due to microbial attack and strand breakage will negatively affect Polymerase Chain Reactions (PCR), thus resulting in the inability to produce a full genetic profile.

Nuclear DNA in human bone will also be affected by DNA degradation and the degradative effects of water. Skeletal tissue consists of inorganic calcium phosphate and organic collagen protein. The inorganic calcium phosphate mostly consists of hydroxyapatite, which is where skeletal DNA binds [1].

> "As the bone degrades, the ratio of the inorganic to organic portions change while the bone exchanges chemical constituents with the depositional environment. The rate of *bone diagenesis*, the complex process of degradation and modification to the chemical and/or structural properties of bone, is based on the leaching of the various inorganic and organic components of the osseous tissue, whose rate of loss is heavily dependent upon depositional environment and intrinsic bone factors" [1].

Once the hydroxyapatite degrades, DNA is consequently released and is no longer protected and or stabilized, which allows it to be easily degraded [1]. Since bone is denser and has a lower water content than soft tissue, it can shield DNA from degradative effects. However, when bone is exposed to water for long periods of time, the water can enter the bone through a process called *bone dissolution*. This occurs when the pores of the skeletal material become enlarged and allows the water to enter through hydrolytic flow [1]. The water that enters the bone material can further degrade the bone itself along with the DNA. Due to hydrolysis that occurs in bone and soft tissues DNA can become damaged and unavailable for further investigation and analysis.

Previous studies have been done that analyzed methods of DNA recovery from skeletal remains from bone that has been exposed to specific aqueous environments. In particular, the research done by Mamelo et al. resulted in an accurate method of recovery of a genetic profile from remains that were exposed to seawater for roughly eight months. In this new method, they experimented with using more bone to increase the overall DNA yield [4]. The research found that it was not possible to recover enough quality DNA to produce a full genetic profile using the standard DNA extraction protocols utilized in forensic laboratories. It was found that by increasing the bone powder used for extraction by 36 times the suggested amount, using 36 extraction columns and adding an additional step to concentrate the DNA, 200 pg of amplification-quality DNA was obtained. This yielded a genetic profile of 12 short tandem repeat (STR) loci. Though it is possible to obtain a genetic profile via this new method, by increasing the amount of tubes and steps used during the extraction process, the potential for contamination is increased. Especially when using low-template DNA samples, any source of contamination may be amplified exponentially. This method also requires large starting quantities of bone to be used. In some cases not much bone is available to be processed or only small amounts of bone can be given to be consumed in the DNA analysis because the majority of the bone recovered needs to be used for forensic anthropological evaluation. Therefore, even though this method was successful, it may not fully be applicable in forensic investigations, and thus it is crucial to further investigate the decomposition rate and effects of water immersion on bone and tissue samples.

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). 1200-1400mLs of water each were 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 diffuser. Human ribs were cut in to 1-2 inch sections and 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®. Ethylenediaminetetraacetic acid (EDTA) 0.5M at a pH of 8.0 was added to 0.3g – 0.7g of bone powder and was then incubated 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" [5]. 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 [6]. DNA was then extracted from the tissue using the "isolation of total DNA from tissues" protocol from the Qiagen QIAamp® DNA Investigator Handbook [7].

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 using Foundation Collection Software v.3.0. Samples were injected for five and ten seconds at 3kV. The data produced from the BioSystems® Prism 3130x1 Genetic Analyzer was analyzed using the Applied BioSystems® Genemapper ID v.3.2.1 software.

Results

DNA loss was observed in the bone samples immersed in all three water environments. The average starting quantity of DNA in the bone (not subjected to decomposition) was approximately 7.44 ± 14.30 ng/µL. The average quantity of DNA that was detected for bone samples that were incubated in freshwater for 72-hours was approximately 0.0209 ± 0.0231 ng/µL. This was a significant loss of DNA; ~350 fold. The average quantity of DNA that was detected for bone samples that were incubated in salt fen water for 72-hours was approximately 0.0275 ± 0.0341 ng/µL; ~270 fold. The average quantity of DNA that was detected for bone samples that were incubated in saltwater for 72-hours was approximately $4.87x10^{-4}$ ± $4.24x10^{-4}$ ng/µL; ~15,270 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; ~0.243 ± 0.439 ng/µL of DNA (~30 fold).

The tissue from the rib samples closely resembled the findings from that of the bones. The average starting quantity of DNA in the soft tissue (not subjected to decomposition) was approximately 404.88 ± 342.65 ng/µL. The control tissue sample (dry) yielded on average approximately 218.89 ± 72.33 ng/µL of DNA, ~1 fold. On average approximately 151.37 ±147.65 ng/µL of DNA was detected for tissue samples that were incubated in freshwater for 72-hours; ~2 fold. The average amount of DNA was detected for tissue samples that were incubated in salt fen water for 72-hours was approximately 52.98 ± 90.28 ng/µL; ~7 fold. The average amount of DNA was detected for tissue samples that were incubated in saltwater for 72-hours was 0.315 ± 0.245 ng/µL; ~1,280 fold.

When assessing the quality of DNA from bone samples, DNA degradation and loss were observed. The average allele drop-out was 24.6 ± 4.16 alleles of the alleles detected (10.9% ± 15.3% of potential alleles detected) for bone samples that were immersed in freshwater for the 72-hour incubation period. As for bone samples immersed in salt fen water for 72-hours, the average allele drop-out of detected alleles was 25.2 ± 3.42 alleles and 8.8% ± 12.2% of potential alleles were detected. For bone samples that were immersed in saltwater a genetic profile was unable to be obtained and no alleles were detected resulting in the average drop-out of 27.6 ± 0.55 detected alleles and 0% of alleles detected.

Soft tissue samples that were subjected to 72-hours of freshwater immersion had an average allele drop-out of 9.2 ± 7.2 alleles of alleles detected and on average, detected 66.4% ± 26.5% of potential alleles. Allele drop-out was more prevalent in higher molecular weight loci, which was indicative of substantial DNA degradation. Soft tissue samples that were immersed in salt fen water for 72-hours had an average allele drop-out of 6.8 ± 9.73 alleles and on average, 75.4% ± 35.0% of the potential alleles were detected. Full genetic profiles were obtained at this time period. Degradation was observed, but not as substantial as that observed in soft tissue samples subjected to freshwater immersion. Soft tissue samples that were immersed in saltwater for a 72-hour period had an average allele drop-out of 1.4 ± 3.13 alleles of and, on average, detected 95.0% ± 11.2% of potential alleles. Full genetic profiles were obtained for all duplicate experiments except one (4/5).

For both soft tissue and bone, additional peaks were detected that were consistent with the expected genetic profile but they were below the analytical threshold and thus deemed unreliable. Though many of the samples were below the stochastic threshold, allele drop-in was not observed.

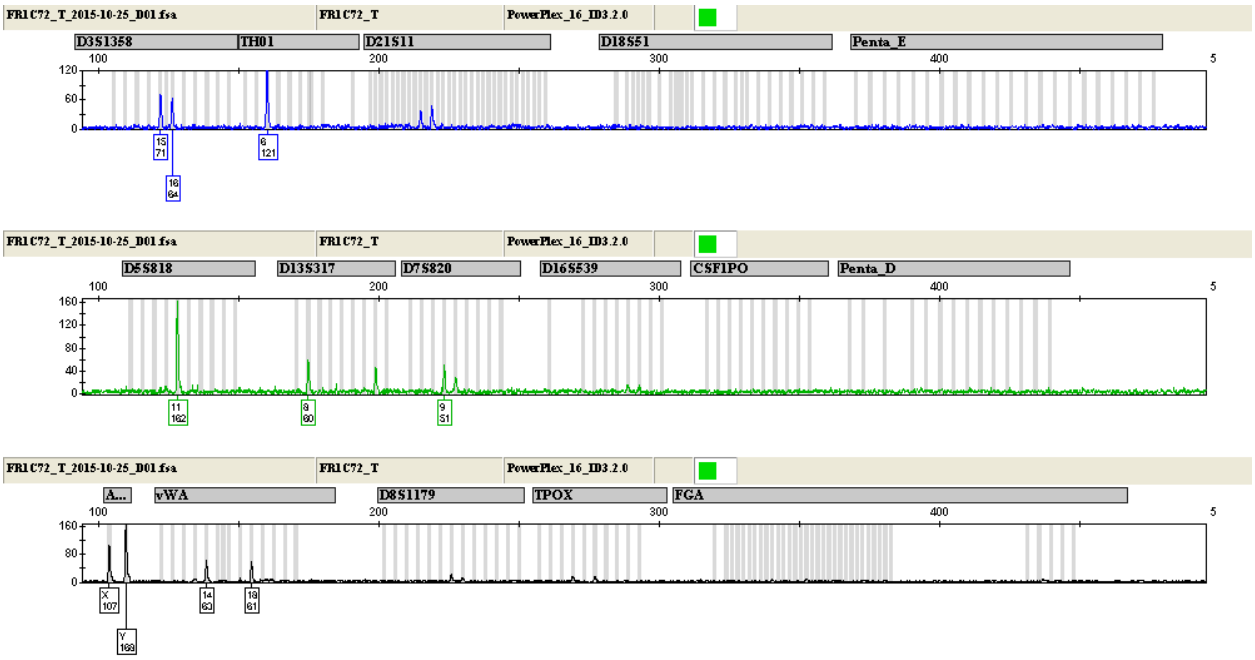

Figure 1: Electropherogram of DNA from soft tissue that was subjected to a 72-hour incubation period in freshwater. Substantial DNA degradation is observed.

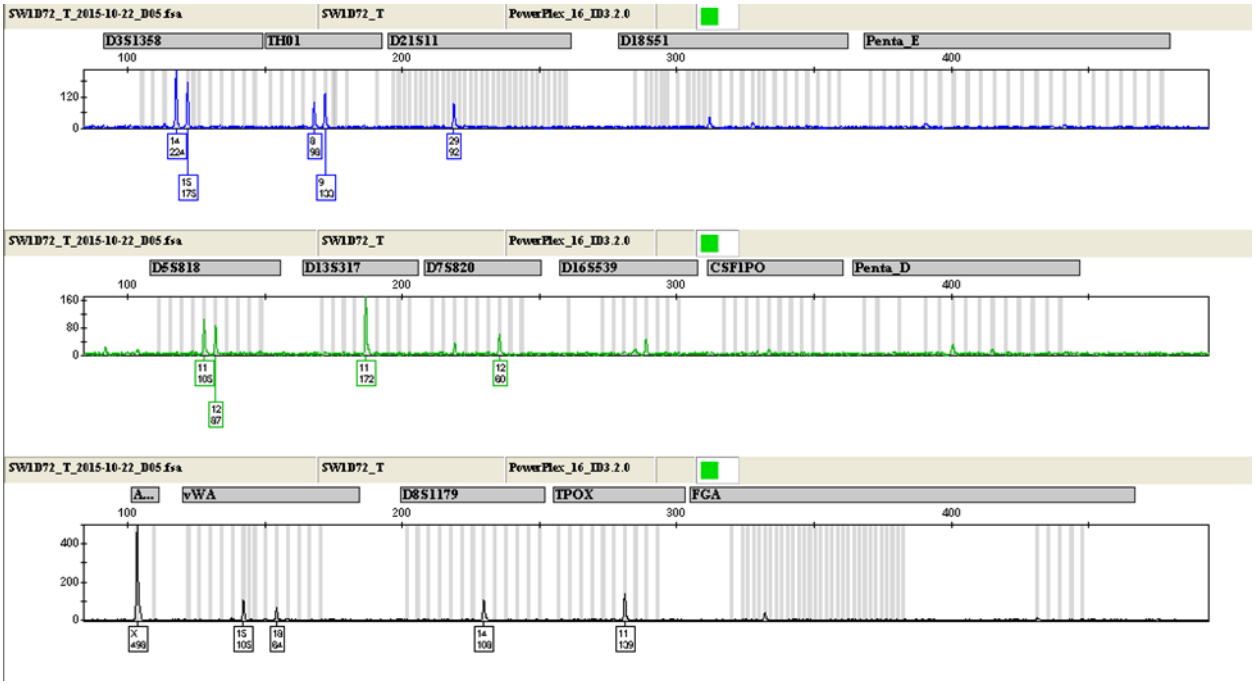

Figure 2: Electropherogram of DNA from soft tissue that was subjected to a 72-hour incubation period in salt fen water. DNA degradation is observed.

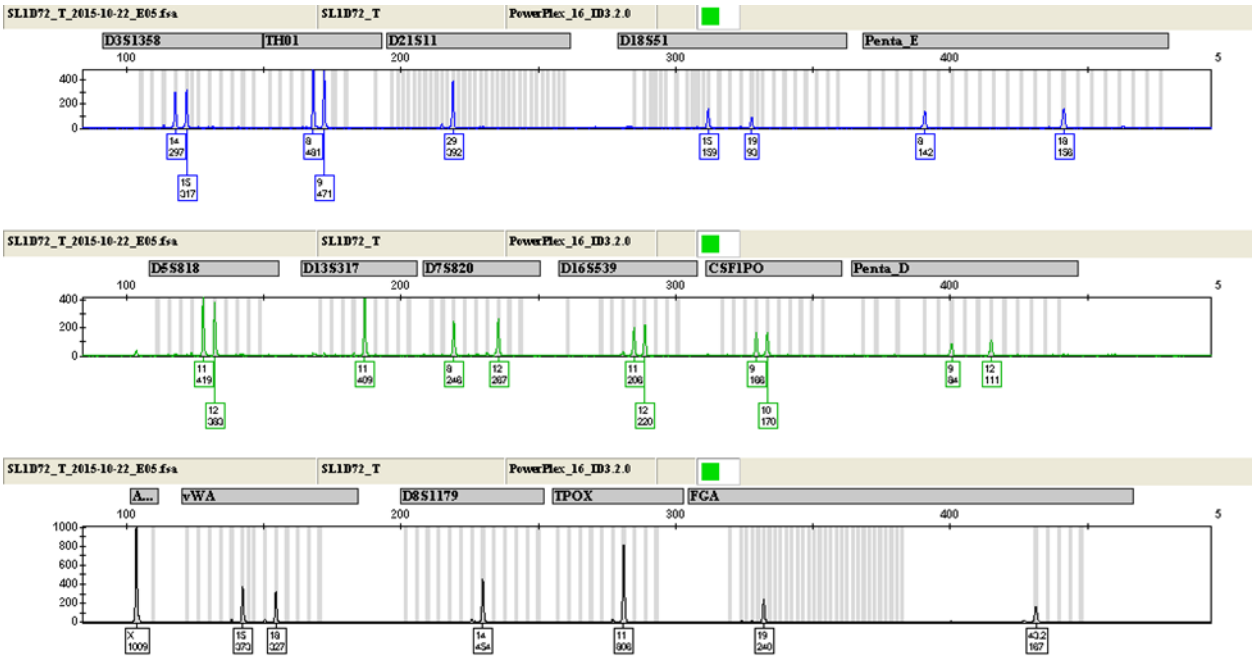

Figure 3: Electropherogram of DNA from soft tissue that was subjected to a 72-hour incubation period in saltwater. A full genetic profile was developed.

	Freshwater Tissue	Salt Fen Water Tissue	Saltwater Tissue	Freshwater Bone	Salt Fen Water Bone	Saltwater Bone
Average Quantitative DNA Recovery	151.37 ± 147.65 ng/µL	52.98 ± 90.28 ng/µL	0.315 ± 0.245 ng/µL	0.0209 ± 0.0231 ng/µL	0.0275 ± 0.0341 ng/µL	$4.87x10^{-4}$ ± $4.24x10^{-4}$ ng/µL
Average Allele Drop-out	9.2 ± 7.2 alleles	6.8 ± 9.73 alleles	1.4 ± 3.13 alleles	24.6 ± 4.16 alleles	25.2 ± 3.42 alleles	27.6 ± 0.55 alleles
Average Percent of Detection of Potential Alleles	66.4% ± 26.5%	75.4% ± 35.0%	95.0% ± 11.2%	10.9% ± 15.3%	8.8% ± 12.2%	7.44% ± 14.30%

Table 1: The average quantitative DNA recovery, average allele drop-out, and average percent of detection of potential alleles for bone and tissue immersed in different water types.

Discussion

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 samples showed much more extensive DNA loss than that of soft 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 aqueous environments, saltwater exhibited the greatest DNA loss. This was consistent in both the bone samples and the tissue samples. From these results it is indicative that water immersion for 72 hours does have a

substantial effect on the quantity of DNA from human remains.

The quality of the DNA was also effected by the type of water environment in which the sample was immersed. Overall, water had a detrimental effect on DNA from bone samples, especially in bone samples exposed to saltwater. The loss in bone immersed in saltwater was so large, that after 72 hours the quantity of DNA was so low that alleles were undetectable by the instrumentation. In saltwater the DNA is either becoming so highly degraded or lost that it is not being detected. In freshwater bone less loss was observed, but substantial degradation was seen, as indicated by the recovery of smaller molecular weight loci.

Tissue samples that were immersed in freshwater displayed the least amount of allele recovery, despite having the highest quantitative recovery of DNA of the three water environments. The freshwater had the most degradative effect on the DNA itself out of the water types. Tissue samples immersed in saltwater showed larger total losses of DNA, but barely any degradation. As the salinity of the water increases it is causing the DNA from bone and tissue to be lost at a much more substantial rate. On the other hand, when the salinity decreases, the rate of degradation is increased. This can be explained by the increased salinity inhibiting the effects of hydrolysis, but increasing cell lysis and bone diagenesis.

Conclusion

Water immersion has a substantial effect on the ability to recover usable 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 [8], it was found that there was a critical loss of DNA in between the time periods of 24 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 obtained reliably from samples using less than one gram of material. 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. It was also found that saltwater induces more DNA loss, whereas freshwater generated higher degradative damage to the DNA, proving that the type of water environment has different effects on the DNA.

Literature Sited

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Biography

Ema Graham is currently a senior at the University of New Haven double majoring in Forensic Science with a concentration in Biology and Biology with a concentration in Pre-Medical, along with a minor in Chemistry. Ema plans on continuing her research on this topic for the rest of her time at the University of New Haven. After college she aspires to pursue a Ph.D. in Molecular Biology or Human Genetics.