Research Proposal: The Effect Of Water On DNA Degradation

Introduction

DNA profiling is a technique used in the identification of human remains. Advantages include a low minimum sample size and high evidential value (de Boer 2018). Common applications include disaster victim identification (DVI), homicides and missing persons cases. INTERPOL (2018) recommends DNA profiling alongside friction ridge analysis and odontology as the most reliable methods of identification. DNA profiling is particularly useful in cases where these other techniques are not viable due to decomposition or damage, and as such is considered the ‘gold standard’ of DVI (Zietkiewicz et al. 2012, Mameli et al. 2014).

A key limitation is obtaining a profile when the DNA itself is decomposed (Hughes-Stamm 2010; de Boer et al. 2018). The rate of degradation is affected by many factors; including the decomposition of remains, presence of bacteria, pH, heat, salinity, and water (Campos et al. 2012 Zietkiewicz et al. 2012; Butcher et al. 2014). This can result in profiles being incomplete or unobtainable (Primorac et al. 2014).

I will be focussing on how fresh and saltwater effects the degradation of DNA. Research into this area is important for several reasons. Firstly, DNA profiling of submerged remains has not been extensively researched, and no set methodology exists (Courts and Madea 2011, Mameli et al. 2014). Analysis of DNA from samples submerged in water has also been said to be ‘challenging’ (Courts and Madea 2011). In several studies, the effect of water has not been distinguished from ions, bacteria, and other natural contaminants. Meta-analysis can collate and review the data available to establish any correlation more strongly, and with consideration to other variables. Secondly, this research may help in decision-making and selection of extraction and analysis methods. Mameli et al. (2014) showed that larger samples were needed to obtain a profile from submerged tissues. Dawnay et al. (2018), stated that the effectiveness of their field-based analysis was limited by degradation. Further research can help in identifying areas where methods may be unsuccessful or require alteration. Another benefit of this research is the potential to estimate the post-mortem interval (PMI). Xiji et al. (2005) stated that DNA degradation can be used effectively to estimate PMI. This study did not involve water, however research into this area could expand the range of cases in which this method could be used. Conversely, a known exposure time to water may be used to estimate quality and amount of DNA, which could also help in choosing appropriate analysis methods.

Aim & Objectives

The aim of this independent research project is to establish how fresh and saltwater may affect DNA degradation. This aim will be achieved by:

- Retrieving and reviewing existing data on DNA degradation in water
- Performing a meta-analysis of collected data
- Analysing and interpreting the findings of the meta-analysis

Background

DNA Degradation

DNA degradation is shown to be an issue that can affect victim identification outcomes. Courts et al. (2013) created a grading system for measuring the putrefaction of human remains comprising of 19 tiers. The degradation of DNA within samples was measured, and a correlation was found between specific stages of putrefaction and STR success, although these are not stated in the report. It was also found that the specific impact of degradation was variable dependant on tissue type, with kidney tissue least impacted by degradation. All samples were taken from tissues and not bone, so applications of this study are limited in cases of significant degradation where these tissues may be unusable.

Fresh and saltwater exposure has been linked to low DNA profiling success rates in several studies. Campos et al. (2012) looks at DNA extraction from cow bone submerged in bogs and seabed. The study found that after a year, the quantity of extracted mtDNA from all locations was less than 10% of that of the control. The mtDNA levels then stabilised for the following 3-4 years. This suggests that fresh and saltwater both have a detrimental effect on the amount of retrievable mtDNA present, and that this affect is strongest within the first year of submersion. This study used an mtDNA assay to determine approximate DNA levels within the samples. Schwarz et al. (2009) argues that approximating DNA in this way is incorrect due to variations in observed ratios. The use of drilling to extract bone samples is also discussed as a limitation, as the mtDNA is disturbed by this process, which can cause higher quantities to be reported. The salinity, temperature, pH and exposure to sediment were described, however the presence of bacteria and contaminating particles is not discussed. Whilst these would be present in a DVI or death investigation scenario, it also makes it unclear whether the effect is due to water or other conditions. The study does not reflect ‘real life’ conditions due to the use of cow bone and the handling of samples. Samples were washed and air dried, and marrow was removed before submersion. Therefore, this is not applicable to a genuine DVI or death investigation case.

Sirker et al. (2016) performed a study on DNA degradation within human bodily fluids. A comparison was made between freshwater and dry conditions. However, this was in terms of humidity with no contact between the sample and the water source, so real-life conditions were not reflected. The study found significant reduction in DNA amplification success rates after 47 weeks of exposure to 100% humidity. However, the study was limited by random mould growth which may have accounted for this reduction. It is not possible to distinguish the effect of the humidity from the effect of the mould, and the dry and humid samples were incomparable. Therefore, this study is of limited value.

A study by Musse et al. (2009) on human teeth showed that the effect of saltwater on DNA degradation was greater than that of freshwater. This study included a survey and a laboratory study. A range of ions and organic particles were found in both water sources, which are unaccounted for as a contributor to rate of degradation. As part of the survey, it was found that 14.74% of the drowning victims studied were identified by forensic dentists, highlighting the importance of DNA profiling in victim identification. Of the samples, only 37.5% yielded a DNA profile. The majority of these were fresh water, supporting the link between saltwater and greater levels of degradation. Control samples showed greater success rates, indicating the effect of water exposure. The effects of other conditions (temperature, bacteria etc.) were

discussed but not accounted for in this study.

Kapiñska and Szczerkowska (2004) studies the effect of long-term sea and freshwater exposure on DNA extraction from significantly decomposed human remains. It was found that the degree of decomposition of the remains and environmental factors both had an effect on the quantity of extracted DNA. Four different extraction methods were compared, a profile was only obtained using the phenol-chloroform method. More DNA was extracted from seawater than freshwater, however this does not indicate a higher quality, as it is stated that contaminating bacterial and fungal DNA may be present. The PMI and exposure time to water were unknown, so cannot be considered in interpreting the results of this study. The sample size was also very small, with only four human remains used.

The above studies agree that salt and fresh water do have an affect on DNA degradation. However, there are many other variables within a natural aquatic environment which may also contribute to the rate and extent of degradation. It is therefore challenging to determine the extent of the impact of water alone. Many studies (Kapiñska and Szczerkowska 2004, Musse et al. 2009) involved samples taken from submerged human remains, which is advantageous as it more accurately reflects DVI or sudden death conditions.

Impact on Victim Identification

Submersion in both fresh and saltwater has been shown in several studies to be a challenge to DNA profiling. Degradation of DNA can prevent certain techniques from being successful, as the amount of DNA extracted is not sufficient. In Mameli et al. (2014) no measurable DNA was retrieved from remains submerged in seawater for 8 months in two of three trials, and 200pg was extracted when a larger sample size was analysed. This is problematic as it may limit the success of certain techniques.

An example of this issue Courts and Madea (2011), where a new method of DNA extraction is tested. A challenge with degraded DNA is said to be finding an ‘optimum compromise’ between yield and quality of DNA, in order to retrieve a sufficient profile from fresh water submerged bone. A mild extraction process was used to obtain the highest possible DNA yield without further degrading the DNA. This study was successful, however with limited reliability as only one bone was analysed.

Dawnay et al. (2018) is a notable example of a technique limited by DNA degradation. Whilst this study used air-exposed remains rather than water, it does illustrate the challenges caused by DNA degradation. A field-based victim identification method was used to analyse human remains and pork. The study found a rapid decline in profile quality as the accumulated degree days (ADD; a measurement of heat and time) increased. Furthermore, no profiles could be obtained from exposed and surface areas where decomposition was more extensive. The studies’ use of ADDs to precisely measure decomposition allows for more accurate comparison of results. The results when using pork are both less applicable and less reliable than those using human remains, as pork was obtained from a supermarket and refrigerated.

De Boer et al. (2018) is another example of the challenges created by DNA degradation. This study of a technique used in the MH17 DVI operation claims a 98.2% success rate after a disaster in which remains were comingled and damaged. However, over half of the fragmented

remains were not sampled due to their condition and were therefore not included in this number. It is therefore unknown how successful this technique would be when including significantly decomposed and damaged remains.

Methodology

The background section of this research proposal was created by using online databases to search for relevant literature. Physical resources could not be reviewed at this point due to COVID-19 restrictions. Searches were completed using the following keywords:

- DNA degradation
- Saltwater
- Fresh water
- Human
- Disaster victim identification

The databases searched were:

- Academic Search Ultimate
- Google Scholar
- JSTOR
- Medline Complete
- PubMed
- Science Direct
- Scopus
- Web of Science

The resources retrieved were read critically to establish the main arguments, considering relevance, advantages and limitations. These were then collated to create a summary of existing literature.

The independent research project will be completed by performing a meta-analysis of existing data in order to identify any existing trends. Data will be collected by searching for relevant literature through the aforementioned databases, including physical resources if accessible. An inclusion criterion will be applied; studies should be accessible, available in English, and should involve the exposure of human or animal tissues to water. Unpublished studies may be included to avoid publication bias. The effect size for each dataset will be calculated to determine strength of conclusions, and the weighted average will be determined. The results of the meta-analysis will then be interpreted and discussed.

As meta-analysis involves data from a range of literature, a larger and more diverse dataset is analysed. This means the conclusions drawn are stronger than in a single empirical study. Complex and conflicting datasets can be analysed and summarised in order to identify trends and contributing factors. Meta-analyses are also less likely to be affected by bias, as the results are not interpreted by individuals involved in the original research.

Limitations

The meta-analysis may be limited by availability of adequate, comparable data for analysis, as it has been stated that there is limited research in this area (Courts and Madea 2011, Mameli et al. 2014). Publication bias may cause overestimation of effect size, as insignificant findings are less likely to be published. I will include unpublished resources that meet the inclusion criteria in order to reduce the effect of publication bias. Another limiting factor may be application of results to real-life situations. Whilst this study aims to identify the effects of fresh and saltwater on DNA, in criminal and disaster scenarios many other variables will be present that will also contribute to degradation.

References

1. Butcher, B.A., Bieber, F.R., Budimlija, Z.M., Dennis, S.M., and Desire, M.A., 204. Identification of Missing Persons and Mass Disaster Victim Identification. In: Primorac, D., and Schanfield, M., eds. Forensic DNA Applications: An Interdisciplinary Perspective [online]. Taylor & Francis Group: Florida, USA.
2. Campos, P.F., Craig, O.E., Turner-Walker, G., Peacock, E., Willersley, E., and Gilbert, M.T.P., 2012. DNA in ancient bone- Where is it located and how should we extract it? Annals of Anatomy- Anatomischer Anzeiger [online]. 194(1), pp. 7-16.
3. Courts, C., Madea, B., 2011. Full STR Profile of a 67-Year-Old Bone Found in a Fresh Water Lake. Journal of Forensic Science [online]. 51(s1), pp. s172-175.
4. Courts, C., Sauer, E., Hofmann, Y., Madea, B., and Schyma, C., 2013. Assessment of DNA profilability from putrefied bodies based on a newly developed quantitative grading system for putrefaction. Forensic Science International: Genetics Supplement Series [online]. 4, e262-e263.
5. Dawnay, N., Flamson, R., Hall, M.J.R., and Steadman, D.W., 2018. Impact of sample degradation and inhibition on field-based DNA identification of human remains. Forensic Science International: Genetics [online]. 37, pp. 46-53.
6. De Boer, H.H., Maat, G.J.R., Kadarmo, D.A., Widodo, P.T., Kloosterman, A.D., and Kal, A.J., 2018. DNA identification of human remains in Disaster Victim Identification (DVI): An efficient sampling method for muscle, bone, bone marrow and teeth. Forensic Science International [online]. 289, pp. 253-259.
7. Hughes-Stamm, S.R., Ashton, K.J., and van Daal, A., 2010. Assessment of DNA degradation and the genotyping success of highly degraded samples. International Journal of Legal Medicine [online]. 125, pp. 341-348.
8. INTERPOL, 2018. Disaster Victim Identification Guide [online].
9. Kapiñska, E., and Szczerkowska, Z., 2004. Personal identification based on nuclear DNA extracted from bones of deceased individuals. Problems of Forensic Sciences [online]. 60, pp. 104-115.
10. Mameli, A., Piras, G. and Delogu, G. (2014) ‘The successful recovery of low copy number and degraded DNA from bones exposed to seawater suitable for generating a DNA STR profile’, Journal of forensic sciences, 59(2), pp. 470–473.
11. Musse, J.O., Nardis, A.C., Anzai, E.K., Hirata, M.H., Cicarelli, R.M.B., and Oliveira, R.N, 2009. Freshwater and salt-water influence in human identification by analysis of DNA: an epidemiologic and laboratory study. Brazilian Journal of Oral Science [online]. 8(2), pp 71-75.
12. Primorac, D., Schanfield, M.S., and MARJANOVI?, D., 2014. Basic Genetics and Human Genetic Variation. In: Primorac, D., and Schanfield, M., eds. Forensic DNA Applications: An Interdisciplinary Perspective [online]. Taylor & Francis Group: Florida,

USA.
13. Schwartz et al.
14. Sirker, M., Schneider, P.M., and Gomes, I., 2016. A 17-month time course study of human RNA and DNA degradation in body fluids under dry and humid environmental conditions. International Journal of Legal Medicine [online]. 130, pp1431-1438.
15. Xiji, S., Yaling, L., Liang, R., Fanggang, H, Hongyan, Z., Lijang, L., and Liang, L., 2005. Correlative analysis on the relationship between PMI and DNA degradation of cell nucleus in human different tissues. Journal of Huazhong University of Science and Technology [Medical Sciences] [online]. 25, pp. 423-426.
16. Zietkiewicz, E., Witt, M., Daca, P., ?ebracka-Gala, J., Goniewicz, M, Jarzab, B., and Witt, M., 2012. Current genetic methodologies in the identification of disaster victims and in forensic analysis. Journal of Applied Genetics [online]. 53, pp. 41-60.