Persistence of DNA in water from an actively decomposing cadaver for human identification

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INTRODUCTION

Environmental DNA (eDNA) refers to genetic material from organic elements left behind from interactions with the surrounding environment.[1,2,3] Previous studies have focused on collecting eDNA from blood and pathogens in aquatic settings, notably contributing to Covid-19 research.[1,3] However, the inadvertent collection of human DNA, known as human genetic bycatch, can occur while gathering eDNA samples.[3] Despite the extensive research of eDNA for environmental monitoring, the use of eDNA in forensic capacities has great potential, but limited studies have been conducted in regard to collecting human DNA, especially concerning the collection and identification of submerged human remains

The objective of this study was to assess if enough DNA could be recovered for STR analysis on eDNA collected from a freshwater tub containing a decomposing cadaver submerged for 30 days. Water samples were periodically collected from the tub, both with the cadaver in situ and for 30 days after its removal. Concurrently, tissue samples from the rectus femoris muscle were collected during the same interval for comparison. The eDNA extracted from the water samples was examined to determine if enough DNA was present to positively identify the cadaver.

MATERIALS AND METHODS

Cadaver Placement

In the summer of 2023, a cadaver housed at the Southeast Texas Applied Forensic Science Facility (STAFS) was placed in a 228 x 85 x 57 cm tub and filled with approximately 500L of fresh water (Fig. 1). The body was submerged for 30 days, and samples of the surrounding water (30mL) and muscle tissue from the thigh muscle (rectus femoris) were collected 3 times a week. Once the initial 30 days had passed, the cadaver was removed, and water and tissue samples were collected 3 times a week for another 30 days.

Fig. 1. Picture of water vault placement on May 5, 2023.

Tissue Preparation

From the rectus femoris samples, 10mg of tissue was incubated overnight in 190 µl of G2 Buffer (Qiagen), and 10 µl of ProK at 900 rpm at 56°C (Fig. 2).

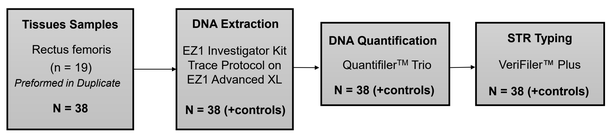

Fig. 2. Workflow for tissue preparation and downstream processing.

RESULTS & DISCUSSION

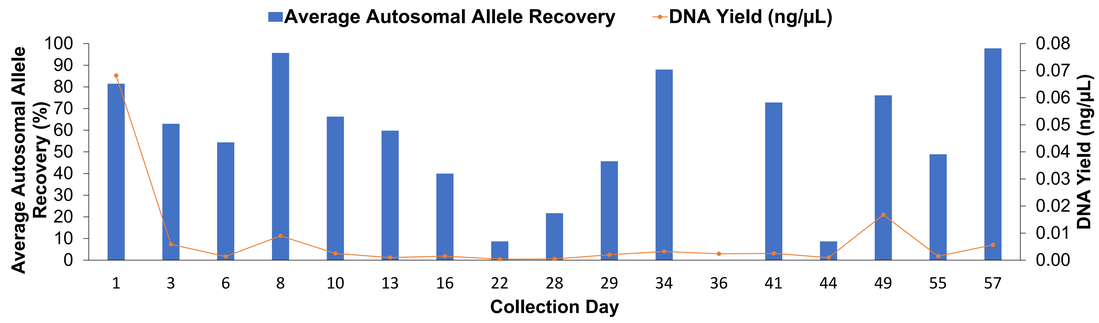

Fig. 7. Average Autosomal Allele Recovery and DNA yield of tissue samples from submerged cadavers collected over 2 months.

Table 1. DNA Yield and Degradation Index (DI) of water samples from over 2 months.

Collection Date	Water DNA Yield (ng/µL)	Degradation Index (DI)
0	0.0745	1.9
1	0.0099	6.0
3	0.0024	9.2
6	0.0004	18.1
8	0.0016	7.6
20	0.0002	2.1
31	0.0016	7.0
55	0.0005	Undetermined

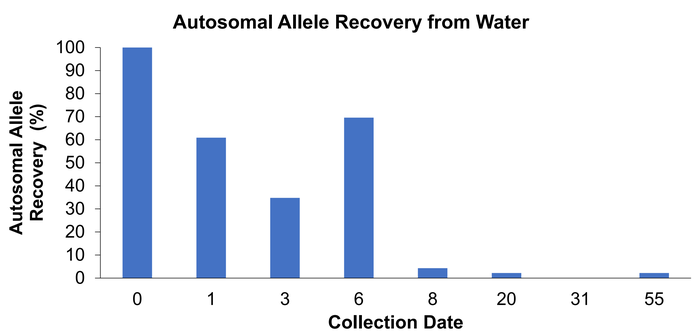

Fig. 8. DNA Yield and Degradation Index (DI) of tissue samples from submerged cadavers collected over 2 months.

- In general DNA yield decreased from tissue samples over time (Fig. 7). The highest DNA yield was 0.0681 ng/µL on Day 1.
- STR allele recovery was variable over time from tissue samples (Fig. 7).
- Only 8 water samples yielded quantifiable amounts of DNA; the most recovered DNA was 0.0745 ng/µL on Day 1 (Table 1).
- After one week of water submersion, water samples produced STR profiles with less than 10% of expected alleles.
- The autosomal allele recovery from water samples decreased over the first few days, with a spike on day 6, but then plateaued for the days preceding it (Fig. 8). This spike could be correlated to the purging stage of decomposition.
- Partial STR profiles could be obtained throughout the study; however, DNA degradation and loss of loci increased markedly over time for both tissue and water samples.

REFERENCES

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MATERIALS & METHODS

Water Procedure

The water samples (Fig. 3) were filtered through nitrocellulose filter paper on a Buchner funnel system (Fig. 4). The filter was halved, cut into pieces, and placed in an Investigator® Lyse&Spin Basket (Fig. 5). Downstream processing is outlined in Fig. 6.

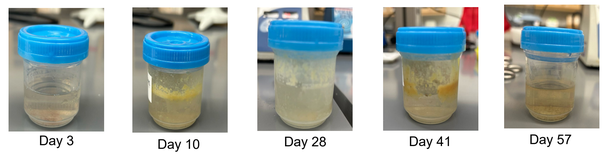

Fig. 3. Five of the collection cups containing the water collected for the study

Fig. 4. Buchner Funnel diagram, nitrocellulose paper, and an example of filter paper after the water samples were filtered through.

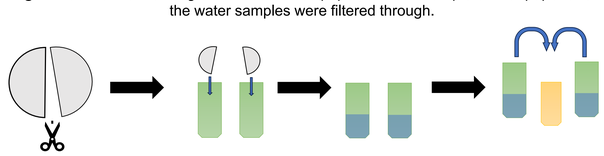

Fig. 5. The process of cutting the filter paper to accommodate the tube size and combining the final elution volumes .

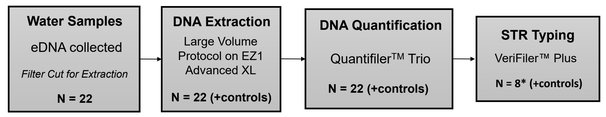

Fig. 6. Workflow for water samples and downstream processing. *Samples with quantifiable amounts proceeded to STR Typing

CONCLUSIONS

- Quantifiable amounts of DNA were recovered from tissue samples collected throughout the study, but STR completeness was variable.
- After one week of submersion, limited DNA was recovered from water samples to produce low quality partial STR profiles.
- The collection of a larger volume of water (1L) should be considered (compared to 30mL in this study) in order to retrieve more eDNA from the water surrounding human remains.
- The authors suggest optimizing the filtration method to maximize eDNA recovery.

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