A87 Predicting the Postmortem Submersion Interval (PMSI) From the Microbiome of Bone in a Fresh Water Lake

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After attending this presentation, attendees will have a greater understanding of the use of microorganisms in estimating the PMSI for skeletal remains recovered from a freshwater lake.

This presentation will impact the forensic science community by providing information concerning a novel area of research, the use of longitudinal succession of microbiomes on skeletal remains submerged in a freshwater lake to predict PMSI.

When individuals die in or are disposed of in aquatic environments, the remains may not be recovered for a period of time, rendering identification difficult. Water turbulence, exposure to aquatic scavengers, as well as accumulated temperatures contribute to tissue decomposition. Once remains are skeletonized, microorganisms endogenous to both the environment and the skeletal remains themselves participate in microbioerosion of bone, increasing the rate of bone diagenesis in water.[1,2] Being able to estimate time since death or, in the case of water-related incidents, the PMSI is pertinent to medicolegal death investigations. Knowing the PMSI assists investigators in identifying remains, validating eye witness statements, and narrowing suspect pools. Because microorganisms are present throughout decomposition, this study proposes the use of longitudinal succession (including aspects of richness, diversity, and indicator taxa) of the microbiome of submerged skeletal remains to estimate PMSI. Advancements in metagenomics approaches (e.g., next-generation sequencing and pipeline analysis software) have demonstrated that bacterial communities can be a useful tool for estimating PMI and PMSI; however, none of these studies have focused on skeletal remains in aquatic environments.[2,3]

In this study, fresh pig (*Sus scrofa)* bones (*N*=100 rib and *N*=100 scapula samples) were purchased from a butcher. Beginning in November 2016 through November 2017, the bones were in cages attached to a flotation device and submerged in Henley's Lake in White Hall, VA (38º 05' 11.7" N, 78º 41' 02.8" W). Water temperature was recorded hourly using waterproof loggers. Every 250 Accumulated Degree Days (ADD), five scapulae, five ribs, and water samples were collected, photographed, and stored at -80ºC until processed. Water samples were filtered; meanwhile, bone samples were cut and ground into a powder using liquid nitrogen in a mortar and pestle. DNA was extracted and purified using ChargeSwitch® gDNA Plant Kit. Following parameters set forth by Kozich et al., extracted samples were used to conduct sequencing-by-synthesis of microbial 16S recombinant DNA (rDNA) variable region 4 using the Illumina® MiSeq® 2X300 paired-end sequencing.[4] The resulting data were analyzed via MiSeq® SOP Mothur version 1.36.1.[5]

Preliminary Analysis of Molecular Variance (AMOVA) encompassing collections 0–1,250 ADD indicated a significant difference in the bacterial structure between rib-scapula, (p <0.0002), rib-water (p <0.0002), and scapula-water (p <0.0002). Therefore, samples were analyzed by sample type. Phylum and family level changes were observed for each sample type across ADD. In addition, rib (R^2=0.48) and scapula (R^2=0.64) samples demonstrated a positive relationship between Shannon species diversity and logADD, whereas the water samples showed a negative relationship (R^2=0.48). The decrease in diversity observed in water samples may be related to changes in temperature between the seasons, with bacteria able to survive well on a bone nutrient substrate, but not in colder water.

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PMSI, Waterlogged Bone, 16S rRNA Gene

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