



H30 Postmortem Submersion Interval (PMSI) Estimation From the Microbiome of Bone in a Freshwater Lake Across 4,750 Accumulated Degree Days (ADD)

Claire M. Cartozzo, MS*, Virginia Commonwealth University, Richmond, VA 23220; Baneshwar Singh, PhD, Virginia Commonwealth University, Richmond, VA 23284; Tal Simmons, PhD, Virginia Commonwealth University, Richmond, VA 23284

Learning Overview: After attending this presentation, attendees will understand how changes in microbial communities across ADD can be used to predict the long-term PMSI for skeletal remains recovered from a freshwater lake.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing proof of concept concerning a novel area of research, the use of microbial succession on skeletal remains submerged in a freshwater lake to predict PMSI.

Aquatic decomposition, the process of remains progressing from submerged fresh remains, to remains floating in a stage of decay, and finally sunken remains, varies due to water temperature, salinity, depth, current, scavengers, three-dimensional movement, and other extrinsic factors. Being able to estimate time since death, or in the case of water-related incidents the postmortem submersion interval (PMSI), is pertinent to Medicolegal Death Investigations, as it assists in identifying remains, corroborating eye witness statements, and narrowing suspect pools. Because microorganisms are present throughout decomposition, researchers proposed that their succession pattern, like insects, could be used to estimate PMI.¹ Recent studies by Dickson et al. and Benbow et al. have demonstrated that bacterial communities in aquatic environments can be a useful tool for estimating PMSI due to advancements in metagenomic approaches (e.g., next-generation sequencing and pipeline analysis software).^{2,3} Unfortunately, these studies were performed with small sample sizes ($n \leq 3$), a short sampling period (21–42 days), repeated sampling and disturbance of the same remains. In addition, the focus of these studies was not on microbial community structure of skeletal remains, which is addressed by this research.

In this study, fresh pig (*Sus scrofa*) bones ($N=100$ rib and $N=100$ scapula samples) were obtained from a butcher. From November 2016 to June 2018, bones were placed in cages attached to a flotation device and submerged in Henley's Lake (White Hall, VA 38° 05' 11.7"N, 78° 41' 02.8" W). Water temperature was recorded hourly using waterproof loggers. Every 250 ADD, five scapulae, five ribs, and 500ml of water were collected and stored at either -80°C or 4°C until processed. Bone samples were cut and ground into a powder using liquid nitrogen in a mortar and pestle; meanwhile, water samples were filtered on 0.22µm filters. DNA was extracted and purified using ChargeSwitch® gDNA Plant Kit and DNeasy PowerClean® Pro Cleanup Kit, if necessary. Following parameters set forth by Kozich et al., extracted samples were used to carry out sequencing-by-synthesis of microbial 16S rDNA variable region 4 using Illumina's® MiSeq® 2X300 paired-end sequencing.⁴ The resulting data were analyzed via MiSeq® SOP mothur version 1.36.1.⁵

According to preliminary data encompassing baseline through collection 5 (0–1,250 ADD), Analysis of Molecular Variance (AMOVA) indicate a significant difference in the bacterial structure between sample types, specifically rib-scapula, ($p < 0.0002$), rib-water ($p < 0.0002$), and scapula-water ($p < 0.0002$). For each sample type, phylum and family level changes were observed 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 ($R^2=0.48$). Similar differences and trends are expected with the remaining 13 collections.

References:

1. Vass, A.A. Beyond the Grave – Understanding Human Decomposition. *Microbiol Today* 2001; 28: 190-192.
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3. Benbow, M.E., Pechal, J.L., Lang, J.M., Erb, R., Wallace, J.R. The Potential of High-throughput Metagenomic Sequencing of Aquatic Bacterial Communities to Estimate the Postmortem Submersion Interval. *Journal of Forensic Science* 2015; 60(1): 1500-1510.
4. Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied Environmental Microbiology* 2013; 79: 5112-5120.
5. Schloss, P.D., Westcott, S.L., Ryabin, T., Justine, R.H., Hartmann, M., Hollister, E.B., et al. Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied Environmental Microbiology* 2009; 75:7537-7541.

Postmortem Submersion Interval, Freshwater Bone, 16S rRNA Gene