E46 Modeling Postmortem Submersion Interval (PMSI) Estimation From the Microbiome of Bone in a Freshwater Lake

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Learning Overview: After attending this presentation, attendees will understand how microbial community changes across Accumulated Degree Days (ADD) can be used to aid medicolegal death investigators and forensic pathologists when skeletal remains are recovered from freshwater lake environments.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing proof of concept for the use of microbial succession on skeletal remains submerged in a freshwater lake to predict the long-term PMSI.

Understanding decomposition and its associated factors is imperative to aid medicolegal death investigators and forensic pathologists in estimating the Postmortem Interval (PMI) on land or the PMSI in water. Because human remains deposited in aquatic environments are exposed to conditions distinct from those of terrestrial environments (i.e., water salinity, temperature, depth, tides, currents, pH, aquatic scavengers, floating or sunken debris, oxygenation, etc.), traditional PMI methods (i.e., invertebrate succession) are not transferable. With advancements in next-generation sequencing technology, improved bioinformatic pipelines, and the identification of changing microbial communities in animal models, recent studies have sought to utilize bacterial succession as a method for estimating PMSI. While initial studies provide a foundation for understanding microbial communities in aquatic decomposition, their applicability to humans is limited due to the fundamental differences between aquatic (e.g., salmon) and terrestrial organisms.[1] In addition, studies performed with porcine remains have not focused on long-term PMSI estimation extending to the skeletal stages.[2,3]

Fresh pig (*Sus scrofa)* bones (*N*=100 rib and *N*=100 scapula samples) were obtained from a butcher. Bones were placed in cages attached to a flotation device and submerged in Henley's Lake, White Hall, VA, from November 2016 to June 2018. Water temperature and environmental parameters (i.e., pH, Dissolved Oxygen [DO], salinity, etc.) were recorded using waterproof loggers and a YSI® Sonde, respectively. Every 250 ADD, five scapulae, five ribs, and 500ml of water were collected and stored at either -80°C or 4°C until processed. Water samples were filtered on 0.22µm filters. Bone samples were cut and ground into a powder using liquid nitrogen in a mortar and pestle. DNA from filters and powder was extracted and purified using ChargeSwitch® gDNA Plant Kit and DNeasy® PowerClean Pro Cleanup Kit, if necessary. Following the protocol established by Kozich et al., sequencing-by-synthesis of microbial 16S recombinant DNA (rDNA) variable region 4 was performed via Illumina's® MiSeq® 2X300 paired-end sequencing.[4] Data analysis and visualization was completed via the MiSeq® mothur SOP, mothur version 1.35.9, and R studio.[5]

Preliminary analyses indicate significant differences in bacterial communities among sample types (i.e., rib-water-scapula). For rib and scapula samples, phylum-level relative abundances differed significantly across ADD. Similarly, beta-diversity (Bray-Curtis) successional patterns were visible in ordinated space for both sample types. Spatial ordination was correlated with environmental parameters and ADD. Additionally, bacterial communities identified on scapula and rib samples differed significantly with ADD. With regard to alpha diversity (shannon), rib and scapula samples demonstrated a curvilinear relationship with ADD; therefore, fifth and fourth polynomial regression models were applied, respectively. Based on these results, bacterial succession patterns were used to develop a long-term PMSI estimation model.

Reference(s):

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PMSI, Freshwater Lake Bone, 16S rRNA Gene

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