H14 Bone Microbial Community Succession During Multi-Year Decomposition in an Aquatic Habitat

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Learning Overview: After attending this presentation, attendees will better understand how microbial communities colonize bones and shift during long-term decomposition. Attendees will see a microbial community change over the Postmortem Submersion Interval (PMSI). Attendees will recognize that machine learning methods have potential applications to PMSI estimation for aquatic cases with long-term decomposition.

Impact on the Forensic Science Community: This presentation will impact the forensic scientific community by presenting random forest modeling of the postmortem microbiome as a viable PMSI estimation tool for cases with submerged bone evidence.

Microbes are ubiquitous organisms, and the composition of microbial communities are specific to the environments where they reside (e.g., human gut vs. pond water). With advancements in high-throughput sequencing, it is becoming more cost-effective and practical to describe microbial communities with targeted amplicon sequencing (i.e., 16S ribosomal RNA (rRNA) gene amplicon). Characterizing the microbial communities has wide-ranging forensic applications, including answering questions that arise during forensic death investigation such as: when did the decedent die? Identifying the time since death for aquatic cases, known as the PMSI, can be challenging in unattended deaths or cases with concealed bodies. However, the accessibility of high-throughput sequencing technologies allows for microbial evidence to be used in death investigations.

During aquatic decomposition, microbes that colonize carrion or human bodies come from a variety of sources, including the carrion itself, the environment, and insects/scavengers. Bones are subjected to microbial alteration, known as bioerosion, of the bone during decomposition. These bones are a potentially important source of evidence for forensic anthropologists and forensic biologists who routinely extract DNA from bone for decedent identification. Specifically, bone bioerosion by microbes could potentially be used in conjunction with other evidence in cases with longer PMSI estimations (months to years), as PMSI estimation models of microbial community succession on submerged bones have accurately predicted submersion periods.

While microbial models for PMSI estimation are not yet accepted in court, research suggests these models have promise. But, before microbes can be used as reliable evidence, additional studies are needed as knowledge gaps for long-term decomposition in a variety of habitats, such as aquatic ecosystems, exist. Most studies focus on earlier decomposition periods (prior to skeletonization) in terrestrial habitats or sunken remains for short-term (less than one year) decomposition. This study is the first to identify long-term microbial community succession both inside and outside of bones following natural decomposition of carcasses in an aquatic environment to develop PMSI estimation models.

It was hypothesized that the microbial community inside and outside would converge by 18 months of submersion, and that there would be a predictable succession of microbial communities, based on previous success with aquatic decomposition in short-term PMSIs.

To address these hypotheses, replicate human surrogates were submerged (swine [*Sus scrofa domesticus*] carcasses [$N = 5$]) in a natural freshwater aquatic habitat (i.e., pond) to model the PMSI based on the microbial communities that alter the bones' long-term decomposition. Sunken bones were sampled every three months over two calendar years and used targeted gene amplicon high-throughput sequencing (16S rRNA gene amplicon) to describe the microbial communities on (external) and within (internal) the bones. Microbial diversity metrics (alpha- and beta-diversity) varied with microbial community type (internal vs. external), season (spring, summer, winter, fall), and over time (months). Indicator taxa were identified by ANCOM for season, microbial community type, and over time (i.e., Alphaproteobacteria and Bacteroidia). Over time, the "core" microbiome of internal and external communities shifted, but ultimately did not converge to a similar community structure. Random forest models estimating PMSI were accurate (> variation explained > 80%; $R^2 > 0.95$) over this long-term decomposition study.

Overall, the PMSI estimation using microbial communities within and on aquatic bones was a viable tool for application to forensic death investigation. Microbial communities varied over time. Both external and internal microbial communities shifted over time but did not converge to a similar microbial community. Microbial communities could have forensic utility by providing important evidence for unattended death cases, as establishing time since death is essential information for building a case. Moving forward, more studies are needed to expand current knowledge on bone bioerosion as forensic evidence, including additional aquatic environments, longer time points (> 2 years), and with human bones.

Postmortem Submersion Interval, Microbial Succession, Bone Bioerosion