H159 Estimating the Postmortem Submersion Interval (PMSI) From the Microbiome of Bone in Lacustrine and Riverine Environments in Virginia

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Learning Overview: After attending this presentation, attendees will understand how different aquatic environments (i.e., freshwater lake and freshwater river) impact microbial community changes across Accumulated Degree Days (ADD).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing insight into the potential use of universal microbial succession on submerged skeletal remains to predict long-term PMSI.

Water-related deaths, criminal activity, and mass disasters occurring over or in water (e.g., boating accidents, ferry disasters, commercial plane crashes, and natural disasters) result in thousands of deaths every year. In Virginia alone, the Office of the Chief Medical Examiner reported that death by drowning (asphyxia) accounted for 91 accidental deaths in 2017.[1] Bodies recovered from aquatic environments are exposed to diverse and changing environmental conditions (i.e., scavengers, microorganisms, temperature, pH, current, etc.). Furthermore, microorganisms, such as bacteria, algae, and fungi, participate in the decomposition of human remains in an aquatic environment. With recent advancements in technology, studies have identified microorganisms as potential estimators of time since submersion (PMSI).[2,3] Unfortunately, a reliable and universal method for determining long- and short-term PMSI has yet to be developed.

To examine the impact of aquatic environments on microbial succession, a freshwater river (James River, Charles City, VA) and lake (Henleys Lake, White Hall, VA) were selected as field sites. Work at Henleys Lake and the James River occurred between November 2016–June 2018 and November 2017–November 2018, respectively. For both sites, fresh pig (*Sus scrofa*) bones (scapulae and ribs) were submerged in cages that were attached to a flotation device or wharf. Waterproof temperature loggers and a YSI Sonde were used to record environmental parameters. Every 250 ADD, five scapulae, five ribs and 500ml of water were collected and stored at either -80°C or 4°C. Sample processing included filtration on 0.22μm filters for water samples and pulverization via liquid nitrogen in a mortar and pestle for bone samples. Both filters and powder underwent DNA extraction and purification using ChargeSwitch® gDNA Plant Kit and DNeasy® PowerClean Pro Cleanup Kit, if necessary. Library preparation and sequencing-by-synthesis of microbial 16S recombinant DNA (rDNA) variable region 4 followed the established Kozich et al. and was performed via Illumina's® MiSeq® 2X300 paired-end sequencing.[4] Data analysis and visualization was completed using the MiSeq® mothur SOP, mothur version 1.35.9, and R studio.[5]

Preliminary analysis suggests that there are significant differences between locations and among sample types at each location. In regard to relative abundance, changes were observed in rib and scapula samples; furthermore, scapula and rib samples at both locations shared Firmicutes and Proteobacteria as the two dominant phyla across time; however, the remaining taxa varied. Both locations also demonstrated significant differences in ordinated space (Bray-Curtis Operational Taxonomic Units [OUT] beta-diversity) for each sample type across ADD. Additionally, curvilinear relationships between shannon diversity and ADD were observed for scapula and ribs at both locations. The aforementioned results suggest that there may be enough similarities between locations to create a long-term PMSI model for Virginia.

Reference(s):

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PMSI, Aquatic Environments, Bone Microbiome

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