A132 Postmortem Submersion Interval (PMSI) Estimation From the Microbiome of Bone in a Freshwater River

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

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 river to predict PMSI.

For victims who die in and/or are recovered from water, PMSI estimation has been limited to instances when invertebrates have been found on remains or when the victim was last seen alive, making it applicable in cases of known accidental water deaths only, rather than deposition of homicide and unidentified/unknown victims in water. Since the 2014 National Institute of Justice (NIJ) Forensic Science Technology Working Group highlighted the use of metagenomics to aid investigative leads and the advancement in technologies (i.e., next generation sequencing and bioinformatic pipelines), researchers have explored the use of microorganisms to predict time since death on land and in water, respectively Postmortem Interval (PMI) and PMSI.[1-3] These studies have demonstrated that microorganism colonization of remains occurs in a successional manner, taxa increase over the range of decomposition, and both can be used to predict PMSI. Unfortunately, these studies were conducted over short periods of time, with small sample sizes and repeated disturbance, which are knowledge gaps addressed in this study.

From November 2017 to November 2018, fresh pig (*Sus scrofa)* bones (*N*=125 rib and *N*=125 scapula) were submerged in cages that were attached by a rope to the wharf at the Virginia Commonwealth University (VCU) Rice Rivers Center, located on the James River in Charles City, VA. Water quality parameters were continuously and remotely logged using a permanently deployed sonde. Every 250 ADD, calculated using a 0^{o}C threshold, five ribs, five scapulae, and 500mL of water were collected, photographed, and stored at -80^{o}C or 4^{o}C until processed. Water samples were filtered using a cellulose membrane filtration system; bone samples were pulverized using liquid nitrogen in a mortar and pestle. Both filters and bone powder underwent DNA extraction via ChargeSwitch® gDNA Plant Kit. If necessary, samples were purified with the Dneasy® PowerClean Pro Cleanup Kit. Library preparation followed the Kozich et al. protocol.[4] Specifically, 16S recombinant DNA (rDNA) variable region 4 sequencing-by-synthesis was performed on the Illumina® MiSeq® FGx2X. The resulting data was analyzed and visualized using the MiSeq® mothur SOP, mothur version 1.35.9, and R studio.[5]

Preliminary analyses indicated significant differences in bacterial communities among sample types (i.e., rib-scapula-water-mud). For bone types, differences across Accumulated Degree Days (ADD) were present at the phylum-level. Operational Taxonomic Units (OUT) -based beta-diversity (Bray-Curtis) suggested significant differences in ordinated space between ADD for both scapula and rib samples. Meanwhile, alpha-diversity (Shannon) indices demonstrated a curvilinear relationship with ADD. Overall, these results suggest that patterns in bacterial succession can be used to develop a PMSI estimation model.

Reference(s):

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PMSI, Freshwater River Bone, 16S rRNA Gene

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