A97 Prediction of Minimum Postmortem Submersion Interval (PMSImin) Based on Eukaryotic Community Succession on Skeletal Remains Recovered From an Aquatic Environment

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Learning Overview: After attending this presentation, attendees will have acquired information on a new method designed to aid in the determination of PMSImin, specifically from waterlogged bones.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees how the results obtained from this research may be applied to determine time of death for remains found in cold cases, which may corroborate eyewitness testimony, as well as increase accuracy in the estimation of PMSI in combination with bacterial succession.

Many recent studies were conducted concerning bacterial succession in decomposing animal carrion in terrestrial systems.[1,2] However, not much is known about the microorganisms involved in aquatic decomposition.[3] Dickson et al. presented a study on marine bacterial succession to determine PMSI using partial pig remains; however, this study did not utilize next-generation sequencing technologies.[3] Per research, there are currently no published studies which estimate PMSI based on eukaryotic community succession in aquatic systems. The main goal of this study was to determine the eukaryotic community succession associated with porcine skeletal remains in lentic aquatic environment and to derive a statistical model for PMSImin prediction.

Henleys Lake in Crozet, VA, was the location chosen for this research. Fresh pig bones (rib N=100, scapula N=100) were placed in cages (10x10 inch^2), attached to a floatation device, and submerged using waterproof loggers (to record hourly temperature), and a Yellow Springs, OH Sonde™ (to monitor pH, specific conductivity, dissolved oxygen, salinity, and depth). Every 250 Accumulated Degree Days (ADD), one cage containing five rib and five scapula samples was collected, photographed, and stored at -80°C until processed. Water samples were also collected every 250 ADD and filtered using a cellulose membrane filtration system. DNA extraction was performed using the Invitrogen® ChargeSwitch® gDNA Plant Kit Protocol. Variable region nine (V9) of the 18S rRNA gene was amplified and sequenced using the primers as described by Earth Microbiome Project, including a mammalian blocking primer, and was based on dual-index strategy as described in Kozich et al on MiSeq® FGX sequencing platform.[4] Sequenced data were quality controlled and analyzed via the MiSeq® SOP in Mothur version 1.36.[4] Hierarchical classification of good quality sequences was performed based on SILVA119 database. A phylogenetic approach was utilized for α-and β-diversity estimation. Analysis of Molecular Variance (AMOVA) was used to test statistical differences in eukaryotic colonization of bone types over ADD.

Preliminary results at the Phylum level (level 2) showed that eukaryotic community associated with rib and scapula samples were similar, characterized by the combined presence of Ciliophora, Ochrophyta, and Peronosporomycetes. Scapula samples showed a difference due to the added presence of Euglenozoa (2.1%). The eukaryotic community changed significantly with ADD in both bone types ($p<0.0002$). Samples collected at 250 ADD were characterized by the presence of Peronosporomycetes (74.3%) and Dinoflagellata (1.9%, found highest at this time point). The 500 ADD collection was characterized by the presence of Peronosporomycetes (90.4%). Peronosporomycetes abundance declined from 750 ADD through 3,250 ADD, after which an increase was observed (e.g., 51.3% at 4,250 ADD). Collections spanning the 1,000 ADD to 3,250 ADD interval were characterized by Ciliophora (>20%), and the final collection (4,750 ADD) was categorized by the combined presence of Ciliophora (14.7%), Euglenozoa (3.9%), and Peronosporomycetes (6.8%).

In conclusion, this study highlights the eukaryotic community associated with bones in lentic systems and, in the future, this information may be utilized for the development of statistical models for prediction of PMSImin either alone or in combination with bacterial community succession.

Reference(s):

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PMSI, 18S rDNA Sequencing, ADD

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