H72 Aquatic Decomposition of Vertebrate Remains: An Experimental Test for a Cold Case Investigation

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Learning Overview: The goal of this presentation is to provide attendees with the ability to better understand how conditions in aquatic environments impact the decomposition of vertebrate remains and how such information can be informative to death investigations.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing information on how insects and microbes colonize and change on remains in aquatic environments and how decomposition progresses under environmental conditions in a way that informed a cold case death investigation.

Decomposition of remains in aquatic environments remains an understudied area of forensics, especially under conditions with direct relevance to death investigations. The objectives of this study were to determine how terrestrial and aquatic invertebrates and bacteria colonize and change on vertebrate remains over the course of decomposition and compare those findings to a cold case in a similar habitat that occurred in 2005 (referred to as the Aquatic Case [AC]).

At a similar time of year to the AC (July-August), swine carcasses (*N*=5, approx. 45kg) were euthanized and transported to the study location before being dressed in clothing to resemble the apparel found on the AC. The carcasses were introduced to the pond and evaluated for invertebrate activity at three-day intervals. In addition to evaluating the carcass for invertebrate activity and visual changes, cotton swabs were used to collect DNA samples from several different regions on the carcasses. DNA present on these swabs was extracted using a commercially available kit and quantified fluorometrically. Targeted amplicon sequencing was performed to amplify the 16S ribosomal RNA (rRNA) region (V4) using an Illuminia® MiSeq® platform (2 x 250 base pair, paired ends). Resulting sequences were filtered and taxonomically assigned using QIIME™ 2 and the SILVA database before open source statistical tools, including machine learning algorithms, were used to identify bacterial indicators of decomposition stage.

During the experiment, there was not a significant difference in air temperatures at the experimental pond compared to those recorded during the 21 days when the decedent of the AC was missing in 2005. Within three days of carcass placement, there was considerable bloating and large numbers of larval and adult blow flies (Diptera: Calliphoridae) present on all experimental carcasses as well as multiple taxa of aquatic invertebrates. When comparing the experimental results to the lack of any insect evidence reported in the AC autopsy and death investigation reports, the findings suggested that the AC body had a low probability of being at or near the surface for several days; these findings matched autopsy photos and medical examiner notes indicating only moderate decomposition. While the experimental results suggested extensive colonization of a carcass at the surface by both terrestrial and aquatic invertebrates would occur quickly, the potential remains that the AC body could have sunk and remained neutrally buoyant for 20 days at a depth where decomposition was slowed. Significant changes in the bacterial communities were found across decomposition with distinct bacterial communities present at different decomposition stages. Several diversity metrics including Faith's Phylogenetic Diversity (PD) and observed richness significantly differed among decomposition stage, with freshly submerged carcasses showing the highest bacterial diversity. Several bacterial genera, predominately from *Proteobacteria* and *Firmicutes*, were identified by as indicator taxa for decomposition stage by machine learning models. Future aquatic death investigations could take advantage of taking bacterial swabs that could be used as a "microbial clock" of decomposition in the aquatic habitat using the methods tested in this experiment.

In conclusion, this research highlights that the decomposition of vertebrate remains in aquatic environments is a complex process mediated by both biotic and abiotic factors and that both microbes and invertebrates change over the course of decomposition and have the potential to be used as forensic indicators.

Aquatic Decomposition, Microbiome, Cold Case