H93 Forensic Implications of Multi-Species Blow Fly Larval Masses and Their Associated Microbiomes

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After attending this presentation, attendees will be more familiar with previously unexplored aspects of postmortem larval insect communities, which include complex microbial communities, and how both insect and microbial evidence can offer insight into time-since-death estimates. Analysis of blow fly (Diptera: Calliphoridae) larvae and how they form masses throughout decomposition, as well as their associated microbiomes, will be presented to document interactions between necrophagous insects and microbial communities, thus providing new data that can potentially be used together for more accurate Postmortem Interval (PMI) estimates. Microbes that colonize cadavers are still largely unstudied and it is unknown how they interact with insects that forensic entomologists commonly use to estimate a minimum PMI (minPMI). A more thorough collection of entomological evidence and a greater understanding of how these communities interact could help investigators calculate a narrower PMI range.

This presentation will impact the forensic science community by offering new information on the community composition of postmortem blow fly colonizers and related microbes, which could be used by investigators to devise better methods for collecting evidence and improve accuracy of estimating the minPMI. Current common forensic entomology practice is to concentrate collections on the largest specimens: most often, only a few larvae are collected from just a single location on the body or within a larval mass. While this method seeks to include the most developed (presumed oldest) larvae in order to estimate the minPMI, it could be excluding other valuable information. By only collecting the largest larvae from a single location, investigators could be missing other species that may be in a different area of the body or smaller in size but of the same developmental stage, if not older, than the largest and most developed specimens. Knowledge of the variation in blow fly larval communities is important for forensic investigations, but it is also necessary to understand the reasons behind this variation. Microbial communities could be responsible for the fluctuation in presence, abundance, and size of blow fly larvae on cadavers.

To better understand insect larval community dynamics throughout decomposition, a survey study was conducted using six swine carcasses (*Sus scrofa* L.) in an open field in Indiana. Samples from the following areas were collected from each carcass: individual larvae from larval masses and their associated internal microbiota; the microbiome of the larval mass; and epinecrotic microbial communities of the skin. Samples were aseptically collected using sterile cotton-tipped swabs every 12 hours for eight days, which was the amount of time needed for the carcasses to fully decompose and larvae to migrate from the resource for pupation. Third instar blow fly larvae from multiple masses and within the same masses were identified as *Phormia regina*, *Lucilia coeruleiviridis*, and *Cochliomyia macellaria* with *P. regina* being the most abundant taxon collected and used for internal microbiome

analysis. Using 16S rRNA V4 gene region amplicon sequencing, this study characterized the relative abundance and taxon richness of each microbial community over decomposition time. Relative abundance of blow fly species within the larval mass shifted as decomposition progressed and the relative abundance of microbial taxa followed trends over time as well. These findings suggest significant interactions between the environment, microbes, and blow fly larvae. Further, in support of recent studies showing that microbes associated with cadaver decomposition affect blow fly behavior, the temporally changing microbial communities may be driving the presence of certain blow fly species during the decomposition process.

There have been relatively few studies that have addressed the microbial ecology of postmortem blow fly larval masses, but understanding these interactions is becoming recognized as potentially important for forensic death investigation. Forensic entomology relies on incorporating numerous complex variables in order to draw a conclusion about time since death. The more that is understood about the ecology of postmortem consumers, the more precisely investigators can estimate the minPMI.

Decomposition, Microbiome, Postmortem Interval