H98 Profiling of Marine Microbial Communities Associated With Decomposing Remains Can Indicate Postmortem Submersion Interval

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After attending this presentation, attendees, will have a better understanding of the role that extrinsic microbial communities play in the rate and pattern of decomposition of remains in New Zealand coastal marine environments and how such information may enable forensic investigators to more accurately determine the postmortem submersion interval (PMSI).

This presentation will impact the forensic science community by extending the currently limited knowledge of aquatic decomposition of bodies and/or body parts mediated by marine microorganisms while introducing a novel framework through which PMSI may be determined by analyzing the marine microbial community present on submerged remains at the time of body recovery.

Microbial communities play a central ecological role in the recycling of nutrients and organic matter in aquatic ecosystems. Despite the clear importance of bacteria in the decomposition process, a detailed understanding of the postmortem microbiology of decomposing remains in aquatic environments is currently lacking. A previous study in a coastal marine environment showed that marine microbes display successional colonization patterns which can be linked to particular submersion intervals, but highlighted the need to develop a more high- throughput methodology, if this concept is to be utilized in a forensic setting.

One such methodology is terminal restriction fragment length polymorphism (TRFLP) analysis. TRFLP is a molecular fingerprinting technique that examines the 16S rRNA genes of virtually all bacteria in a microbial community, producing a community profile or "fingerprint" which consists of different length fragments for different bacterial phyloypes. TRFLP has the advantage of being a fast, reliable and relatively quick and inexpensive technique that produces digital output and could potentially be used for forensic analysis.

This study aimed to identify, using TRFLP analysis, successional changes in microbial community composition on submerged pig remains as they decomposed in the sea, and to assess whether unique components of the community existed for particular stages of decomposition or periods of submersion.

Adult domestic pig (*S. scrofa* L.) carcasses, used as models for partial human remains, were placed in cages encompassed by mesh, so as to deny larger scavengers access and achieve the longest postmortem submersion interval possible for the collection of colonizing bacteria. Cages were submerged in the Otago Harbour in water 5-7 m deep in January (summer) 2009 and July (winter) 2010, and in Wellington Harbour in water 8-10 m deep in February (summer) 2009. The study was performed in two geographic coastal locations, and in one of these locations, during two seasons, in order to explore the general applicability this technique would have across time and space. Bacterial samples were taken by swabbing the skin of the carcasses before entry into the water, after 1, 2 and 3 days, then at 2-4 day intervals until skeletonization. DNA from the bacterial community present on the carcass at each time point was extracted from the swabs and subjected to TRFLP analysis. On sampling days, observations of gross

decomposition changes and the presence of any small marine scavengers in or on the cage were also noted. During the course of the experiments, environmental data such as seawater temperature and pH were also monitored.

Clustering analysis of TRFLP microbial community profiles from early, mid and late periods of submersion formed distinct clusters and could be distinguished based on the presence of certain bacterial phylotypes. There appeared to be very little difference in the pattern of community change over time between carcasses deployed in the two different geographic locations, suggesting this concept has broad spatial applicability. Many phylotypes present on the skin before submersion disappeared within the first few days, indicating significant and rapid disruption of the original skin microbiome following submersion of the remains in salt water. Extrinsic marine microbes colonized the remains immediately. Dynamic shifts in the structure of the microbial community present on the remains were seen during the early submersion period (Fresh to Early Putrefaction stages), with a number of short-lived, time-specific phylotypes observed. The mid-submersion period (Advanced Putrefaction) was characterized by much more gradual shifts in community composition, with colonization by unique phylotypes not observed before particular submersion intervals, but which persisted on the remains for longer periods of time. As well as the continued presence of mid-phase colonizers, the late submersion period (Advanced Decay and Skeletonized Remains) saw the arrival and

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subsequent disappearance of many short-lived, time-specific phylotypes; thus the microbial community present on the decomposing remains once again underwent noticeable and rapid compositional changes. Preliminary results from a comparison of decomposition events during summer and winter in Otago Harbour found substantial differences in bacterial phylotypes within TRFLP profiles, suggesting there is a strong seasonal aspect to colonization. However the overall pattern of compositional change over time was similar.

The use of TRFLP as a microbial community profiling tool has enabled the first characterization, at a community level, of the postmortem microbiology of submerged mammalian remains over the course of an aquatic decomposition event. This reproducible, high- throughput technique is cost-effective and could be easily implemented in the modern forensic laboratory. Microbial community presence, abundance and successional dynamics, coupled with temperature data, have the potential to provide detailed information regarding length of submersion time of immersed bodies and/or body parts recovered from coastal marine waters of New Zealand and beyond in cases where a specific PMSI is in doubt.

Decomposition, Postmortem Submersion Interval, Microbial Communities

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