THE MICROBIOME OF THE EASTERN OYSTER, *Crassostrea virginica*, IN HEALTH AND DISEASE

by

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ABSTRACT

The eastern oyster, *Crassostrea virginica*, is a keystone species in estuarine environments along the east coast of North America. Oysters are ecosystem engineers that provide habitat for native species and increase biodiversity, improve water quality, influence biogeochemical cycling, and structure trophic levels. To the detriment of estuarine environments, oyster populations today are a fraction of their historic levels due to anthropogenic and disease pressures, and climate change and pathogens pose serious threats to future populations. Next-generation, high-throughput sequencing technologies have led to an appreciation for the diversity of host-associated microbial communities (microbiomes) and their role in the health and physiology of their host; yet, little is currently known about the diversity of the oyster microbiome, the factors that shape the microbiome community, or the role of the microbiome in oyster health and fitness. In the work described herein, bacterial and viral populations within *C. virginica* extrapallial fluid (EF) – fluid produced by the oyster that initiates shell formation – were investigated using high-throughput, cultivation-independent approaches. The EF microbiome was characterized over time and between locations to identify temporal and geographic differences in community composition. In addition, the bacterial communities of healthy and diseased individuals were compared to identify changes in the normal microbiota in response to infection by *Perkinsus marinus*, a major oyster pathogen and the etiological agent of oyster disease Dermo.

The EF bacterial community was distinct from the surrounding water and seasonally dynamic. Water and EF bacterial communities were both influenced by temperature, but the same taxa in water and EF often responded differently to changing environmental conditions. The EF bacterial community was also influenced by geographic location, and oysters from the same sample site had more similar EF communities to each other than to oysters from other sites. In contrast, the EF community was largely unchanged by infection with *P. marinus*, though several individual taxa were associated with health status.

A number of bacterial taxa were enriched in EF, notably Deltaproteobacteria, which are capable of catalyzing calcium precipitation and are found in caves and lithifying microbial mats where calcification is common. Given that the EF is the site of shell formation, which occurs via calcium precipitation, it is possible that the EF microbiome plays an unappreciated role in oyster shell formation. The abundance of Deltaproteobacteria in oyster EF decreased with higher water temperatures and may be an important consideration as average water temperatures increase in the future.

Lytic phage populations were investigated in EF via ribonucleotide reductase (RNR), a diverse, abundant, and ecologically important marker gene of viral diversity in aquatic environments. The RNR assemblage as a whole was seasonally dynamic, although many of the most abundant populations were persistent over one year. The most abundant populations were also endemic to the Delmarva region. These endemic populations belonged to the dsDNA tailed bacteriophage family *Podoviridae* and likely infected members of the Proteobacteria, the most abundant bacterial phylum in oyster EF. Thus, these abundant and persistent lytic phage populations may play an important role in the top-down regulation of EF bacterial communities. Ultimately, a

better understanding of the EF microbial community, its role in oyster health, and how that role might be impacted by various threats will enable more targeted and effective conservation efforts in the future.