

INVESTIGATIONS INTO THE ECOLOGY, MORPHOLOGY, AND GENETIC
DIVERSITY OF VIRUSES IN SALINE ENVIRONMENTS

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Abstract

Viruses are the most numerous biological entities in aquatic environments and have been shown to have a significant influence on the ecology and evolution of aquatic microorganisms. They serve variously as agents of microbial mortality, phenotypic conversion, and horizontal gene transfer. For the last two decades, the field of aquatic virology has been propelled by investigations of viruses in diverse ecosystems coupled with advances in methodologies used to investigate the ecology and diversity of viruses in aquatic environments. This dissertation includes the study of viral ecology and morphological diversity in a relatively unusual environment and explores a new methodological approach to the study of aquatic viral genomic diversity.

The seasonal study of the ecology of viruses and viral infections in the moderately hypersaline, seasonally stratified, Mono Lake, which contains the highest concentration of viruses and prokaryotes of any previously studied natural aquatic environment, revealed that dynamics in the concentrations of viruses and prokaryotes, as well as the frequency of visibly infected cells (FVIC), were primarily driven by the pronounced seasonality of chemical and physical forcing in the lake. Extraordinarily high concentrations of viruses and prokaryotes in Mono Lake, which were estimated to result in exceptionally high contact rates, did not, however, result in high FVIC. This paradox may be a result of high diversity of viruses and prokaryotes or large-scale resistance of prokaryotes to co-occurring viruses in the lake.

Investigation of the morphological diversity of viruses in the stratified layers of Mono Lake revealed that the viral assemblage in the lake differed with respect to the

distribution of viral capsid diameters and the proportion of phage families, compared to other aquatic ecosystems. While the concentrations of viruses and prokaryotes were not found to differ significantly in the oxic and anoxic layers of the lake over a seasonal cycle, viral morphological parameters were found to differ in the stratified layers of this lake, most likely as a result of differing microbial community structures. Further investigation of viral ecology and diversity through isolation of virus-host systems and metagenomic analyses of the viral assemblage will be useful for further interpretation of the ecological and biogeochemical consequences of viral infections in Mono Lake.

The extraordinarily high diversity of viruses in aquatic systems has prevented the reassembly of viral genomes from metagenomic analyses. One potential solution to this problem is to break it into a set of smaller problems by separating small subsets of viruses from the larger assemblage. In this dissertation, cesium chloride (CsCl) equilibrium buoyant density gradients, Sephacryl gel-filtration chromatography, and strong anion-exchange chromatography were found to be effective for fractionating viruses in natural aquatic assemblages based on their differing buoyant densities, sizes, and surface charges, respectively.

Two of the most effective fractionation procedures (buoyant density gradients and strong anion-exchange chromatography) were employed in series to fractionate a viral assemblage collected from Kāneʻohe Bay, HI. Based on genome size analysis, the resulting fractions appeared to be highly enriched in a small number of virus types and one fraction was selected for metagenomic analysis. Pulsed field gel electrophoresis showed that the fraction contained only three detectable genome sizes and electron microscopy showed only four distinguishable morphologies. A shotgun clone library was

constructed from this fraction and 1923 sequences were obtained. The proportion of the sequences that could be assembled and the size spectrum of the resulting contigs were much greater than has been reported for any previous metagenomic analysis of an unfractionated natural DNA viral assemblage. Comparisons among assembled contigs using mosaic graphs, a new analytical tool for bioinformatics, indicated that the sample contained related viruses that were genomic mosaics of one another. This approach of fractionating natural viral assemblages dramatically improved the genomic assembly process and is anticipated to bring the field closer to the goals of investigating uncultivated aquatic viruses and connecting the genomes of viruses with their ecology.