SUMMARY PROPOSAL FORM

PROJECT TITLE: DEVELOPMENT OF DIGITAL RT-PCR METHODS TO QUANTIFY HUMAN-ASSOCIATED BACTERIOPHAGE IN STORM WATER AND COASTAL RECREATIONAL WATERS

OBJECTIVE:

Our overall goals are to adapt and further develop a rapid, sensitive water quality monitoring and source-tracking tool that can be used to track the movement of viruses in coastal recreational waters and can be used to track human-associated contamination. Specifically we aim to 1) to develop a sensitive, robust droplet digital RT-PCR assay to measure and distinguish human-associated and non-human-associated F+RNA coliphage genogroups; and 2) apply this assay as microbial source tracking tool in coastal recreational waters and storm waters.

METHODOLOGY:

We will develop and test sensitive (potentially detecting a single gene copy) and robust (resistant to PCR inhibition) droplet digital RT-QPCR methods adapted from recently developed multiplex Reverse Transcriptase-QPCR assays applied to wastewater and environmental samples. These assays can distinguish multiple F+RNA coliphage genotypes at once by targeting shared coat protein and RNA replicase genes. Using this assay, we will quantify F+RNA coliphage genotypes collected from storm water, estuaries, and marine waters in the coastal zone in Southern California. Specifically we will target beaches likely to suffer from aging, leaky infrastructure and collect and filter 1-5L of seawater to capture the viruses by adsorption onto electronegative mixed cellulose ester filters. We also will draw on a sample archive previously collected by SCCWRP and the Martiny lab at UC Irvine consisting of large (20L) and small (0.5-1L) volume storm water samples from San Diego and Malibu, and near-shore beach samples from Ocean Beach and Tourmaline Surfing Park (San Diego), Doheny State Beach, Newport Beach (Orange County), Avalon, and Malibu (Los Angeles County).

RATIONALE:

Levels of fecal indicator bacteria (FIB) are used to monitor the recreational water quality to protect swimmers from exposure to pathogens found in fecal material, but are an imperfect indicator. The main limitation is that concentrations of FIB have been shown to be poorly correlated with the presence of human enteric viruses (e.g. Human Norovirus, Enterovirus or Adenovirus) that are responsible for the majority of gastrointestinal illnesses in swimmers. Other limitations include discerning the source of FIB (human or non-human), the dilution and degradation of FIB in the environment, and the physical removal of bacteria as they are transported through groundwater. Viruses and bacteriophage (i.e. viruses that infect bacteria) are not filtered out by sand or soil at the same rate as much larger bacteria. Further, bacteriophage are more abundant than human viruses (since their bacterial hosts are much more abundant) which makes them more attractive water quality indicators at beaches where the source of contamination is leaking infrastructure, rather than acute inputs, such as storm water pulses or sewage spills. F+RNA coliphage (i.e.
viruses infecting E. coli), bacteriophage infecting human-associated Bacteroides bacteria (e.g. Bacteroides GB-124 phage), and a bacteriophage discovered from human gut microbiome metagenomes (crAssphage) have been proposed as potential fecal indicators.

Traditional cultivation techniques are slow, taking up to 18-24 hours to quantify the number of phage, followed by molecular analysis to identify phage genotypes. Adding to these difficulties, bacteriophage abundance is variable and can go undetected by culture methods, requiring non-quantitative enrichment cultivation in order to enhance detection. Molecular quantification of bacteriophage directly from environmental waters avoids the lengthy cultivation process and, in the case of F+RNA coliphage, measures genotypes associated with human (genotypes II and III) and non-human (genotypes I and IV) fecal sources.

DATA SHARING PLAN:

All data generated by the project will cleaned, formatted, and be made publicly available through the California Environmental Data Exchange Network, or on SCCWRP’s website. In addition, the publications will be open-access and data and protocols generated by this project will be housed on a publicly accessible website either in an open sharing site such as GitHub.