USING MOLECULAR METHODS FOR UNDERSTANDING IMPACTS OF NON-POINT SOURCE RUNOFF ON ECONOMICALLY IMPORTANT COASTAL RECREATIONAL AND SHELLFISH HARVESTING WATERS

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Microbiological water quality of beaches and coastal zones is typically determined by measuring indicator bacteria, such as total and fecal coliforms, and enterococci, to infer the presence of microbial pathogens. Bacterial indicators are used to infer the presence of human pathogens associated with fecal contamination, based upon the assumption that the bacteria are present in waters with fecal contamination, and not in those without. However, the use of bacterial indicators is limited in that they sometimes fail to successfully predict the presence of pathogens (bacterial, viral, and protozoan). Several studies have revealed that several dangerous types of viruses can be contracted by swimming in contaminated ocean waters, and that outbreaks of gastroenteritis have been caused by swimming in water with acceptable coliform counts. Also, bacterial indicators are not always a useful predictor of the presence of human fecal contamination, as they can come from a variety of sources. A relatively new molecular technique, Quantitative PCR (Q-PCR), is a novel primer-based molecular technique that combines the specificity of "traditional" PCR with the quantitative measurement of fluorescence for determination of presence of specific types of nucleic acid in environmental samples. We perform filtration, concentration, and RNA/DNA extraction steps paired with Q-PCR, employing TaqMan™ assays, to detect enteroviruses, adenoviruses, and specific types of enterococci (fecal streptococci) species. In addition, we have also designed primers for the detection of both canine parvovirus and bovine enterovirus as indicators of different types of animal fecal contamination. Our results are being analyzed as part of a large study that includes flow-weighted measurements of runoff, bacterial indicators, and the use of other microbial source tracking approaches to define sources of fecal contamination stemming from non-point source runoff from 3 different land-use types: silviculture, residential, and agriculture. We aim to use this information in conjunction with other available water quality data to build a hydrological model in a small coastal watershed in Eastern North Carolina, USA. These molecular approaches are also being used in conjunction with Total Maximum Daily Load (TMDL) implementation, and Bayesian modelling approaches to mitigate causes of poor recreational water quality in coastal and estuarine environments of both the Atlantic and Pacific coasts of the United States. Results of virus/bacterial detection using Q-PCR approaches will be presented, and related to other currently used water quality parameters. In addition, we will discuss the use of new molecular techniques for model development, and relevance of model development to mitigation of runoff and public health risk.