

Research Category III: Water Quality

Development of a Quantitative Detection Method for Enumerating Host-Specific Fecal Bacteria Based on Real-Time, Quantitative Polymerase Chain Reaction

Principal Investigator:

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Executive Summary:

Fecal pollution continues to be among the leading contaminants of our nation's waters. In California, the closure of popular recreational water bodies as well as shellfish harvesting areas due to concentrations of indicator organism that exceed regulatory limits has brought the issue into the public eye. Unfortunately, current detection methods and monitoring approaches are inadequate for developing successful and cost-effective strategies for reducing fecal pollution. One of the main limitations of the current culture-based detection methods is that they do not distinguish between bacteria that originate from different hosts, such as a human, cow or bird. Because any particular water (e.g., lake, river, estuary, or beach) is likely to receive pollution from multiple point and non-point sources, the inability to distinguish host-specific indicators significantly limits the ability to identify and target the main sources, placing a major constraint on the management of fecal pollution.

Currently, a variety of new approaches that exploit host-specific fecal bacteria to conduct microbial source tracking (MST) studies are being explored. While many of the methods may eventually provide powerful tools for identifying the wide range of human and animal point and non-point sources that contribute fecal pollution to a water body, none of the molecular methods currently has the potential to provide a direct quantitative measure of the fractional contribution from each of the sources to the total concentration of fecal pollution. Quantitative methods are needed to identify and target the dominant sources of pollution, to monitor changes in the concentration of fecal pollution and its sources over time, to assess the effectiveness of specific mitigation strategies, and to provide more information for evaluating the true public health risks.

The goal of the proposed research is to develop and evaluate a quantitative method for calculating the fractional contribution of fecal pollution from human and animal sources by measuring host-specific fecal indicator bacteria using real-time, quantitative polymerase chain reaction (qPCR). The method will be based on human and cow-specific nucleic acid sequences that have been published in the literature. Development and validation of the method will be conducted using pure cultures of bacteria as well as fecal samples, with final evaluation using field samples collected from Tomales Bay. It is expected that the new qPCR method could be used independently or in combination with other MST methods that are under development. Ultimately, a combination of methods may be needed to develop long-term solutions for managing fecal pollution.