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

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To effectively manage watershed quality requires identifying the dominant sources of fecal contaminants, both spatially and temporally, such that the effectiveness of mitigation strategies can be assessed. Toward this end, we are developing a quantitative method for determining the relative contribution to fecal pollution concentrations from different animals. Our technique relies on using real-time quantitative polymerase chain reaction methods developed to identify several host-specific bacteria at the low concentrations typically encountered in the field. These lab developed methods are then being applied in the field to allow comparison to traditional water quality measurements.

Fecal pollution continues to be among the leading contaminants of our nation's waters. 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 experimental methods include both laboratory and field research. In the laboratory, we are developing QPCR methods for total *E. coli* as well as several host-specific target sequences in fecal indicator bacteria (other than *E. coli*). In the field, we are characterizing the sources of fecal pollution in a watershed by combining our QPCR methods with traditional water quality measurements, such as culturable *E. coli*, nutrients, and BOD.

First we focused on developing a QPCR method for total E. coli. The main challenge was the elimination of trace levels of contamination in commercial preparations of the polymerase enzyme, which interfere with detection of low levels of E. coli. A novel DNase treatment step was developed that may have widespread relevance for many QPCR-based applications. Next we developed QPCR methods for several host-specific targets that we have identified from the literature and discussions with colleagues.

This research will provide improved tools for managing fecal pollution, ultimately increasing our ability 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.

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