Enhancing the Bacterial Source-Tracking Toolkit: A Collaborative Approach

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Introduction

Sewage contamination, as demonstrated by elevated concentrations of fecal-indicator bacteria (e.g., *E. coli*, fecal coliforms, and enterococci) is one of the leading causes of water-quality impairment in the surface waters of Massachusetts and many other states. Identifying and correcting chronic and episodic bacterial contamination events in rivers, streams, lakes, and coastal areas is an important public-health and environmental challenge for federal and state regulators, municipal officials, and watershed advocates. Recognizing the increasing need to find and eliminate sources of bacterial contamination in surface waters, the Massachusetts Department of Environmental Protection, Division of Watershed Management (MassDEP DWM), the MassDEP Wall Experiment Station (MassDEP WES), the Massachusetts Office of Coastal Zone Management (CZM), the U.S.Geological Survey (USGS), and the U.S. Environmental Protection Agency (EPA) have begun a collaborative investigation aimed at developing a set of reliable and cost-effective tools for identifying sources of bacterial contamination in Massachusetts surface waters.

Objectives

- Develop and test a new approach for identifying sources of bacterial contamination in rivers, streams, and other waterways that may receive sewage contamination
- Incorporate the approach into a practical and cost-effective bacterial source-tracking protocol
- Apply the protocol to sites with known bacterial contamination to demonstrate the ability to distinguish human from non-human

Approach

- Select tributary and mainstem sites in the Shawsheen River drainage basin in eastern Massachusetts for dry- and wet-weather sampling
- Collect water samples during five dry-weather periods and three storm events
- Measure *E. coli* and enterococci numbers; compare results with Colilert and Enterolert methods
- Measure fluorescent whitening agents; compare with fluorometry measurements for detergents
- Measure caffeine and acetaminophen as indicators of sewage
- Use polymerase chain reaction assays to test for the presence of human-specific genetic markers in *Bacteroidetes* and *Enterococcus* species of intestinal bacteria

Storm Sampling-Three levels

- Passive (first flush) stormwater samplers at 5-6 sites
- Grab samples (first flush, peak flow, falling limb) during storms at 3-4 sites
- Automated stormwater sampling at 1-2 sites

Automated water sampler

Dry-weather data collection at a storm drain that enters the Shawsheen River in Bedford, Mass.

Culverted section of Vine Brook, a tributary to the Shawsheen River, in Lexington, Mass. (Stations VBSD and VB0A)

Passive "first flush" water sampler

Enterococcus faecium magnified 33,370 X

Bacteroides fragilis magnified approx. 17,000 X

Automated stormwater and wet-precipitation collector installed at Vine Brook, Lexington, Mass. (Station VB0A).

Legend

- Log *E. coli* (MPN)
- Log Enterococci (MPN)
- Log *E. coli* (CFU)
- Log Enterococci (CFU)
- Detergent concentration
- No Evidence of Sewage
- Inconclusive Evidence of Sewage
- Weak Evidence of Sewage
- Strong Evidence of Sewage
- Presence of Sewage Indicator

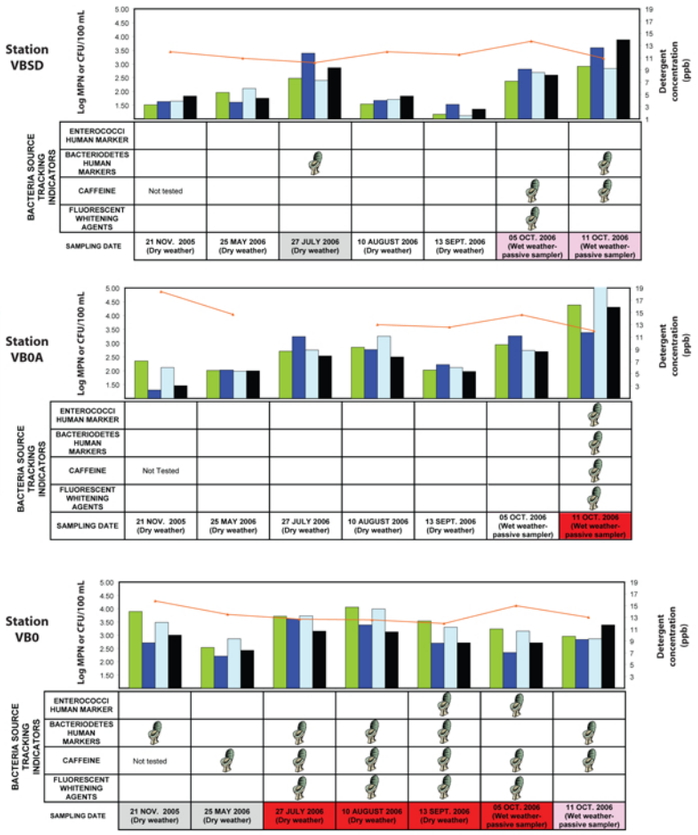
Collaborative Research Approach

- **USGS**-Collects fecal samples from known sources; conducts single-blind QA challenge of PCR assays; collects representative dry-weather and stormwater samples for analysis
- **MassDEP DWM**-Analyzes water samples using Colilert, Enterolert, and fluorometric methods for measuring detergent concentrations
- **MassDEP WES**-Measures fecal-indicator bacteria numbers, tests for fluorescent whitening agents, caffeine, & acetaminophen, conducts PCR assays for human-specific genetic markers
- **CZM**-Supplies automated stormwater samplers; assists in deployment, operation
- **USEPA**-Conducts additional water-quality monitoring at 32 sites on Vine Brook and the lower Shawsheen River mainstem; measurements include temperature, specific conductance, pH, dissolved oxygen, fecal coliforms, and *E. coli*

Preliminary Results

Bacterial DNA assays–Sensitive library-independent PCR assays for detecting human-specific genetic markers in fecal *Bacteroidetes* and *Enterococcus faecium* have been developed in our laboratory. The assay for the *Enterococcus* marker is very specific for human sewage but may produce false negatives in samples contaminated by individuals that lack the marker. The *Bacteroidetes* assay was 80 percent successful at distinguishing human sources from a variety of wild and domestic birds and animals.

Dry-weather and storm sampling–Dry-weather sampling was conducted at 12 tributary and mainstem sites in the Shawsheen watershed between November 2005 and September 2006. Sampling also was conducted during two wet-weather events in October 2006 at three Vine Brook stations (VB0, VB0A, & VBSD) in Lexington, MA. Three methods of sample collection were used during each storm: manual grab, passive sampler, and automated sampler. Samples were tested at WES for evidence of human sewage using the following indicators: *E. coli*, *Enterococcus*, fecal coliforms, fluorescent whitening agents, caffeine, acetaminophen, and the presence of two human-specific bacterial genetic markers. DWM analyzed split samples for *E. coli* and *Enterococcus* using the IDEXX system, and measured fluorescence. The graphs below show log bacteria counts, and presence/absence of the indicators at three stations during each of the sampling events. Colors in the table indicate the "weight of evidence" for the presence of human sewage as determined by the suite of toolkit parameters. The toolkit approach was 100% successful at identifying human sewage in six quality-assurance samples.



Results of bacterial and chemical source tracking indicators sampled for five dry weather and two wet weather events on three sites along Vine Brook.