Quantitative PCR Assays for Specific Host Sources of Fecal Pollution in Watersheds

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The purpose of this study is to develop Quantitative PCR (QPCR) assays for host specific and reference targets and utilize the assays to identify sources of fecal pollution in the San Pedro Creek Watershed (Pacifica, CA) and focus remediation efforts.

The San Pedro Creek Watershed is an important local recreational resource and steelhead rookery, but has levels of fecal pollution that contribute to coastal pollution and beach closures. Watershed sampling focused on previously untested, potentially polluted sites feeding into the North Fork above the Park Mall culvert (Fig. 1, upper right) and two areas associated with tributaries feeding the main stem from the South, i.e. the vicinity of Adobe Drive at Higgins Way and of Perez Drive (Fig. 1, lower left).

A total of 27 sites were sampled for up to one year, many in wet and dry conditions. Ten sites were sampled on 17 to 21 events, 4 sites on 6 to 9 events, and 13 sites on < 3 events. Enterococcus levels are reported; however, the levels of other fecal indicators (E. coli and total Coliform) paralleled the reported data.

Five sites had extensive fecal pollution, with mean Enterococcus levels >530 CFU/100ml. Twelve sites had mean Enterococcus levels from 90 to 290 CFU/100ml. Nine of the remaining 10 sites had mean Enterococcus levels <35 CFU/100ml. (CA State Standards for coastal water pollution are >35 and >104 Enterococcus CFU/100ml for 30 day geo-mean and single sample, respectively). Of the 5 most polluted sites, two were reference sites, i.e. the North Fork culvert and the Creek mouth. The remaining three sites, all near the intersection of Higgins and Adobe, were the most heavily polluted (mean Enterococcus levels > 948 CFU/100ml) and included surface runoff, a storm drain, and a water filled concrete dissipater. This vicinity was investigated further.

Of the 12 sites with intermediate levels of pollution, two sites were near the Higgins and Adobe intersection, seven were on or near Perez Drive, and the remainder were at or near Frontierland Park and drain toward the North Fork culvert. At each area, there appeared to be several potential sources of fecal pollution.

The heavily polluted Higgins Way concrete dissipater (4’ x 3’) was found to run perennially even in extended periods of dry weather. Runoff from the dissipater drains down a trail used by dog walkers, children and hikers, through a subdivision, toward San Pedro Creek. Numerous above ground

Figure 1. San Pedro Creek Watershed

water pollution sources potentially feeding into the dissipater were investigated and eliminated. The City of Pacifica drained the dissipater, and cleaned and scoped the underground storm drainpipe found to connect the dissipater to an uphill storm drain. This pipe was in good condition, suggesting that the primary source of fecal contamination for the dissipater arises from storm drain input. Subsequently, the subdivision, onto whose property the dissipater outflow drains, committed to return the outflow from the dissipater to the storm drain system, which would remediate the surface flow of waterborne pollution at this site.

Quantitative PCR (QPCR) assays for the Esp gene of *Enterococcus faecium*, as a specific marker for human fecal pollution, and the 16S rRNA reference gene target diagnostic for total Enterococcus and total fecal pollution were previously developed and validated (see 2006 annual report). DNA from mixed Enterococcus cultures from over 50 watershed samples with elevated Enterococcus levels were analyzed. Twenty eight were local watershed samples, with Enterococcus isolated by the San Mateo County Public Health Laboratory (SMCPHL) and DNA isolated by the PI's laboratory. Although the 16S reference assay reproducibly quantified significant levels of Enterococcus DNA across all standard and watershed samples (C_ts of 12 to 17 for neat samples), the human specific Esp gene target amplified only in standard positive control samples (C68 strain of Esp (C_t 12) plus *E. faecium* and sewage samples from BCS) and not significantly (C_ts > 38) in any of the 28 watershed samples. In contrast, for over 20 watershed and reference samples where Enterococcus and DNA were prepared by BCS of North Florida, the Esp gene target amplified in ca. 50% of the samples (C_ts of 28 to 35) and the reference gene amplified in 100% of samples. It was concluded that the isolation of Enterococcus cultures from IDECC plates by the SMCPHL routinely used for all CA watershed samples artificially skewed the Enterococcus population. Enterococci isolated directly from watershed samples (BCS method) were not subject to this artifact.

At this point, new human specific and reference QPCR assays were developed and compared to the existing assays across numerous positive controls and watershed samples. Based on these preliminary studies, it was concluded that the new human specific and reference assays showed increased sensitivity compared to existing assays. Using the new assays, 2 of 5 watershed samples from Northern California were found to contain significant human fecal pollution, including one sample from the North Fork Culvert of the San Pedro Creek.

In summary, the second year of the project has demonstrated significant progress in assay development and validation, identification of sites in the San Pedro Creek Watershed with highly elevated levels of fecal pollution, and development of strong collaborative ties for remediation efforts.

Publications

Ivanetich, Kathryn, Pei-Hsin Hsu, Kathleen M. Wunderlich, Evan V. Messenger, Ward G. Walkup IV, Troy M. Scott, Jerzy Lukasik, Jerry Davis, Microbial Source Tracking by DNA Sequence Analysis of the *Escherichia coli* malate dehydrogenase gene. *Journal of Microbiological Methods* 2006, 67:507-526.

Professional Presentations

Ivanetich, Kathryn, Overview of Northern California Water Quality Research Projects, 18th World Molecular Engineering Network, Cabo San Lucas, May 2007.

Collaborative Efforts

Troy Scott and George Lukasik of Biological Consulting Services of North Florida, and University of Florida, Dept. of Microbiology and Cell Science, Gainesville, FL; Evan Messenger, Biosearch Technologies; Joel E. Baldwin II, Earth Investigations Consultants.

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