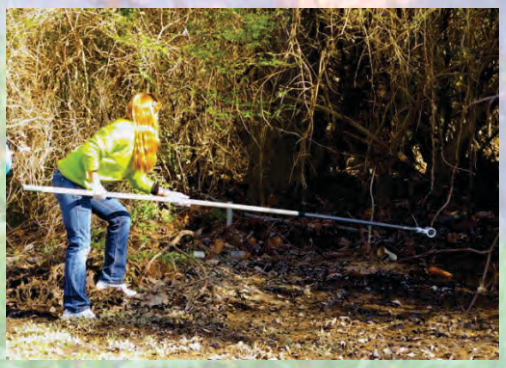
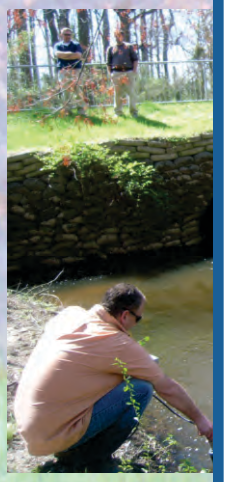
Assessing Sources of Fecal Contamination in High Priority Creeks in the Hampton Roads Region





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January 2013

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REPORT DOCUMENTATION

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Assessing Sources of Fecal Contamination in High Priority Creeks in the Hampton Roads Region

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ABSTRACT:

Elevated levels of fecal indicator bacteria (FIB, e.g. total coliforms, fecal coliforms or E. coli, and Enterococcus) have adversely affected water quality in many of the Hampton Roads localities. Many of the water bodies are listed as impaired under Section 303(d) of the CWA for indicator bacterial standards. To develop a long-term plan for improving water quality in the region, the Hampton Roads Planning District Commission (HRPDC) developed a microbial source tracking study and selected three watersheds for the initial assessments, Moores Creek, Shingle Creek, and Mill Dam Creek.

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This report was prepared for the Hampton Roads Planning District Commission (HRPDC) by the team of Drs. Noble, Hagedorn, and Harwood following field investigations and laboratory analysis that began in the Spring of 2010. Local government staff from Suffolk, York County, and Virginia Beach assisted in the collection of samples. The Hampton Roads Sanitation District (HRSD) assisted with both sample collection and laboratory analysis.

This Study was contracted by the Hampton Roads Planning District Commission in cooperation with member localities. Funding was provided by HRSD, Isle of Wight County, James City County, Norfolk, Portsmouth, Suffolk, Virginia Beach, and Williamsburg. Preparation of this report was first included in the Hampton Roads Planning District Commission Unified Planning Work Program for Fiscal Year 2010 approved by the HRPDC at its Executive Committee meeting of June 17, 2009.

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Assessing Sources of Fecal Contamination in High Priority Creeks in the Hampton Roads Region

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1.0 Project Summary/Introduction

Many of the aquatic systems, including rivers, creeks, estuaries, and bays, in the Hampton Roads area are impaired due to contamination with fecal pollution. This causes both negative impacts on tourism and the reduced ability to protect public health for those using the waters for recreation, shellfish harvesting and boating. In 1999, the U.S. Environmental Protection Agency (EPA) entered into a consent decree with complaintants who charged that the Clean Water Act was being violated in Virginia. This consent decree established a total maximum daily load (TMDL) development schedule through 2010 that can be observed at this URL (http://www.deq.state.va.us/Portals/0/DEQ/Water/WaterQualityAssessments/IntegratedReport/2012/ir12_Appendix9_TMDL_Status.pdf). The Virginia Department of Environmental Quality (VA DEQ) administers development and implementation of this TMDL program. To date, Virginia DEQ has completed almost 800 TMDL assessments. However, not all of these have been conducted in or near the coastal reaches of Virginia.

Virginia's Water Quality Monitoring, Information, and Restoration Act (WQMIRA) requires that TMDL assessment be followed up by development of an implementation plan, which describes the measures necessary to remediate the impaired status of each water body. Starting in the 1990's, VA DEQ led the nature in the first set of microbial source tracking exercises, then using library-dependent methods, including studies of antibiotic resistance analysis of *E. coli* (http://www.epa.gov/region10/pdf/tmdl/mst_for_tmdls_guide_04_22_11.pdf).

Library-dependent MST methods rely on the creation of databases, or "libraries," of the target organisms, in most cases *E. coli* or *Enterococcus,* by a typing or fingerprinting approach. Typing may discriminate among strains based on phenotype (e.g. antibiotic resistance analysis from growth of colonies of specific bacteria on petri dishes with varying types of antiobiotics) or genotype (e.g. the pattern of DNA bands observed when using pulsed field gel electrophoresis). The target fecal indicator bacteria is isolated from known sources of fecal contamination, i.e. feces/scat of animals, including humans, that have potential to impact water quality in the watershed. The bacteria are then typed, and these "known" types form the library. Samples are then collected from the water bodies of interest, and the same procedure is conducted, except these "types or fingerprints" are "unknowns" until they are successfully matched to known types or fingerprints in the library to infer the source of the water bacteria. The analysis is frequently set up to assign bacteria to relatively broad categories of fecal contamination, e.g. human, pet, wildlife, or agriculture.

Libraries that cover a very wide geographic area tend to be less accurate than those focused on more very small regions, which mean that many regional libraries had to be created at relatively great expense. Furthermore, since specificity is compromised for very large areas, in the past it has been difficult for localities interested in a single watershed to rely on the results from a regional comprehensive library. In many cases, library-dependent MST methods produced estimates of source allocations that were questioned by local government staff and citizens with knowledge of the TMDL watershed. Regardless of perceived accuracy, since localities were expected to address the sources of bacteria assigned by the MST analysis, these estimated source allocations had real-world consequences. That is, localities were often responsible to design mitigation strategies for specific types of fecal contamination, even though they were not

necessarily convinced that the fecal source represented a majority of the problem. Additionally, the library dependent methods, while conducted only during specific seasons or during specific conditions, were often widely applied.

In the past decade, a new set of library independent approaches has emerged, removing the scientist and the localities from the guesswork of interpreting library dependent methods. These new tools are largely based upon the quantitative PCR based technology, and often yield quantitative information about targets with specific attributes related to fecal contamination inputs. The localities view these tools as a means to determine where human sources of bacteria where entering the system, and to assist them in prioritizing and justifying expensive public works/utilities projects. While the qPCR based methods suffer from some issues related to sensitivity and specificity, when used in a tiered approach (e.g. Noble et al. 2006) combining specificity and sensitivity with appropriate analysis of relevant markers for the watersheds, the approach can be highly successful.

Elevated levels of fecal indicator bacteria (FIB, e.g. total coliforms, fecal coliforms or *E. coli*, and *Enterococcus)* have adversely affected water quality in many of the municipalities, especially those impacted by stormwater contributions to receiving waters. For a number of years, elevated FIB levels have concerned several of the municipalities. Many of the water bodies are listed as impaired under Section 303(d) of the CWA for indicator bacterial standards. To develop a long-term plan for improving water quality in the region, the Hampton Roads Planning District Commission (HRPDC) developed a microbial source tracking study and selected three watersheds for the initial assessments, Moores Creek, Shingle Creek, and Mill Dam Creek.

To address the issue of the source of fecal contamination in the area's tributaries, at the request of the Hampton Roads Planning District, we assembled a team of Drs. Noble, Hagedorn, and Harwood. We proposed a study of 12 months in duration as a preliminary assessment of the sources of water contamination in Hampton Roads/Virginia Beach, beginning in the spring of 2010, with the understanding that supplemental work might be needed depending upon project success.

Virginia's coastal area in the Tidewater region is an ecological treasure and a lynchpin of the state's economy. In 2005 it was estimated that recreational tourism generated $17 billion annually in the region, recreational fishing $6 billion, and commercial fishing almost $3 billion (including providing ~15% of the nation's oysters). Attractive living and working conditions in the region have resulted in a burgeoning human population and concomitant development. This growth threatens to increase the extent of water quality degradation throughout the area, including microbial impairments which are based on elevated levels of FIB. In the greater Tidewater area many streams and embayments are listed as impaired (due to FIB) by the Virginia Department of Environmental Quality (DEQ, http://www.deq.virginia.gov/tmdl/), and sporadic beach closures have been persistent problems at several of the ocean and many of the bay beaches (http://www.vdh.virginia.gov/Epidemiology/dee/beachmonitoring/ and http://hamptonroads.com/node/440611). Stormwater was identified as the contamination source in 24% of closures; but the greatest fraction of closures, 76%, was caused by unknown sources.

The greatest threat to beach water quality at the national level is currently considered stormwater, followed by sewage pollution (USEPA, 2003a).

The overall goals of the project were to:

- Identify a potential suite of methods and approaches that will be useful in conducting microbial source tracking work in the Hampton Roads area;
- Identify sources and quantify the loading of bacterial contamination using a multi-tiered approach at three locations within the Hampton Roads Planning District region, focusing specifically on Poquoson River/Moore's Creek in York County, Mill Dam Creek in Northern Virginia Beach, and Shingle Creek in the City of Suffolk.
- Characterize patterns of bacterial concentrations and identify "hot spots" of high FIB levels during both dry and wet weather;
- Determine the presence of and quantify markers of human fecal contamination, and establish whether such contamination at specific sites is chronic or intermittent;
- If non-human contamination is found, attempt to partition the fecal contamination signal stemming from animal populations, particularly birds and pets;
- Provide a framework for recommending appropriate actions to reduce sources of fecal contamination in impaired waterways.

We used a tiered approach that included (1) a "tracer screen", i.e. a preliminary assessment of the most appropriate FIB and library independent approaches to apply in a particular watershed or area of interest, (2) quantification of FIB using traditional methods to determine the concentrations of bacteria in the system of interest, in addition to identification of "hot spots; " (3) application of quantitative molecular MST methods such as *Bacteroides* spp., and human polyomavirus using quantitative PCR (QPCR). We focused first on human contamination, with some later characterization for the presence and quantification of gull specific fecal markers.

2.0 Multi-Tiered Analytical Approach Applied in Three Selected Watersheds

The three study areas included in this study plan are Poquoson River/Moore's Creek in York County, Mill Dam Creek in Northern Virginia Beach, and Shingle Creek in the City of Suffolk. The study sites are fully described in Section 3.0. A specific sampling plan was developed and implemented in tight collaboration with the water quality management personnel in the City of Suffolk, York County, and the City of Virginia Beach. Sample frequency, site selection and other study details were identified during the development of each plan and took into account personnel resources of each group, the capabilities of the Hampton Roads Sanitation District (HRSD) analytical laboratories, and the sampling frequencies needed to conduct a scientifically rigorous assessment of each system.

2.1 Fecal Indicator Bacteria

Fecal indicator bacteria (FIB) are surrogates used to measure the potential presence of bacteria, fecal material, and associated fecal pathogens. Fecal indicator bacteria such as fecal coliform and *Enterococcus* are part of the intestinal flora of warm-blooded animals. FIB have long been used to protect swimmers from illnesses that may be contracted from recreational activities in surface waters contaminated by fecal pollution. These organisms often do not cause illness directly, but have demonstrated characteristics that make them good indicators of harmful pathogens that may

be present in water bodies (RWQCB, 2007). The USEPA recommends fecal indicator bacteria quantification for monitoring of ambient waters because studies have demonstrated that *E. coli* and *Enteroccocci* are better predictors of the presence of gastrointestinal illness-causing pathogens than fecal and total coliforms, and, therefore, provide a better means of protecting human health. Some states, includingVirginia, utilize different FIB groups for different monitoring purposes, i.e. recreational waters are monitored using Enterococcus sp. and shellfish harvesting waters are assessed via quantification of fecal coliforms.

The microbial source tracking (MST) component of this project was conducted to quantify markers of human fecal contamination, and evaluate whether contamination at specific sites was chronic or intermittent. The multi-tiered analytical approach utilized an array of conventional FIB analyses (conducted by the microbiology laboratory at HRSD) and quantitative molecular assays for microbial source tracking in urban environments. We followed a multi-tiered analytical approach for this project. Water samples collected during a range of seasons and weather conditions were initially analyzed for the FIB *E. coli* and *Enterococcus.* All FIB analyses were conducted by the Hampton Roads Sanitation District using methods described in Appendix A. For molecular analyses, subsamples were filtered and frozen for follow-up molecular analyses by the laboratories of Project Team members Noble and Harwood.

2.2 Multi-Tiered Analytical Approach

Before the multi-tiered approach could be initiated, it was first important to select the appropriate molecular markers to analyze in the Hampton Roads region, and conduct sampling over relevant time and spatial scales for adequate assessment of natural systems.

Tracer Screen:
In order to best differentiate fecal contamination sources in the three identified watersheds of concern in the Hampton Roads area, we first conducted an initial site examination. This had already been conducted for Mill Dam Creek, where a specific project funded to PI Noble initially examined patterns of contamination in Mill Dam Creek sampling locations (see Preliminary Report). At Shingle Creek and Moore's Creek we initiated this process through a stakeholder meeting and initial site walks to select sampling locations. We discussed pertinent information with local officials regarding size and duration of recent sewage spills and functioning/failing septic tanks, walked the system looking for specific fecal inputs, examined flow during rainfall and during dry periods, and conducted sampling to do an initial tracer screen using the *Bacteroides* and human polyomavirus based analysis. The "tracer screen" involved sampling in Moore Creek, Shingle Creek, and Mill Dam Creek at sites, because of their past contamination levels, mutually selected by the Project Team and the local government staff.

For the tracer screen, we started by sampling in the spring of 2010 for two consecutive days for the three watersheds. To the best of our ability, sampling took place as close to low tide as possible. Once the initial tracer screen was conducted the research team met again with stakeholders to discuss results, including quantification of targets, sampling location placement. It was initially thought that flow measurements would be possible throughout this project, but such measurements were not within the capacity of the sampling teams. Therefore, no flow information is available for these systems. During this period of time, three other steps were

taken to ensure that the molecular markers being employed in this study were useful in this region. First, a set of raw sewage influent samples was sent to the laboratories of Noble and Harwood, where they verified the usefulness of the trio of Bacteroides spp. markers and the Human Polyomavirus marker. Second, a blind study was conducted of 42 water samples prepared with different mixtures of human, gull, and/or dog fecal contamination and these samples were sent blind to the laboratory of Noble. Her laboratory, using the trio of Bacteroides spp. markers, was able to 100% correctly identify the contamination in the samples. Third, additional wastewater samples were sent from the HRSD to the laboratory of Harwood to verify the methods used for quantification of the Human Polyomavirus.

Quantification of FIB and Assessment of Hot Spots during Wet and Dry Weather:
Once the tracer screen was conducted, we conducted sampling focusing primarily on wet weather events over a range of seasons. The sampling team was generally mobilized upon reports of a minimum of 1 cm of rain.

2.3 Microbial Source Tracking

Bacteroides spp. comprise approximately one-third of the human fecal microflora, considerably outnumbering *Enterococcus* and *E. coli*. The *Bacteroides* group belongs to a group of non-spore-forming, gram-negative, obligate anaerobes, so there is little concern over regrowth in the environment. QPCR methods were used to conduct the *Bacteroides* assays:

- *Fecal Bacteroides QPCR* assay (Converse et al. 2009) relies on Taqman chemistry and all the reagents are in a liquid formulation, except the OmniMix. The assay quantifies a cohort of bacteria found in high concentrations in the human gut, including *Bacteroides thetaiotaomicron*, *Bacteroides distastonis*, and *Bacteroides fragilis*. However, the method is not human specific. The assay has been tested against a range of different fecal samples types, and has been shown to be capable of quantifying over a wide range of concentrations, and to be sensitive at concentrations relevant to water quality source tracking studies. When using the QPCR approach for fecal *Bacteroides*, strong relationships have been observed with a wide array of human sewage collected from areas on both east and west US coastlines. The assay is highly sensitive and the target bacteria that are enumerated have been shown to be a predictor of human health in both sand and recreational waters (Wade et. al. 2011, Heaney et al. 2011) during large-scale EPA-run epidemiology studies. This is a fully quantitative QPCR-based assay that is being used in an array of studies in stormwater contaminated areas and that, with the use of other additional confirmatory methods, can be used to both identify potential hot spots of human fecal contamination (Converse et al, 2009).
- BacHum Human Marker: A separate QPCR assay was utilized to quantify the BacHum molecular markers reported by Kildare et al., 2007. The assay has been widely tested for specificity against a range of fecal sample types and has shown high capacity for discrimination against human and animal fecal types (Ahmed et al., 2009). The assay is conducted in conjunction with a specimen processing control for full quantification, even on samples that demonstrate inhibition of QPCR amplification.
- HF (human fecal) 183: Human specific marker by QPCR has been conducted previously by Bernhard and Field (2000) and updated by Seurinck et al., 2006. This assay is specific

to a region of ribosomal rDNA within the *Bacteroides spp*. that is found almost exclusively in human feces. The assay has been tested repeatedly in a range of different environments for cross reactivity with other types of fecal material, and various researchers have found either a 90- to 100-percent ability to discriminate between human and animal feces when using this assay. The assay, however, can be problematic when used alone, because the target copy concentration in fecal material contributed to receiving water environments can be quite low due to dilution and the assay has a relatively low sensitivity.

- The human polyomaviruses (HPyVs) qPCR assay targets the two most common HPyVs species, the JC and BK viruses. Both viruses are have a DNA genome, are genetically stable, and are found in high rates in human population worldwide. The asymptomatic primary infection, which generally occurs during childhood, is followed by latent infection in renal tissue. In the human body, HPyVs can persist indefinitely and are generally non-pathogenic. High tiers of viral particles are excreted in feces, as well as in the urine of healthy individuals (McQuaig et al, 2006, 2009, 2012). The TaqMan qPCR assay, which targets a conserved T-antigen portion of the viral DNA genome, has been successfully tested in number of laboratory and field studies (Ahmed 2010, Harwood 2009, 2011). HPyVs are among the most specific MST markers. In a recent study focused on comparing the specificity of human associated markers, human polyoma viruses were found 100% host specific, whereas the specificity of the human-associated *Bacteroides* HF183 was 98% (Ahmed, 2009). However, HPyVs are present at lower concentration in sewage than HF183. The concentration of the viruses in untreated sewage is generally 3-4 orders of magnitude lower than that of HF183 (Ahmed 2009, Harwood 2009, 2011). Nevertheless, their prevalence in environmental waters under dry, as well as wet, conditions is comparable to other bacterial and viral indicators (Sidhu,2012). Their similarity in terms of size and metabolic requirements to enteric viruses may also better reflect the fate and transport of enteric viruses than bacterial tracers. Because of their lower concentration, and their high host specificity, HPyVs should generally be used in conjunction with targets that are more concentrated as a confirmation of the human source pollution.

Another host-specific QPCR assay that was utilized in this study is specific for fecal contamination from seagulls and other shore birds, including pelicans. The assay targets the gram-positive, catalase-negative bacterium *Catellicoccus marimammalium* (Lu et al. 2008, Lu etl al. 2011). The assay has now been widely tested throughout the USA and Canada. The QPCR assay was designed by Santo Domingo, and has been used as a marker to quantify the presence of gull fecal material (not specific to other water fowl species, however, see Lu et al. 2011). The assay is highly specific and sensitive, and is a strong complement to the other human specific assays employed.

Selection of Sample for Molecular Marker Analysis:
Based on results of the initial FIB analyses, samples were selected for further MST marker analysis if they exceeded regulatory standards for recreational waters (i.e. *Enterococcus* >104 MPN or CFU/100 ml, and *E. coli* >320 MPN or CFU/100 mL). The molecular analyses conducted consisted of Fecal *Bacteroides spp*. (Converse et al. 2009), *Bacteroides* HF183 Marker (Seurinck et al. 2006), BacHum Marker (Kildare et al. 2007), General *Bacteroides* spp.

(Shanks et al. 2010), and human polyoma viruses BK and JC (McQuaig et al., 2009). Analysis for the gull-specific marker, *Catellicoccus marimammalium*, was performed on a subset of samples (Lu et al. 2011). Details on the various analyses and methods conducted by the laboratories can be found in peer reviewed literature cited for each method.

3.0 Watershed Specific Results and Discussion

3.1 Mill Dam Creek

Mill Dam Creek is a tributary to the Broad Bay, which is part of the Lynnhaven River watershed. The Lynnhaven River, including Broad Bay, is on the 303 (d) list as impaired for fecal coliforms for shellfishing. The original bacterial TMDL for this watershed was developed in 2004. An implementation plan was developed in 2006, along with a modeling study conducted in Mill Dam Creek/Dey Cove/Broad Bay, which was conducted by researchers at the Virginia Institute of Marine Sciences. Subsequent monitoring and studies, conducted in part by Dr. Noble, a member of the project team here, has identified Mill Dam creek as a potential significant source of fecal indicator bacteria to Broad Bay. Since 2010, monitoring and assessment efforts began to focus on identifying, and quantifying specific sources of fecal contamination in Mill Dam Creek through the use of molecular microbial source tracking markers (see image 1 below and map on page 9).

Image 1: Selected major sampling sites on Mill Dam Creek

Image 2: Map of Sampling Locations within the Mill Dam Creek Watershed

Based upon a previously conducted Mill Dam Creek molecular marker study, six sites were identified as being prioritized for intensive sampling during this study. The selected sites were sampled up to 23 times over the course of the study from 5/27/2010 to 1/05/2012. See Table 1 in Appendix B for a full inventory of samples collected and analysis conducted on the samples.

Fecal Indicator Bacterial Analysis

Figures 1.1 and 1.2 provide an overview of mean *E. coli* and *Enterococcus* levels, respectively, and MST marker detection at the Mill Dam Creek sites. Only sites A, D, F, G, I, and J were included in this analysis. Marker symbols are shown if they were detected at least once during the study. Regulatory limits shown on graphs are single sample standards set by USEPA, which are higher than the geometric mean limits. Even though Mill Dam Creek is not heavily used for recreation, it is still helpful to assess the magnitude of exceendance of the FIBs in a highly contaminated stream that impacts shellfish waters in Broad Bay.

Enterococci concentrations showed a similar trend compared to *E. coli*, i.e., levels are particularly elevated at Sites A, D, and F (Figures 1.3 – 1.5). For statistical analysis, the enterococci and E. coli concentration data, along with some rainfall data needed to be log transformed prior to analysis. Rainfall and FIB concentrations were significantly correlated for two of the sites, Site A (Fig. 1.3) and Site D (Fig. 1.4), and weakly correlated at the other locations. Antecedentrainfall 24 hr and 48 hour rainfall was significantly and similarly correlated with *E. coli* concentrations and enterococci concentration, respectively. The adjusted r^2 for correlations in Mill Dam Creek were the strongest observed for the study at nearly 0.44, i.e. 44% of the variability observed in the *E. coli* concentrations at Mill Dam Creek sites could be explained by $\log_{10}$ 24 h rainfall. The relationships observed for all sites pooled with rainfall were significant and strong no matter what the combination, i.e. *E. coli* or *Enterococcus* paired with 24 hr, 48hr, or 5 day rainfall amounts. Since this system is heavily developed we expected to see these types of relationships, where stormwater is dominating the concentrations of *E. coli* and *Enterococcus* in Mill Dam Creek.

E. coli and enterococci concentrations were significantly and strongly correlated for data pooled from all the sites (r=0.84). Although some sites FIB levels did differ by site, both *E. coli* and enterococci concentrations were consistently elevated throughout the Creek. This strong coupling between *E. coli* and enterococci is higher in Mill Dam Creek than observed for the other two systems. This indicates that there is a strong likelihood that the fecal contamination to the system is fresh, rather than aged, which would have a decoupling of *E. coli* and *Enterococcus* concentrations.

Microbial Source Tracking Molecular Marker Analysis

As expected, the general *Bacteroidales* (GenBac) and Fecal *Bacteroides* (FecBac) markers were detected most frequently at all sites in Mill Dam Creek, as they are general fecal markers and are not indicative of a specific host group (Table 2). GenBac was detected in 100% of samples at levels ranging from 537 to 159,000 gene copies/100 ml. Fecal *Bacteroides* detection rates at the various sites were nearly 100% in samples without problems with QPCR due to inhibition, reaching a maximum concentration of 671,786 cell equivalents at Site D on 2/14/2011. The fecal *Bacteroides* spp. concentrations exceeded 1 $x10^4$ cell/ equivalents/100 ml in 57% of the samples. The value of 10,000 cell equivalents/100 ml has been postulated previously as an “action

threshold" for Fecal *Bacteroides* spp. QPCR (Coulliette et al. 2008). That is, a sample containing that concentration of Fecal *Bacteroides* spp., paired with high concentrations of fecal indicator bacteria, in a natural system, deserves further investigation.

The BacHum marker was designed to be human-specific (Kildare et al., 2007), but is known to cross-react with a variety of host species including dogs. HF183 is known to be a more human-specific marker than BacHum, and it was detected in fewer samples than BacHum at the Mill Dam Creek Sites. HF183 was detected 3 times at Site A, 6 times at site D, and 7 times at Site F (Table 2). In addition, sites G, I, and J demonstrated HF183 positive results 2, 3, and 2 times, respectively over the course of the study. There were several times, particularly at sites A, D, and F, where all three markers of human fecal contamination were positive at the same time, indicating human fecal contamination to be present and quantifiable in the system. However, there were more instances when two markers were positive, with one marker negative. There are multiple possible reasons for this. First, the BacHum assay is known to have a greater cross reactivity with non human contamination than the HF183 assay. Second, discrepancies in detection of BacHum and HF183 could be due to greater robustness of the BacHum assay with respect to QPCR inhibition. Third, the reasoning for using multiple markers is that not all markers of human fecal contamination will be present in consistent concentrations in human fecal contamination. Our results at this site support the multiple marker approach, as contamination signals indicating human contamination, for example, would have been missed if using the HF183 marker alone.

Human polyomaviruses are the most human-specific of the MST markers used in this study. Like HF183, they were only detected in a few Mill Dam Creek samples at sites A, D, and I. Concentrations were low, with a maximum of 143 gene copies/100 ml. Any quantification of human polyomavirus in the system, however, is a concern, due to the fact that these viruses would be expected to mirror the presence of other enteric viruses.The *Catellicoccus* gull/shorebird marker was also sporadically detected; twice at Site A and once each at Sites D, F, G, I, and J (Table 2).

<u>Mill Dam Creek Summary:</u> Mill Dam Creek has the highest percentage of impervious surface coverage of the watersheds studied. This area had the strongest correlation between rainfall and FIB concentrations, with 44% of the FIB concentrations in the system explainable by rainfall amounts. Furthermore, over the course of the study site A and site D exhibited very strong relationships between 5 day precipitation totals and both *E. coli* and *Enterococcus* concentrations (ranging from r= 0.80 to 0.69 respectively at Site A). When rainfall and FIB concentrations are strongly correlated, it is suggested that overland transport of stormwater is dictating contamination of the receiving water body. Given the strong connection between rainfall and FIB concentrations observed at Sites A, D, and F, and the confirmation of human fecal contamination at these sites over the course of a previous preliminary study, and during this study, these sites have been identified as the locations of current loading assessments. In order to conduct mitigation of the FIB signal in Mill Dam Creek, given the contamination scenarios observed at sites A through J, it will be necessary to prioritize actions and mitigation strategies. To this end, flow-paced sampling is being conducted with automated flow and level recorders and refrigerated ISCO samplers at Sites A, D, and F. This storm assessment has already proven to be useful in determing that there is an important delivery of FIB over the course of storms (i.e.

that first flush is not dominating the delivery patterns of FIB). This project will generate important information on the delivery of FIB and microbial contaminants of concern in a highly populated, intensely developed area of the City of Virginia Beach.This type of assessment can provide important information as to the location and the magnitude of the sources of contamination. For example, if overland stormwater runoff is the only source of contamination to a system, you expect that microbial contaminants will be delivered early in a storm, following the patterns observed such as "first flush". Conversely, if leaking sewage or septage infrastructure is implicated in the delivery of contamination to a storm, the contamination levels will increase over the course of the storm hydrograph, given rising groundwater levels and increased groundwater transport of subsurface contaminants.

In Mill Dam Creek, a previous small program had been conducted to focus the effort as far as sampling locations. This previous effort had demonstrated that continued investigation of Mill Dam Creek Sites A, D, F, I, G, and J was important. These sites were included over the course of this study, and it has become evident that the human fecal contamination signal was stronger over the course of this assessment at Sites A, D, and F. Continued work in this watershed, paired with flow-paced loading assessments, has already commenced at these three sites.

3.2 Moore's Creek

Moores Creek is a tidally-influenced tributary to the Poquoson River located in York County. Its waters are included in the upper Poqoson River TMDL developed in 2006 for shellfish and primary contact recreational use impairments due to elevated fecal coliform concentrations. It is a small watershed with a mix of older and newer residential development, a small amount of agricultural land, and several homes still on septic systems for wastewater disposal. Some large animals, such as horses, have direct access to the stream at certain points. Wildlife, including birds, raccoons, is numerous. Sanitary sewer overflows have been documented. Not every home is in a neighborhood, some homes are located along roads and these vary from smaller older homes to larger newer homes. Some residences are on septic, but most of the area is served by sewers. Wildlife/birds are possibilities at every site in this tidal zone stream and its tributaries. Twenty-two Moores Creek sites were sampled during this study, with a frequency as low as one time and as high as 21 times (e.g. MC1 and MC3) (Table 4). Sampling occurred over the time period of 01/25/10 to 01/23/12. Table 4 in Appendix B presents an inventory of the sites, dates sampled, and analyses performed (see Image 2 on page 15 and map on page 16).

Fecal Indicator Bacteria Analysis

Figure 2.1 provides an overview of mean *E. coli* levels and MST marker detection at the Moore's Creek sites. Only sites sampled eight times or more were included in this analysis. Marker symbols are shown if they were detected at least once during the study. Analysis of variance (ANOVA) found a significant difference in *E. coli* concentrations by site, with the highest levels at MC5, MC9 and MC10 (P=0.002). Regulatory limits shown on graphs are those for one-time grab samples, which are higher than the geometric mean limits. Figure 2.2 shows *E. coli* levels for all of the sites, regardless of the frequency of sampling. MC3B and MC8 have *E. coli* levels similar to MC5, MC9 and MC10; however, these results must be interpreted cautiously because of the small sample size (see Table 3).

Enterococci concentrations show a similar trend compared to *E. coli*, i.e., levels are particularly elevated at MC3 (Figure 2.3). ANOVA also found a significant difference in enterococci levels among sites ($P<0.001$). Among the infrequently-sampled sites, MC2, MC4 and MC8 stand out as the most elevated in terms of enterococci; however, as previously noted, these data are based on a very small number of samples (Table 3). Rainfall and FIB concentrations were significantly correlated for data pooled from all sites. Antecedent rainfall 24 hr, 48 hr and 5 days prior to sampling was significantly, and similarly correlated with $\log_{10}$ *E. coli* concentrations (Pearson r = 0.334, 0.397, and 0.407, respectively. Log_{10} enterococci concentrations were significantly correlated with 24 hr antecedent rainfall (Pearson r = 0.346), but were better correlated with 48 hr and 5 day antecedent rainfall (r = 0.631 and 0.565, respectively). Figure 2.3 shows a graph of *E. coli* and enterococci concentrations at MC3 as an example of the relationship, with rainfall levels superimposed for the most frequently sampled Moore's Creek sites.

E. coli and enterococci concentrations were significantly correlated for data pooled from all the sites (Pearson r = 0.695). Although some sites FIB levels did differ by site, both *E. coli* and enterococci concentrations were consistently elevated throughout the watershed, even at well-flushed sites such as MC7 and MC9. The chronic state of elevated bacterial levels suggests that the sediments are harboring a reservoir of FIB, and that these fecal bacteria are surviving and perhaps slowly growing in the sediments (Fig. 2.3).

Microbial Source Tracking Molecular Marker Analysis

As expected, the general *Bacteroidales* (GenBac) and fecal *Bacteroides* (FecBac) markers were detected most frequently at all sites (Table 3), as they are general fecal markers and are not indicative of a specific host group. GenBac was detected in 100% of samples at levels ranging from 240 – 370,000 gene copies/100 ml. Fecal *Bacteroides* detection rates at the various sites ranged from 0% (MC7) to 100% (MC1B, MC3, MC3B, and MC6), at levels up to 6.8×10^6 gene copies/100 ml. Overall, FecBac was detected in 79% of samples in which the analysis was conducted.

The BacHum marker was designed to be human-specific (Kildare et al., 2007), but is known to cross-react with a variety of host species including dogs. BacHum not detected at some sites, such as MC9B, but was detected in 90 to 100% of samples from some sites (MC3, MC3B, MC5, MC7) at others. Concentrations ranged from undetectable (0) to 1.75×10^6 gene copies/100 mL. Overall, BacHum was detected in 76% of samples in which the analysis was conducted.

HF183 is known to be a more human-specific marker than BacHum, and it was detected in far fewer samples than BacHum. HF183 was detected only twice throughout the study in 87 samples, once at MC1 and once at MC9A, at concentrations of 5,000 to 6,000 gene copies/100 ml. The discrepancy between detection of BacHum and HF183 could be due to greater robustness of the BacHum assay with respect to PCR inhibition.

Human polyomaviruses are the most human-specific of the MST markers used in this study. Like HF183, they were only detected in a handful of samples (n=47), including one at MC3, one at MC3A, one at MC9, one at MC9A, and one at MC10 (overall detection rate 6%). Concentrations were low, with a maximum or 40 gene copies/100 ml. Because they are viruses, which are smaller than bacteria, their fate and transport in the environment may differ substantially from

that of bacteria. This difference may be useful for predicting human health impacts from contaminated water, as over 50% of the incidents of gastroenteritis are estimated to be caused by viruses.

The *Catellicoccus* gull/shorebird marker was also sporadically detected, twice at MC7, once at MC9 and twice at MC10. Overall, the gull marker was detected in 11% of samples, all of which were located on or very near the Poqoson River. The highest level measured was ~36,000 gene copies/100 ml at MC10.

Response to precipitation events: For Moore's Creek, all the three priority sites (MC1, MC3, and MC9) are sensitive to precipitation events and further intensive sampling before, during, and after precipitation events is appropriate at these locations. For MC9, this should include 9A and 9B as well as at least one point in between (9E and 9F). During a precipitation event, any sampling of flowing water that can be obtained above 9A or 9B and at 9F will be important. Site MC9 should also be included for sampling any time these other sites above MC9 are sampled. Further sampling should be done on the outgoing and low tide if at all possible.

Moore's Creek Summary: The neighborhoods on Moore's Creek are mostly upscale and relatively new, and nothing appears that is clearly obvious from site visits in either dry or wet weather that indicates the possible presence of human contamination. Livestock (horses) contamination is possible at a few locations due to the presence of small farms. At the time of this study, horse fecal contamination markers were not well developed. However, currently, new QPCR-based markers have emerged in the literature that are appropriate for the quantification of horse fecal contamination. Quantification of this marker in the future may be important in Moore's Creek to assess the importance of this signal.

Overall analysis of the results for Moore's Creek indicates that intensive sampling should be focused on MC3 and MC9 as high priority, MC1 and MC5 as important but not high priority, and MC6, MC7, MC8, and MC10 as low priority. The FIB results indicated that additional sites were needed for more intensive sampling and sites 3A and 3B, 9A and 9B, and 1A and 1B were established for this purpose. There was no need to further sample some lower priority sites on a regular basis (MC11, MC12, MC13, MC14, MC15, and MC16), and the FIB results indicated the origins of the pollution could most likely be found between MC9 and 9A and 9B, between MC3 and 3A and 3B, and between MC1 and 1A and 1B. In these aspects, the sampling design was a success, as some sites emerged as priorities and others were found to be no longer useful, and the length of the stream and its tributaries that should be targeted for further sampling efforts has been reduced, permitting a more focused expenditure of resources.

Image 3: Selected Major Sampling Sites on Moore's Creek

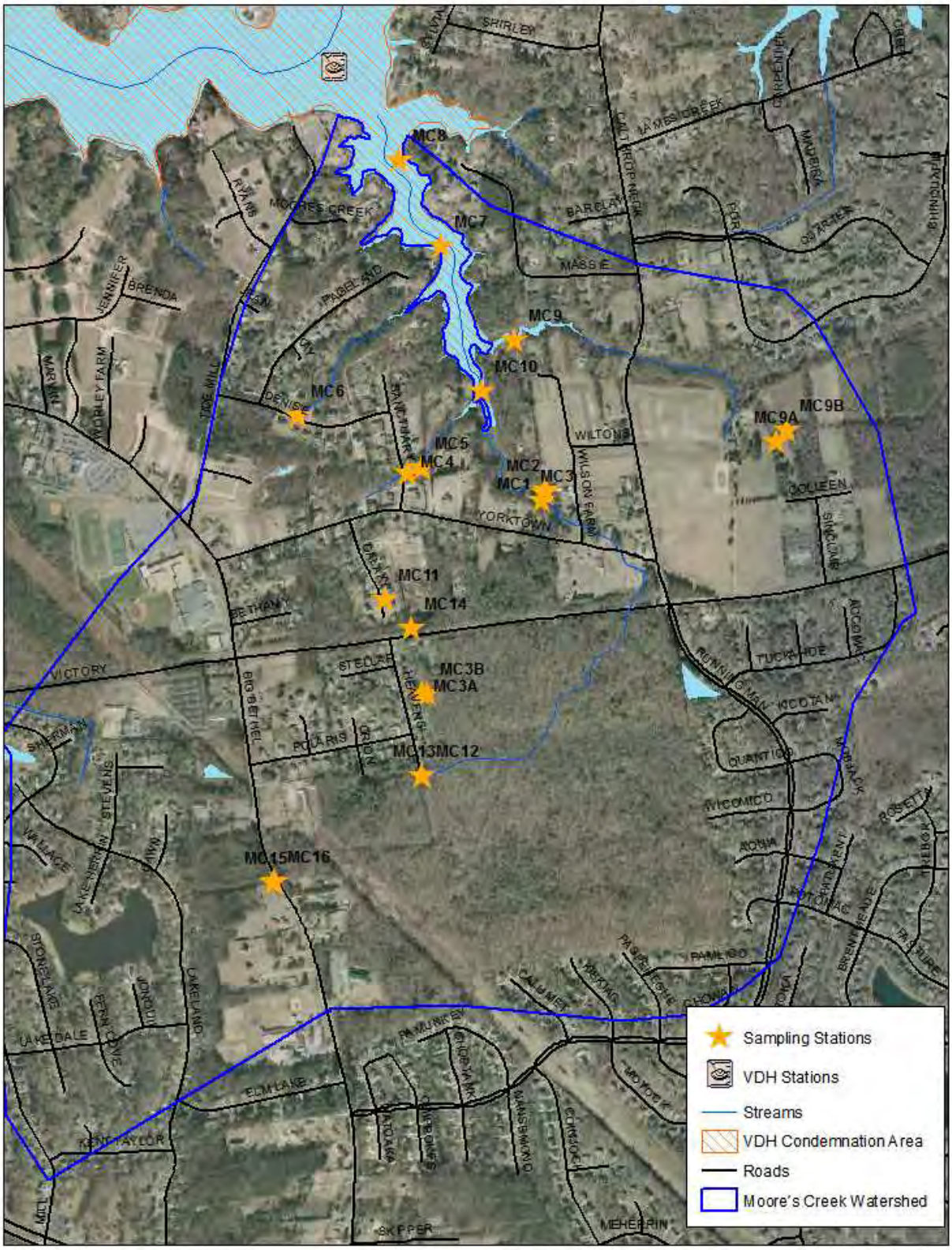

Image 4: Map of Sampling Locations within the Moore's Creek Watershed

3.3 Shingle Creek

Shingle Creek is included in the Nansemond River Bacteria TMDL developed in 2006. It is impaired by for shellfishing and primary contact recreation by high fecal coliform and enterococci levels, respectively. The Shingle creek watershed is primarily a built-out residential area with development that predates stormwater best management practices. There is also some light industrial activity located in the watershed. Properties within this watershed are generally serviced by the municipal sewage system. An implementation plan to reduce fecal bacterial loads had been developed for the Nansemond River that included discussion of this study and well as an intention to follow up on study recommendations (see image 5 on page 19 and map on page 20).

Fifteen Shingle Creek sites were sampled during this study, with a frequency as low as one time and as high as 15 times (SC11) (Table 6). Sampling occurred over the time period of 01/26/10 to 11/23/11. Table 6, Appendix B, shows the sites, dates sampled, and analyses performed.

Fecal Indicator Bacteria Analysis

Figure 3.1 provides an overview of mean *E. coli* levels and MST marker detection at the Shingle Creek sites. Unlike the situation at Moores Creek, *E. coli* levels did not differ significantly by site. Note that the mean *E. coli* concentration at each site exceeded the single sample maximum for recreational waters. Figure 3.2 shows mean *E. coli* concentrations at all Shingle Creek sites.

Rainfall and FIB concentrations were significantly correlated for data pooled from all Shingle Creek sites. Log_{10}-transformed *E. coli* concentrations were significantly correlated with antecedent rainfall 24 hr, 48 hr and 5 days prior to sample (Pearson r = 0.593, 0.547 and 0.460, respectively). In contrast, log_{10}-transformed enterococci concentrations were significantly correlated only with 24 hr antecedent rainfall (r = 0.406). Figure 3.5 shows mean *E. coli* concentrations at Shingle Creek sites with rainfall levels superimposed on the graph. Figures 3.3 and 3.4 provide an overview of mean *Enterococcus* levels and MST marker detection at the frequently sampled Shingle Creek sites (Fig. 3.3) and all sampled sites (Fig. 3.4).

Microbial Source Tracking Marker Analysis

As expected, the general *Bacteroidales* (GenBac) and fecal *Bacteroides* (FecBac) markers were detected most frequently at all sites (Table 5), as they are general fecal markers and are not indicative of a specific host group. GenBac was detected in 100% of samples at levels ranging from 1190 – 5.7×10^6 gene copies/100 ml. FecBac detection rates at the various sites ranged from 40% (SC7) to 100% (at eight of eleven sites in which the analyses were performed), at levels up to 1.4×10^8 gene copies/100 ml. Overall, FecBac was detected in 92% of samples in which the analysis was conducted.

The human-associated BacHum marker, which shows some cross-reactivity to certain other host species including dogs, was detected at all sites at frequencies ranging from 80% to 100%. Concentrations ranged from undetectable (0) to 1.9×10^5 gene copies/100 mL. Overall, BacHum was detected in 90% of samples in which the analysis was conducted.

The more human-specific HF183 was detected in far fewer samples than BacHum; however, it was detected in 16 samples (25%) at SC2, SC5, SC8, SC9, SC11, SC12, SC14 and SC15.

Detectable concentrations ranged from 335 – $2.7x10^7$ gene copies/100 ml. The discrepancy between detection of BacHum and HF183 could be due to greater robustness of the BacHum assay with respect to PCR inhibition, and/or to greater human specificity of the HF183 assay.

Human polyomaviruses, the most human-specific of the MST markers used in this study, were detected on eight occasions (13% of all samples). HPyVs were detected at SC2, SC4, SC8, SC11, and SC12 at concentrations ranging from 60-450 gene copies/100 ml. Because they are viruses, which are much smaller than bacteria, their fate and transport in the environment may differ substantially from that of bacteria. This difference may be useful for predicting human health impacts from contaminated water, as over 50% of the incidents of gastroenteritis are estimated to be caused by viruses.

The *Catellicoccus* gull/shorebird marker was also sporadically detected, once at SC4, SC5, and SC14. Overall, the gull marker was detected in 6% of samples; however, high levels of the marker were noted in these samples ($4.5x10^8$ -$2.2x10^9$ gene copies/100 ml), indicating recent and concentrated contamination from bird sources.

Shingle Creek Summary: This tidal zone stream and its tributaries are more heterogeneous, much larger, and more complex thanMoore's Creek in many ways. There are older economically depressed neighborhoods as well as newer upscale housing developments, and human-origin pollution appeared likely at some locations on initial sampling trips throughout the watershed. Some tributaries and sections of the stream showed evidence of substantial nutrient enrichment that could be either septic or sewage in origin. The older neighborhoods are on sewer, but that may or may not be the case for every single home. Dogs are also a possible contributor to fecal contamination, but that was not quantified over the course of this study. Livestock is a very minor potential contributor to Shingle Creek. Wildlife/birds are strong possibilities at every site in Shingle Creek (especially the tributaries that originate in the Dismal Swamp), and there was evidence of beavers, raccoons, and deer at certain sites outside the boundaries of the swamp. At some locations the stream was in bad condition from accumulated trash and junk, a problem throughout much of the stream watershed. It could be possible, but is unlikely, that homeless populations are playing a role in the human fecal contamination quantified in this system. Lastly, two tributaries emerge from the Dismal Swamp, and these tributaries are tea colored due to tannins in the water, which can be a complicating factor with microbiological and source tracking methods. Tannic and humic acids can cause inhibition of the QPCR reaction used for quantification of the molecular markers, thereby hampering quantification of the intended target.

Overall analysis of the results indicated that priority intensive sampling should be focused on SC8, SC9, and SC11. Lower priority but still important for future sampling are SC2, SC4, SC5, SC7, SC12, SC14, and SC15, and low priority (reduced sampling) are SC1, SC3, SC6, SC10, and SC13. Until SC8, SC9 and SC11 are addressed, there appears to be little need to sample sites downstream that are impacted by SC8, SC9, and SC11 (SC2, SC4, SC5, and SC7). Repeated stream walks and intensive sampling above and around SC8, SC9, and SC11 (along the sewer line right-of-way) has resulted in a complicated situation regarding potential sources of pollution. These efforts have eliminated several possibilities such as the stormwater pipes under County Road and numerous seeps between Locust Drive and Factory Road. There is a gravity main in the area of SC9 and the stream disappears under an older neighborhood a short distance above

SC9. There is a small wetland area on the far side of the neighborhood but it does not contain running water during dry weather. The watershed and possible sources of pollution in the general vicinity of SC8 and SC9 should have the highest priority in the future.

Image 5: Selected Major Sampling Sites on Shingle Creek

Image 6: Map of Sampling Locations within the Shingle Creek Watershed

4.0 Lessons Learned and Recommendations

A need for a tailored approach: On virtually any watershed-level project, it is necessary to conduct sampling over a range of weather conditions as groundwater, surface waters, and tidal flux will all behave differently over the course of seasons. Stakeholders should be involved in the project as early as possible to gain support, identify potential volunteers, benefit from their local knowledge, and obtain assistance and access to private property for sampling if necessary.

This project involved three very different tidal stream systems, Mill Dam Creek, Moore's Creek and Shingle Creek, and the necessity of sampling over a range of both seasonal weather and tidal conditions became evident over time as results accumulated, please see the individual system sections for a full set of results and discussion.

Involve stakeholders and the public early: Another reason to involve stakeholders in watershed projects is property access. Samples collected at public access areas or right-of-ways will likely not be sufficient once high FIB count sampling sites are identified and intensive sampling at locations upstream of those sites is needed. Water quality agencies can expect to do the initial screening and the later, more intensive sampling, over both wet and dry events. Once this is accomplished, the areas with low FIB concentrations can be given lower priority and more intensive sampling can then be conducted on the areas with high concentrations of FIB.

Mine FIB data for relationships to external forcing agents such as rain, wind and tide: It is vital to return to existing FIB data and mine it for existing relationships to rainfall, tidal stage, or events. This can help identify how strongly high FIB concentrations are correlated with rainfall or tidal events, and precipitation amounts - and help focus on the optimal times to schedule sampling events. In both Moore's Creek and Shingle Creek, precipitation analysis demonstrated a strong, statistically significant correlation between FIB concentrations and rainfall and, in combination with the FIB and source tracking results, was largely responsible for the ranking of sites by priority as described in the above section.

Even instantaneous flow measurements or estimates can be helpful: It is very helpful to have flow information accompanying grab sampling efforts if possible. In a large watershed with many inputs, this can help to prioritize mitigation efforts. The reason that this is important is that for a large body of water such as Shingle Creek, tributaries and freshwater inputs of differing levels of stormwater discharge drive the loading of fecal contamination. A very small, low discharge tributary could have chronically high concentrations of FIB, but low discharge causes it to be unimportant in total loading to the system and subsequent mitigation actions will not be fruitful. Flow estimates, when combined with FIB data, can be used to roughly calculate FIB loadings. This can help eliminate possible sources that are not present in sufficient numbers to account for the loading estimates. On Shingle Creek, plans are underway to install an automated sampler that can also measure flow rates adjacent to either Factory Road or County Road. This will provide information of flow/discharge and also permit collection of samples automatically during storm events. This is very important on Shingle Creek as water levels can rise and overflow the stream banks very quickly, creating dangerous conditions for anyone attempting to sample during those times. Flow rates and FIB data from samples collected over the duration of precipitation events will be very important in pinpointing the sources of the FIB. It will also be

important to analyze these samples for the presence of the human-source markers, specifically over the course of a hydrograph, to indicate the relative importance of overland stormwater flow versus groundwater contamination. Unlike Shingle Creek, the priority sites on Moore's Creek can be safely sampled (with caution) during precipitation events. There is no clear need for automated equipment (or a place to use it) on Moore's Creek for the priority sites (MC1, MC3, and MC9). However, instantaneous flow estimates at these sites would still be very helpful. To do this, hand-held flow meters can be used, and if the stream width and average depth can be measured (or estimated) when flows are measured, then a reasonable figure for flow rates can be determined. Like Shingle Creek, the flow rates, when combined with FIB results, can help eliminate possible sources that are not present in sufficient numbers to account for the loading estimates. Loading assessments are already being conducted on Mill Dam Creek, with flow/discharge assessment currently conducted at all three Mill Dam Creek Sites A, D, and F.

If possible, take multiple samples over the course of storm events: It would be very helpful to take multiple samples through storm events, or optimally to conduct stormwater assessments using ISCOs if at all possible. The most critical period for storm events is prior to the event (for a baseline) and then during the event, it is important to capture data during the rising limb of the hydrograph, the falling limb of the hydrograph, and during the tail discharge. By taking multiple samples over the course of a storm, one can visualize the delivery of microbial contaminants during a range of hydrological conditions, e.g. rising limb, peak, falling limb and tail of the hydrograph. Samples taken during each of these times can be interpreted in a series to describe the means of microbial contaminant delivery. All stormwater BMPs are designed on the premise of a specific capture of a specific part of the hydrograph, so samples captured over the course of a storm can be extremely powerful in eliminating BMPs of a certain design from consideration. For example, if in a specific watershed, the delivery of microbial contaminants is solely during the falling limb and tail of a storm event, a BMP such as a retention pond will not succeed in removing contamination. This is because a retention pond is designed to capture the first portion of the hydrograph (usually designed to capture the first inch of runoff), but is not designed to capture the runoff past this point.

The importance of using molecular tools to generate QUANTITATIVE microbial source tracking data: Quantitative MST analyses, using well chosen molecular markers can permit quantification human and animal fecal contamination, and is most powerful combined with full sanitary survey approaches and cooperation from stakeholders. The differentiation between animal and human in locations with high FIB concentrations can greatly assist in actually identifying and mitigating the source of the FIB. Quantification of a persistent human fecal contamination signal helped identify Shingle Creek sites 8, 9, and 11 as high priority, resulting in further investigation of the gravity main in that location. Quantitative molecular marker data, paired with loading estimates, is the way to conduct assessment of a system for TMDL implementation. This permits prioritization of problem areas, and can be used to conduct load reduction plans in areas with specific large scale contamination problems.

Conduct an assessment of reservoir populations of FIB: Reservoir populations of FIB, which may be particularly elevated in sediments, are likely to play a role in the estuarine and coastal environments studied. Summertime temperatures of these systems, low levels of UV light that penetrate the depths of these systems (because they are shallow and generally well mixed), and

relatively high concentrations of labile DOM in the estuaries of the Virginia Beach region could all contribute to FIB persistence. These populations could play a role in the delivery/resuspension of FIB to estuarine environments in the absence of fresh fecal contamination, thereby causing a disconnect in management needs and health risks, particularly if pathogen survival does not track with that of FIB. It may be appropriate to include sediment sampling in the projects, adding sediment collection to the samplings done at in open areas with high FIB concentrations. This type of work is currently being conducted in the Broad Bay section of the Lynnhaven Watershed, and the results will be made available to stakeholders shortly.

Animal fecal contamination as a major source in the watersheds studied: Birds may be playing a role in contamination of Hampton Roads estuaries, but the contamination levels observed during our study is not likely to singularly explain the high FIB concentrations observed in these systems. Fecal contamination stemming from birds was quantified sporadically during this study, and the quantification of these markers would not have explained much in the way of FIB contamination to the system.

In a fast-paced field, try to keep up with scientific advancements: The stormwater compliance/TMDL development and implementation/ and microbial source tracking fields are moving at a very rapid pace. Additional relevant guidance can be obtained from recent case studies that have been conducted in the MST arena.

The successful application of source-specific markers is very much a developing field of science. For example, the Southern California Coastal Water Research Project (SCCWRP) recently tested 42 source tracking methods at 25 different labs around the country. Standardization of particularly successful methods is also an ongoing effort in the SCCWRP. Be on the lookout for a special issue of the Journal Water Research that will include publications from this important study. The lead article for the special issue (draft form at present) is:

Boehm, Alexandria B., John F. Griffith, Patricia A. Holden, Jenny A. Jay, Orin C. Shanks, Laurie C. Van De Werfhorst, Dan Wang, and Stephen B. Weisberg. 2012. Performance of forty-two microbial source tracking methods: a twenty-five lab evaluation study. Draft form, to be published in Water Research.

Two of the case study chapters within Hagedorn et al., (2011) contain lessons that are pertinent to this Hampton Roads Planning District Commission supported project.

Ch. 19. Case Studies of Urban and Suburban Watersheds, by Cheryl W. Propst, Valerie J. Harwood, and Gerold Morrison.

This chapter described the Weight-of-Evidence Approach (WOE) that allows MST methods to be highly focused, but used only on an as-needed basis. The WOE approach involves categorization of sites by microbial water-quality assessment (MWQA). It includes explanation of how to develop a priority ranking (as was done for Moore's Creek and Shingle Creek), the establishment of most probable source categories, and how recommended management options are arrived at for each sampling site in a watershed-level project. Six sub-basins in the

Hillsborough River watershed (FL) were included examples of the WOA approach. Pertinent conclusions were that local knowledge and agency "buy in" are essential for project success; some sources can be obvious, but many will not be, and it takes a lot of field time and sampling (labor intensive) to trace sources to specific points of origin. One small cross-connection or faulty lift station, or chronic SSO can impact a large area, but there is a high success rate in finding such sources. Lastly, the authors pointed out that there are not many situations in the scientific literature where changes were made and then subsequent sampling was performed to assess the impact of the changes.

Ch. 21. Source Tracking in Australia and New Zealand: Case Studies, by Warish Ahmed, Marek Kirs, and Brent Gilpin.

This chapter describes twelve case studies in Australia and New Zealand. All were expertly done and could serve as models on how to design and implement watershed-level studies. Like the U.S., the chapter illustrates the evolution of MST methods away from library-based approaches to library independent protocols over time. Unlike the U.S. however, the authors use both the fecal stanols and sterols (and their ratios), but admit that the unusual wildlife found in those two countries (and their very different fecal sterols) does enhance the utility of that approach. However the fecal sterols and stanols are also regularly used in Europe. Lastly, the authors found strong regional differences in the various DNA markers that they tested (especially with specificity) – a warning as we continue in the U.S. to search for sets of markers that will be suitable anywhere

5.0 Conclusions:

The conclusions for this project are presented in the context of the original goals stated for the project in the study plan. After each stated goal, a summary of accomplishments is presented.

1. Identify a potential suite of methods and approaches that will be useful in conducting microbial source tracking work in the Hampton Roads region:

As a project team, we were particularly successful in this endeavor. Typically a project team that is applying MST to a new region must spend a minimum of 6 months to identify the appropriate molecular markers for use in a given set of watersheds. The preliminary work conducted by PI Noble in the Mill Dam Creek area, and also the Fecal Contamination Blind Study led by the City of Virginia Beach permitted the project team to hit the ground running with the use of a trio of three carefully selected Bacteroidales based markers (Fecal Bacteroides spp., BacHum, and Human Specific HF183), along with quantification of Human Polyoma Virus, Gull fecal contamination, and the General Bacteroides marker. These selected molecular agents/markers permitted the project team to quantify fecal contamination over a range of sensitivities and specificities. For further information on this topic, please refer to Hagedorn(2011), and citations presented in the methods section.

2. Identify the sources and quantify the loading of bacterial contamination using a multi-tiered approach at three locations within the Hampton Roads Planning District's Jurisdiction, focusing specifically on Moore's Creek, Shingle Creek, and Mill Dam Creek

As a result of the project, we were able to identify and quantify human fecal contamination in all three watersheds using a multi-tiered approach. The first tier was based upon quantification of fecal indicator bacteria (FIB), with only the samples that exceed water quality standards being selected for further analysis using MST based methods. The second tier was the use of the Bacteroidales based markers, and the third tier was quantification and confirmation of human fecal contamination via the Human Polyoma Virus assay, and assessment of a gull fecal contamination signal. In constructing the original study plan, the project team was optimistic about the use of loading assessments, which are vital for prioritization of contaminated systems, and BMP selection. However, during the course of this study, loading was not assessed in any of the three watersheds because of lack of available flow/discharge information. There was a significant attempt made by the project team to assess the existing relationships of the FIB and molecular markers to rainfall amounts, which is presented in the results summary section for each watershed. Currently, work is being conducted in both Mill Dam Creek and Shingle Creek to assess loading and this information will be a vital step towards mitigation of sources. Loading assessments are a vital step toward prioritization of watersheds and BMP selection, or upstream mitigation strategies.

3. Characterize, over relevant time scales, patterns of bacterial concentrations and identify "hot spots" of high FIB levels during both dry and wet weather.

The project team succeeded in characterizing the patterns of FIB based and source based contamination in each of the three watersheds, and those results are presented in the results summary sections. This assessment showed that there are locations within each watershed that are problematic in terms of a chronic human fecal contamination signal. Sites such as Mill Dam Creek Site A, Shingle Creek Sites SC8 and SC9, and Moore’s Creek MC3 are good examples of

locations that exhibited human fecal contamination in a predominant portion of samples tested. The project team succeeded in identifying hot spots, and worked with each watershed sampling crew to try and focus on these problem locations over the course of the project. This was an iterative exercise, due to the ephemeral nature of rainfall and ephemeral nature of over-land delivery of contamination. In the Mill Dam Creek and Shingle Creek watershed, the project successfully permitted honing on a specific set of problem locations (MDC Sites A, D, and F), and Shingle Creek SC8 and SC9, where further work is being conducted. In Moore's Creek the patterns of contamination were less consistent, although there is some indication that further work in the region of MC3 would be prudent as an assessment for the potential impact of failing septic systems during major events.

4. Determine the presence of, and quantify markers of human fecal contamination, and establish whether such contamination at specific sites is chronic or intermittent.

This aspect is addressed in conclusion point #3. The project team was able to successfully quantify human fecal contamination in all three watersheds, and was able to relate that quantitative information to rainfall patterns and to permit prioritization of the watersheds on specific locations. The contamination signal was more consistent in specific locations such as Mill Dam Creek Site A, and Shingle Creek SC8 and SC9, but still showed patterns of delivery matched by size and duration/intensity of storms. To this end, loading assessments will be vital to understanding the actual delivery of contamination at specific sites.

5. If non-human contamination is found, attempt to partition the fecal contamination signal stemming from animal populations, particularly birds and pets.

Since human fecal contamination was quantified, to differing extents, in all three systems, we focused more specifically on quantification and assessment of this signal. However, we did quantify the contamination stemming from gulls in all three watersheds as a component of this project. Gull fecal contamination was not the focus of this project, so analysis was conducted on only a subset of samples. We found that gull fecal contamination was quantified specifically in all three watersheds, but only on an ephemeral basis. Gull fecal contamination was quantified in Shingle Creek at sites SC4 and SC5 in May, 2010, and at SC14 in July 2011. Gull fecal contamination was also quantified in Mill Dam Creek, at sites A, F, J, I, and G, during the spring/summer of 2011. Gull fecal contamination was quantified in Moore's Creek at Sites MC7, MC9, and MC10 during fall 2010, which may have coincided with the appearance of migratory bird populations. None of the gull fecal contamination signals quantified were extensive enough to explain all of the FIB contamination observed at these locations, therefore, there has been no active recommendation made about bird control measures over the course of this project.

6. Provide a framework for recommending appropriate actions to reduce sources of fecal contamination.

We have presented a "Lessons Learned" section (Section 4.0) that actively describes further work that can be conducted in the Hampton Roads region, and how this work can benefit from the groundwork laid by this collaborative effort. The framework that is presented as far as recommendations is location-specific. That is, future loading studies to be conducted at each Mill Dam Creek and Shingle Creek are recommended (and ongoing), because contamination has been quantified at specific locations, permitting focused loading assessments. The framework for action in Moore's Creek is more investigation and assistance based. That is, the project team

will continue to work actively with the Stormwater Personnel from York County and will try to guide their future investigations into this area. At this time, however, there is no specific action that will be taken in this location.

In additional to accomplishing the stated scientific goals for this project, it is also important to document the effort that the project team expended in meeting regularly with the stakeholder groups within the Hampton Roads Planning District Commission, with presentation of up to date research findings. The research team worked hard to interact closely with the members from each specific watershed/agency to address their specific needs and to accommodate their limitations in terms of sampling and resources. This permitted each group to benefit as much as possible from a tailored research implementation approach. The project team regularly conducted site visits and assessments of sampling locations and delivered regular presentations regarding sampling framework and execution. This also permitted modifications to existing study designs.

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APPENDIX A
METHODS

Fecal Indicator Bacteria Methods:
Fecal indicator bacteria were analyzed by the microbiology laboratory at Hampton Roads Sanitation District. Concentrations of *E. coli* and *Enterococcus* spp. were measured using both defined substrate technology using kits supplied by IDEXX Laboratories, Inc. (Westbrook, ME) according to the manufacturer's instructions, and by membrane filtration on defined media, using EPA method 1603 (modified mTEC agar) for *E. coli*. For each sample collected in the three watersheds, 25 ml volumes were filtered through each of four polycarbonate 0.4 um filters, and the filters placed aseptically into 2.0 ml screw cab tubes and immediately frozen at -80 C.

Method 1603: *Escherichia coli* (*E. coli*) in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC)

EPA method 1603 was used to analyze Mill Dam Creek *E. coli* samples. Sample dilutions of either 0.1 mL, 1 mL or 10 mL were filtered through a 0.45 um filter and placed on modified mTEC agar. The agar was incubated at 35°C +/- 0.5^{o}C for 2 +/- 0.5 hours to resuscitate injured or stressed bacteria, and then incubated at 44.5^{o}C +/- 0.2^{o}C for 22 +/- 2 hours. E. coli colonies on mTEC agar are red or magenta in color after the incubation period. The number of colonies on each membrane was counted and calculations were performed to determine the *E. coli*/ 100 mL.

Count per 100 mL = (No. of colonies counted/Volume of sample filtered, in mL) x 100

The targeted range for colonies is 20-80 colonies per membrane. Multiple dilutions were planted for each sample in order to try to meet this criterion. All membranes for each sample that met the acceptable range of 20-80 colonies were counted and averaged if there was more than one. If there were no membranes for a sample that met the acceptable range of 20-80 colonies, the calculation was based on the most nearly acceptable plate count.

The following Quality Control samples were analyzed with each batch of samples according to EPA 1603 requirements:

TSA (Tryptic Soy Agar) Media Sterility
Filter Sterility Check
Filtration Blank
mTEC Method Blank (one analyzed before and one after samples)
mTEC Media Sterility
Positive and Negative Controls

IDEXX – Colilert 18

The IDEXX Colilert-18 method was used to analyze Shingles Creek and Moore's Creek *E. coli* samples. Sample dilutions of either 1 mL or 10 mL were analyzed using the Defined Substrate Technology. The sample was mixed with a Colilert-18 snap pack reagent, shaken until dissolved, poured into a Quanti-Tray/2000 and sealed in an IDEXX Quanti-Tray Sealer. The Quanti-Trays were incubated at 35 +/- 0.5^{o}C for 18 – 22 hours. Using a UV light, the numbers of large and small fluorescent wells were counted. When *E. coli* metabolize Colilert-18's nutrient

indicator (MUG), the sample fluoresces. Based on the number of positive fluorescent wells and sample dilution, the Quanti-Tray/2000 MPN table was used to calculate the number of *E. coli* MPN/100 mL.

The following Quality Control samples were analyzed:

Method Blank (Per Batch)
Positive and Negative Controls (Per Lot)

IDEXX – Enterolert

The IDEXX Enterolert method was used to analyze *Enterococci* samples. Sample dilutions of either 1 mL or 10 mL were analyzed using the Defined Substrate Technology. The sample was mixed with an Enterolert snap pack reagent, shaken until dissolved, poured into a Quanti-Tray/2000 and sealed in an IDEXX Quanti-Tray Sealer. The Quanti-Trays were incubated at 41 +/- 0.5^{o}C for 24 – 28 hours. Using a UV light, the numbers of large and small fluorescent wells were counted. When *Enterococci* metabolize Enterolert's nutrient indicator, the sample fluoresces. Based on the number of positive fluorescent wells and sample dilution, the Quanti-Tray/2000 MPN table was used to calculate the number of *Enterococci* MPN/100 mL.

The following Quality Control samples were analyzed:

Method Blank (Per Batch)
Positive and Negative Controls (Per Lot)

Human Polyomaviruses QPCR

Up to 500 ml of water was filtered through 0.45 µm nitrocellulose filters for HPyVs analysis after the pH of each sample had been adjusted to 3.5 with 1.0 N HCl (Harwood et al., 2009). Using sterile forceps, each filter was carefully folded and placed into a PowerBead tube from the PowerSoil™ DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) and frozen at -80^{0}C to be processed later. DNA was extracted generally following the manufacturer's instructions, except for minor modifications. After adding the first solution for cell lysis (buffer C1), the PowerBead tubes were shaken vigorously using either a bead beater (FastPrep FP120, Thermo Savant) at the speed of 4.0 m/sec for 40s or a Vortex mixer with an adaptor for 2 mL tubes (MO BIO Laboratories # 13000-V1) at maximum speed for 10 min. At each step of the procedure, the entire supernatant volume was carried forward, requiring the volumes of buffers C3, C4 and C5 to be increased to 285µl, 1.2ml and 750µl respectively.

QPCR primers and probes (TaqMan) targeted the conserved T antigen of HPyVs. Primers were SM2 (AGT CTT TAG GGT CTT CTA CCT TT) and P6 (GGT GCC AAC CTA TGG AAC AG), and the probe was (FAM)-TCA TCA CTG GCA AAC AT-(MGBNFQ). Reactions were composed of 12.5 µl TaqMan® 2X Universal PCR Master Mix No AmpErase® UNG, 200 µM BSA, 3 µl of primer/probe mix, and 5 µl template (total volume of 25 µl). PCR reactions were carried out in 96-well plates using the Applied Biosystems 7500 Real Time PCR System

(Carlsbad, CA). All samples were run in triplicate and for each target, three no template controls (NTCs) were included. Thermocycler settings were 50° C for 2 minutes, 95° C for 10 minutes, and 45 cycles of 95° C for 15 seconds and 60° C for 1 minute for all targets. The analytical limit of detection for gene copies within a PCR reaction is 1-5 gene copies, while the limit of quantification is 10-100 gene copies.

Fecal Bacteroides QPCR:

We have the capacity to use the already developed, tested and optimized, rapid QPCR method for fecal *Bacteroides* QPCR (Converse et al. 2009) which is an exciting candidate for an alternative indicator of fecal contamination. *Bacteroides* spp. comprise approximately one-third of the human fecal microflora, considerably outnumbering *Enterococcus* spp. and *E. coli*. The *Bacteroidetes* group belongs to a group of nonspore forming, gram negative, obligate anaerobes, so there is little concern over regrowth in the environment. More importantly, a range of human and animal specific *Bacteroides* sp. markers have been developed increasing the value of this potential indicator (e.g. Bernhard and Field 2000, Carson et al. 2006). The species *B. thetaiotamicron* is highly abundant in human fecal waste, has been demonstrated to be tightly related to the presence of human fecal contamination, and is typically found in very low numbers or not at all in animal feces (Wang et al. 1996). Finally, bacterial markers such as *Bacteroides* spp. have been shown to be potentially useful source tracking tools. In Griffith et al. (2003) the *Bacteroides sp.* markers correctly identified human sources of fecal pollution when present in mixed water samples delivered blind to the laboratory. As a target and potential alternate indicator, the utility of *B. thetaiotamicron* has been previously demonstrated (Carson et al. 2006). In the Noble lab, we have developed and optimized a rapid QPCR assay that is specific, accurate, and quantitative for this target (other publications have generally utilized conventional PCR for this target). We have shared this assay with Rich Haugland at USEPA, where the assay has been tested for specificity and for relation to potential human health effects through the analysis of previously collected epidemiology study samples (from the Great Lakes), showing highly promising results (Noble et al. in preparation). In NC, we originally pursued the use of an alternative *Bacteroides*-related probing system because we identified significant cross-reactivity using the other *Bacteroides sp.* human and cow specific markers developed by Bernhard and Field (2000) with other types of animal scat (Noble et al. 2006). Layton et al. (2006) discussed similar findings when trying to translate existing *Bacteroides* markers into a QPCR format (Layton et al. 2006). In eastern NC, and in urban stormwater outfalls of southern California, *B. thetaiotamicron* has been shown to be present in high concentrations in human impacted areas and at baseline levels in areas where human fecal contamination was demonstrated to not be present (Converse et al. 2009). We have successfully employed the use of two other Human specific Bacteroidales assays in order to use a tiered approach for confirmation of human contamination. These assays include the BacHum assay reported by Kildare et al. (2007) and the original human specific conventional PCR assay of Bernhard and Field (2000). All three assays, the fecal Bacteroides QPCR, the BacHum QPCR, and the human specific conventional PCR assays will be used in conjunction with one another to provide unrivalled power to confirm the presence and quantities of human fecal contamination.

Human Viral Pathogens: (Human enteroviruses):
Of the enteric viruses, human enteroviruses are one of the most commonly detected viruses in polluted waters (Rusin et al. 2000). Enteroviruses are members of the *Picornaviradae* family and are estimated to cause 30 million to 50 million infections per year with 30,000 to 50,000 of these resulting in meningitis hospitalizations (Oberste et al.1999). PIs Fuhrman and Noble have conducted extensive enterovirus research in southern California storm and marine waters, and have demonstrated that enteroviruses are a useful tracer of human fecal contamination in this urban setting. Routine monitoring of FIB in environmental waters has demonstrated weak or non-existent predictive relationships to enteroviruses and other human pathogens, suggesting a need for viral pathogen specific assays in environmental waters (Noble and Fuhrman, 2001, Jiang et al. 2001, Jiang and Chu, 2004). Human polyomaviruses are generally nonpathogenic and are widespread in the human population. These viruses, which have a DNA rather than an RNA genome, are shed primarily in urine but also in feces, and are ubiquitous in sewage worldwide. A PCR assay developed to simultaneously detect the two dominant types, JCV and BKV, was employed with success in a water quality study in Florida, where two other human-associated microbial source tracking markers were found to be correlated with its presence (McQuaig et al., 2006). Recently, a Taqman® QPCR assay for JCV and BKV was developed in our laboratory that has high sensitivity for human sewage and very high specificity (no false positives from 127 animal samples tested) (McQuaig et al 2008). Because this virus is present in over 50% of healthy individuals, it is a much more prevalent target in sewage and contaminated waters than pathogenic viruses. Because it is a virus, it shares fate and transport characteristics with other human viruses, and may be a superior tracer of viral pathogens compared to bacterial or other microbial or chemical targets.

Gull QPCR Method
QPCR primers and probes (TaqMan) targeted a portion of 16S rRNA gene of Catellicoccus marimammalium. Primers were Gull-2F (TGC ATC GAC CTA AAG TTT TGA G) and Gull-2R (GTCAAAGAGCGAGCAGTTACTA), and the probe was (FAM)-CTG AGA GGG TGA TCGGCC ACA TTG GGA CT -(MGBNFQ). Reactions were composed of 12.5 µl TaqMan® 2X Universal PCR Master Mix No AmpErase® UNG, 200 µM BSA, 0.225 µl of forward primer, 0.225 µl of reverse primer, 0.075 µl of probe, and 5 µl template (total volume of 25 µl). PCR reactions were carried out in 96-well plates using the Applied Biosystems 7500 Real Time PCR System (Carlsbad, CA). All samples were run in triplicate and for each target, three no template controls (NTCs) were included. Thermocycler settings were 95° C for 15 minutes, followed by 44 cycles of 95° C for 15 seconds and 62° C for 1 minute for all targets. The analytical limit of detection for gene copies within a PCR reaction is 1-5 gene copies, while the limit of quantification is 10-100 gene copies.

General Bacteroidales QPCR Method
QPCR primers and probes (TaqMan) targeted a portion of 16S rRNA gene of the order Bacteroidales. Primers were GenBacF (GGG GTT CTG AGA GGA AGG T) and gENbACr (CCG TCA TCC TTC ACG CTA CT), and the probe was (FAM)-(CAA TAT TCC TCA CTG CTG CCT CCC GTA -(TAMRA). Reactions were composed of 12.5 µl TaqMan® 2X Universal PCR Master Mix No AmpErase® UNG, 200 µM BSA, 3 µl of primer/probe mix, 2.5 µL of 2mg/ml BSA solution, and 5 µl template (total volume of 25 µl). PCR reactions were carried out in 96-well plates using the Applied Biosystems 7500 Real Time PCR System (Carlsbad, CA).

All samples were run in triplicate and for each target, three no template controls (NTCs) were included. Thermocycler settings were 50° C for 2 minutes, 95° C for 10 minutes, and 45 cycles of 95° C for 15 seconds and 60° C for 1 minute for all targets. The analytical limit of detection for gene copies within a PCR reaction is 1-5 gene copies, while the limit of quantification is 10-100 gene copies.

APPENDIX B
Figures and Tables

Figure 1.1 Results for *E. coli* and source markers by site at Mill Dam Creek

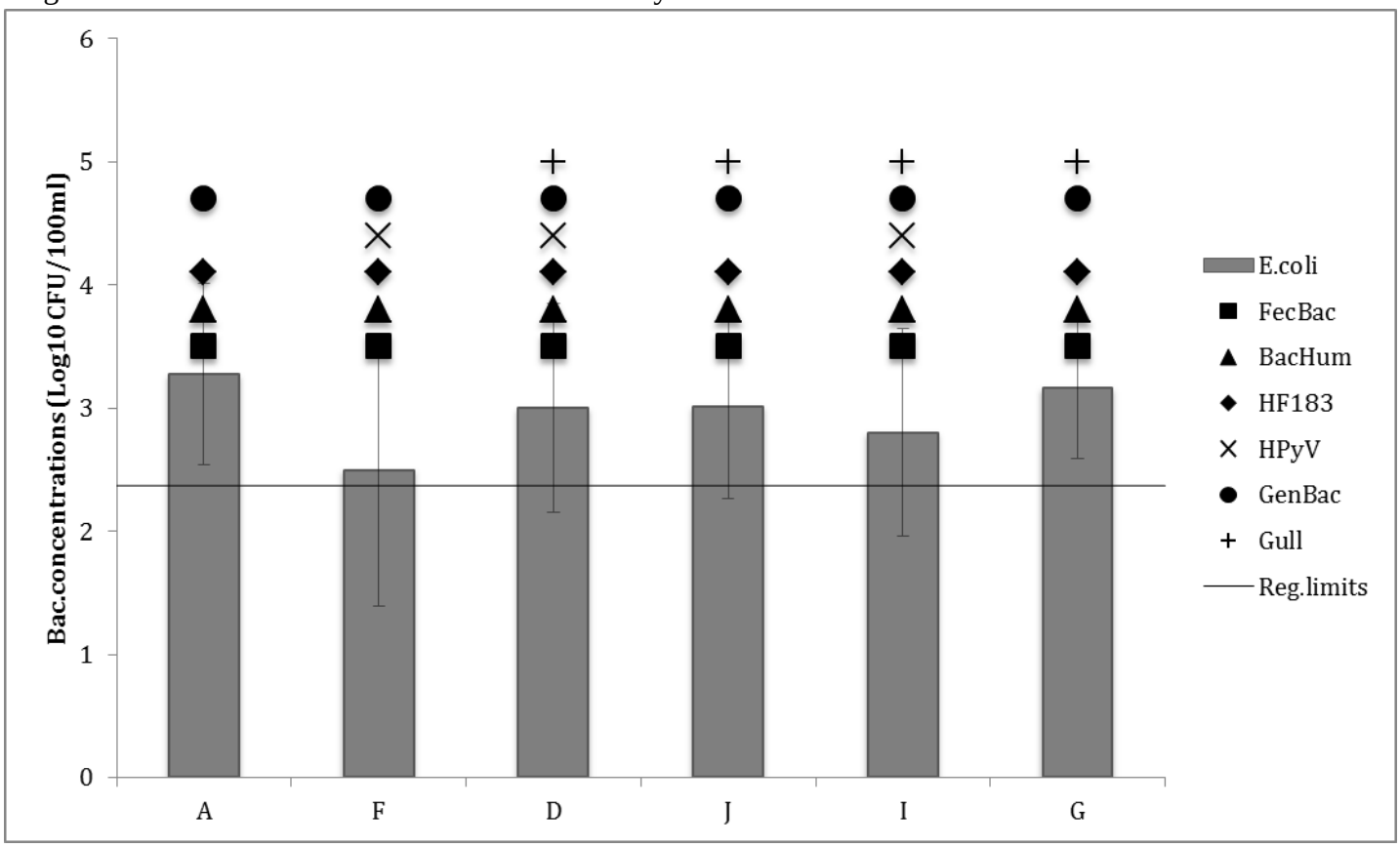

Figure 1.2 Results for *Enterococcus* and source markers by site at Mill Dam Creek

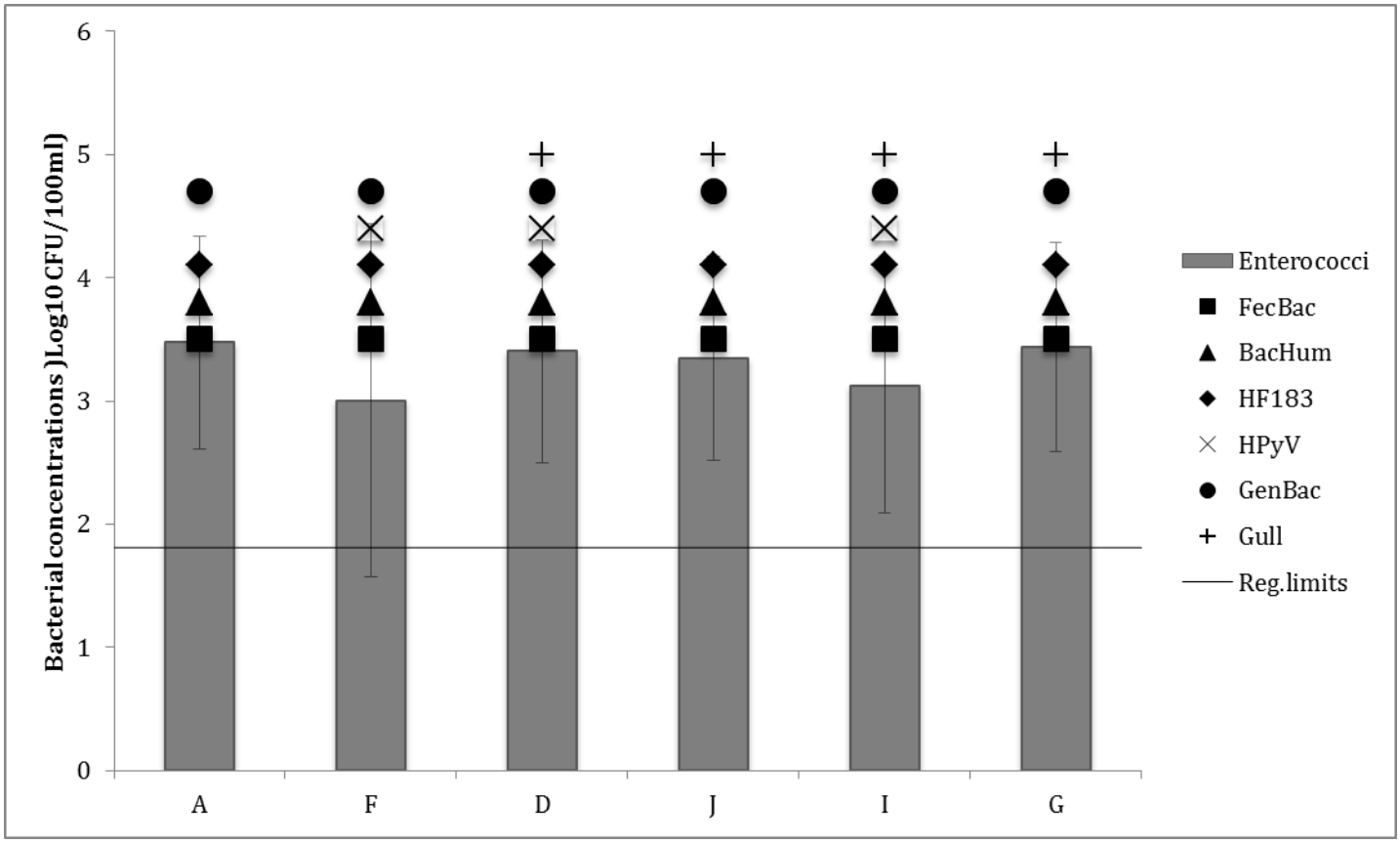

Figure 1.3 Results for *Enterococcus*, *E. coli*, rainfall, and source markers at Mill Dam Creek, Site A

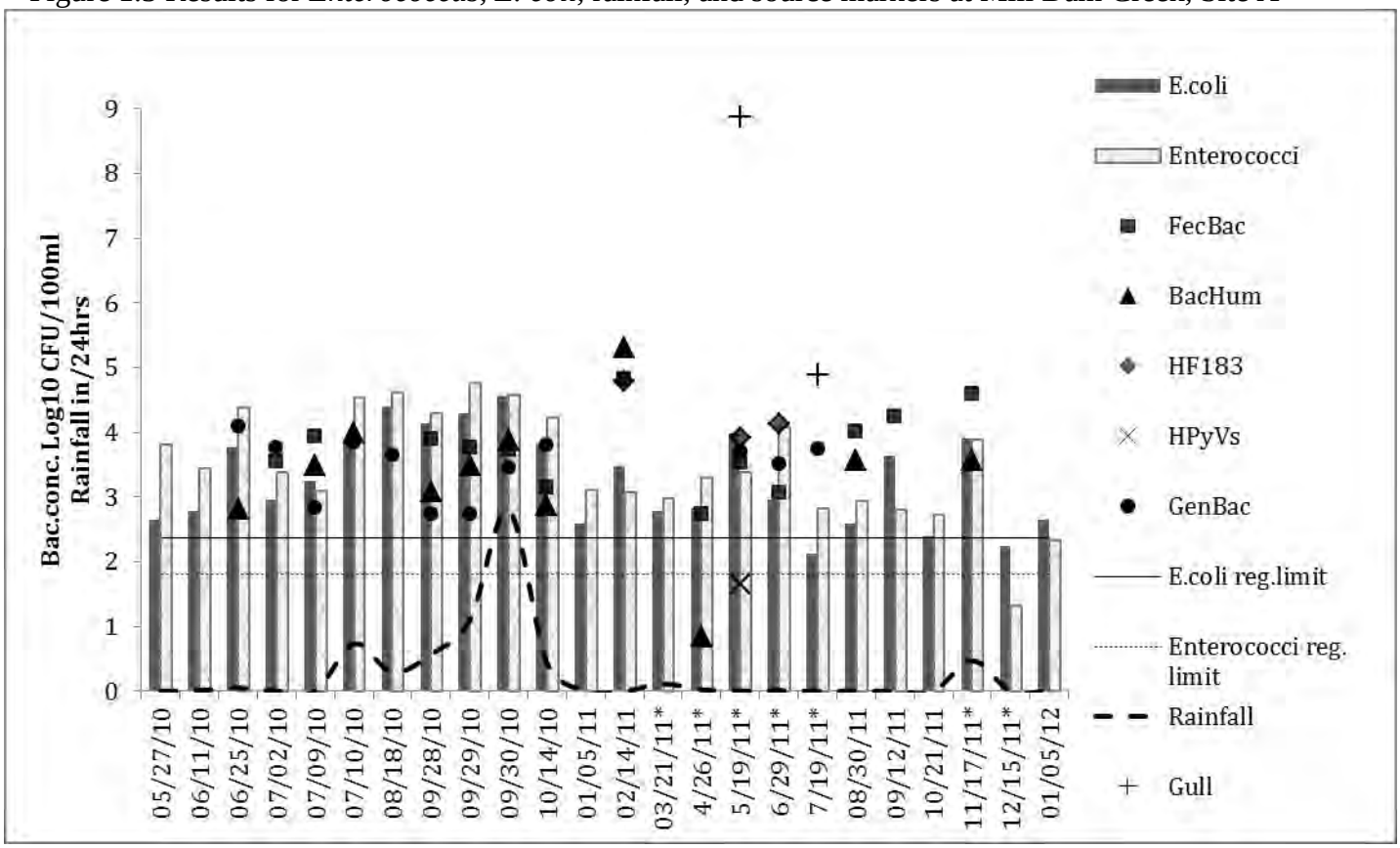

Figure 1.4 Results for *Enterococcus*, *E. coli*, rainfall, and source markers at Mill Dam Creek, Site D

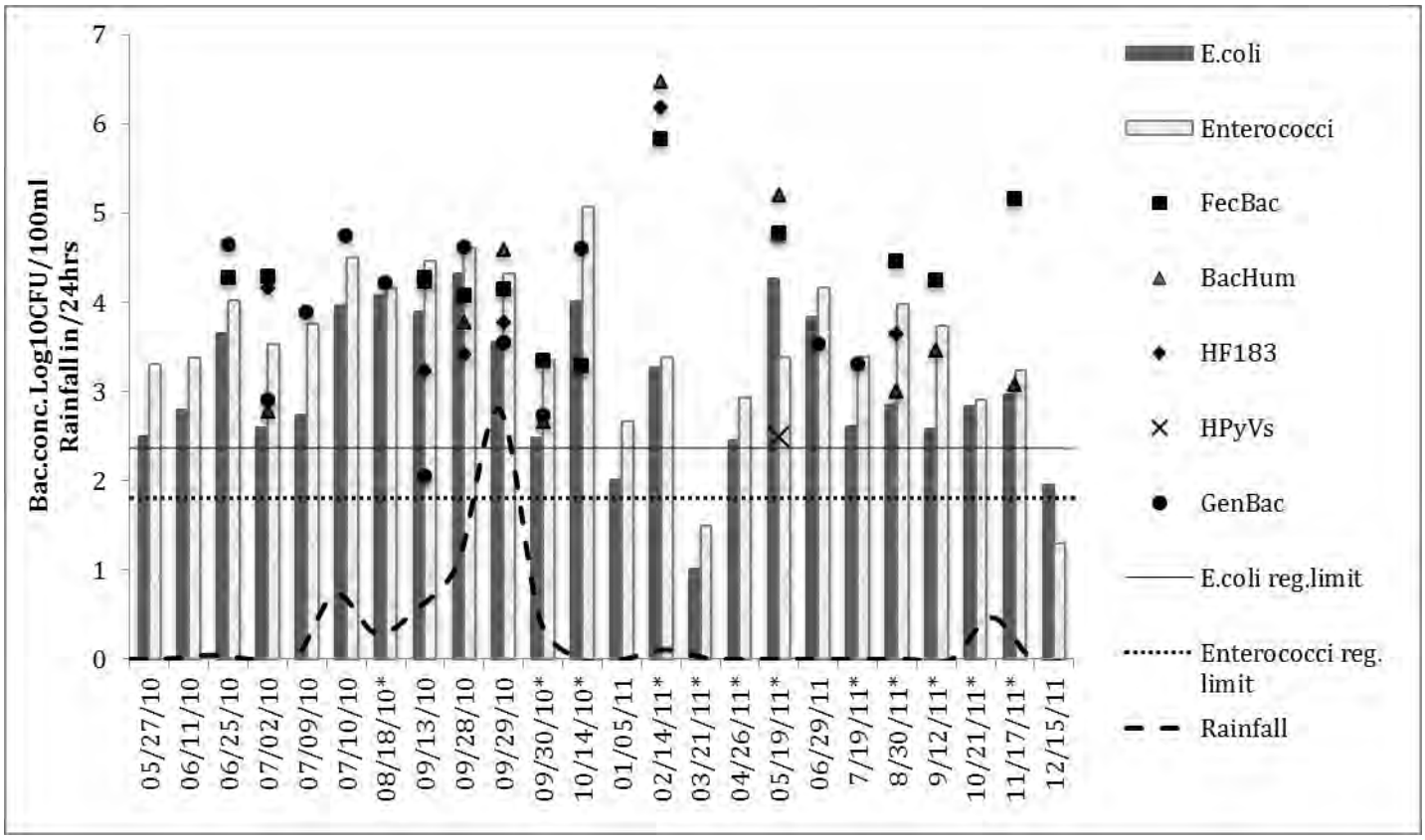

Figure 1.5 Results for *Enterococcus*, *E. coli*, rainfall, and source markers at Mill Dam Creek, Site F

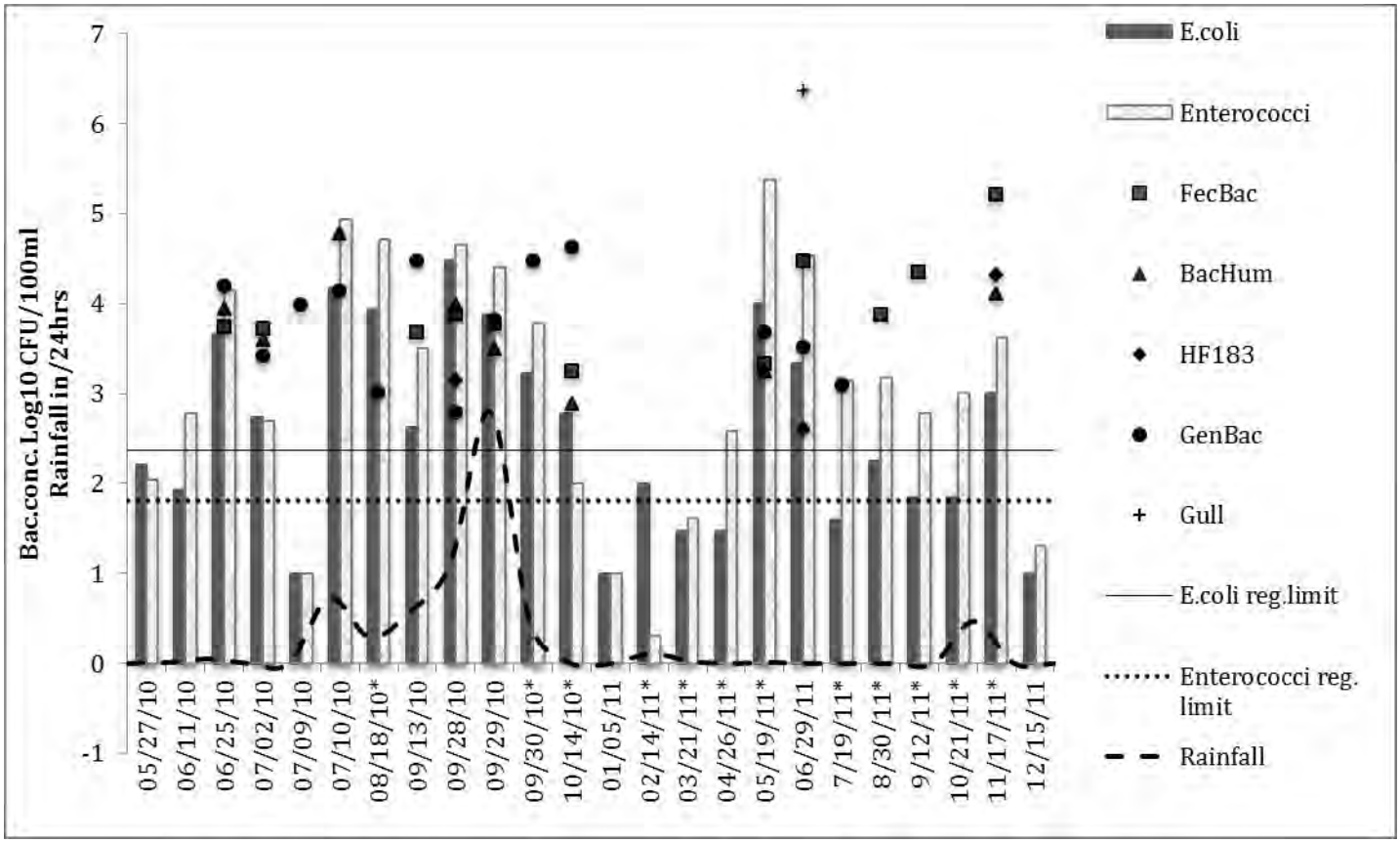

Table 1. Sampling events and analysis performed at Mill Dam Creek sites. Abbreviations: Ec, *E. coli;* Ent, *Enterococcus;* FC, fecal coliforms; FBac, fecal *Bacteroides;* BacHum, *B. thetaiotiamicro;* HF183, human-specific *Bacteroide;* HpyVs, human polyomaviruses; GenBac, General *Bacteroidales;* Gull, *Catellicoccus marimammaliumm*

Site	Date	FIB Analyses	PCR Analyses	Comments
MDC-A	05/27/10	Ec, Ent, FC		
	06/11/10	Ec, Ent, FC		
	06/25/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/02/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/09/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/10/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	08/18/10	Ec, Ent, FC		
	09/28/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/29/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/30/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	10/14/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	01/05/11	Ec, Ent, FC		
	02/14/11	Ec, Ent, FC	FBac, BacHum, HF183	
	04/26/11	Ec, Ent, FC		
	05/19/11	Ec, Ent, FC	FBac, BacHum, HF183	
	06/29/11	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/19/11	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	08/30/11	Ec, Ent, FC	HPyVs, GenBac, Gull	
	09/12/11	Ec, Ent, FC	FBac, BacHum, HF183	
	10/21/11	Ec, Ent, FC		
	11/17/11	Ec, Ent, FC	FBac, BacHum, HF183	
	12/15/1	Ec, Ent, FC		
	01/05/12	Ec, Ent, FC		
MDC-F	05/27/10	Ec, Ent, FC		
	06/11/10	Ec, Ent, FC		
	06/25/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/02/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/09/10	Ec, Ent, FC	HPyVs, GenBac, Gull	
	07/10/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	08/18/10	Ec, Ent, FC	HPyVs, GenBac, Gull	

	09/13/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/28/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/29/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/30/10	Ec, Ent, FC	HPyVs, GenBac, Gull	
	10/14/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	01/05/11	Ec, Ent, FC		
	02/14/11	Ec, Ent, FC		
	04/26/11	Ec, Ent, FC		
	05/19/11	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	06/29/11	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/19/11	Ec, Ent, FC	HPyVs, GenBac, Gull	
	08/30/11	Ec, Ent, FC	FBac, BacHum, HF183	
	09/12/11	Ec, Ent, FC	FBac, BacHum, HF183	
	10/21/11	Ec, Ent, FC		
	11/17/11	Ec, Ent, FC	FBac, BacHum, HF183	
	12/15/1	Ec, Ent, FC		
	01/05/12	Ec, Ent, FC		
MDC-D	05/27/10	Ec, Ent, FC		
	06/11/10	Ec, Ent, FC		
	06/25/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/02/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	07/09/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/10/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	08/18/10	Ec, Ent, FC	HPyVs, GenBac, Gull	
	09/28/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/29/10	Ec, Ent, FC	FBac, BacHum, HF183, HPyVs, GenBac, Gull	
	09/30/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	10/05/10		FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	10/14/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	01/05/11	Ec, Ent, FC		
	02/14/11	Ec, Ent, FC	FBac, BacHum, HF183	
	04/26/11	Ec, Ent, FC		
	05/19/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	

	06/29/11	Ec, Ent, FC	HPyVs, GenBac, Gull	
	07/19/11	Ec, Ent, FC	HPyVs, GenBac, Gull	
	08/30/11	Ec, Ent, FC	FBac, BacHum, HF183	
	09/12/11	Ec, Ent, FC	FBac, BacHum, HF183	
	10/21/11	Ec, Ent, FC		
	11/17/11	Ec, Ent, FC	FBac, BacHum, HF183	
	12/15/1	Ec, Ent, FC		
	01/05/12	Ec, Ent, FC		
MDC-J	05/27/10	Ec, Ent, FC		
	06/11/10	Ec, Ent, FC		
	06/25/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/02/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/09/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/10/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	08/18/10	Ec, Ent, FC		
	09/29/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/30/10 AM	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/30/10 PM	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	10/14/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	01/05/11	Ec, Ent, FC		
	02/14/11	Ec, Ent, FC		
	04/26/11	Ec, Ent, FC		
	05/19/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	06/29/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/19/11	Ec, Ent, FC		
	08/30/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/12/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	10/21/11	Ec, Ent, FC		
	11/17/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	12/15/1	Ec, Ent, FC		
	01/05/12	Ec, Ent, FC		
MDC-I	05/27/10	Ec, Ent, FC		
	06/11/10	Ec, Ent, FC		
	06/25/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/02/10	Ec, Ent, FC	FBac BacHum, HF183,	

			HPyVs, GenBac, Gull,	
	07/09/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/10/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	08/18/10	Ec, Ent, FC		
	09/29/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/30/10 AM	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/30/10 PM	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	10/14/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	01/05/11	Ec, Ent, FC		
	02/14/11	Ec, Ent, FC		
	03/21/11	Ec, Ent, FC		
	04/26/11	Ec, Ent, FC		
	05/19/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	06/29/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/19/11	Ec, Ent, FC		
	08/30/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/12/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	10/21/11	Ec, Ent, FC		
	11/17/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	12/15/1	Ec, Ent, FC		
	01/05/12	Ec, Ent, FC		
MDC-G	05/27/10	Ec, Ent, FC		
	06/11/10	Ec, Ent, FC		
	06/25/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/02/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/09/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/10/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	08/18/10	Ec, Ent, FC	HPyVs, GenBac, Gull	
	09/28/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/29/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/30/10 AM	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	09/30/10 PM	Ec, Ent, FC	FBac BacHum, HF183,	

			HPyVs, GenBac, Gull,	
	10/14/10	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	01/05/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull	
	02/14/11	Ec, Ent, FC		
	03/21/11	Ec, Ent, FC		
	04/26/11	Ec, Ent, FC	FBac BacHum, HF183	
	05/19/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	06/29/11	Ec, Ent, FC	FBac BacHum, HF183, HPyVs, GenBac, Gull,	
	07/19/11	Ec, Ent, FC	HPyVs, GenBac, Gull	
	08/30/11	Ec, Ent, FC	FBac BacHum, HF183,	
	09/12/11	Ec, Ent, FC	FBac BacHum, HF183,	
	10/21/11	Ec, Ent, FC		
	11/17/11	Ec, Ent, FC	FBac BacHum, HF183,	
	12/15/1	Ec, Ent, FC		
	01/05/12	Ec, Ent, FC		
MDC-M	10/05/10	Ec, Ent, FC	FBac BacHum, HF183,	
MDC-X	10/05/10	Ec, Ent, FC	FBac BacHum, HF183,	
MDC-Z	10/05/10	Ec, Ent, FC	FBac BacHum, HF183,	

Table 2 Frequency of detection and quantitative range (gene copies/100 ml) of MST markers at Mill Dam Creek sites

	Detection and Level of MST Markers											
Site	**GenBac**		**FecBac**		**BacHum**		**HF183**		**HPyVs**		**Gull**	
	% (n)[a]	Range[b]	% (n)	Range	% (n)	Range	% (n)	Range	% (n)	Range	% (n)	Range
MDC A	100% (11)		86% (15)		79% (14)	0	20% (15)		9% (11)	0-44	0% (8)	0
MDC D	100% (13)		86% (14)		73% (15)	0						0
MDC F	100% 13		92% (12)		66% (12)							0
MDC G	100% (14)		100% (16)		75% (16)	0	0% (11)	0	0	0	0% (8)	0
MDC I	100% (11)		100% (13)		58% (12)	0	0% (10)	0	0	0-143	ND	0
MDC J	100% (11)		86% (14)		69% (13)		0% (2)	0	0	0	ND	0

Figure 2.1 Results for *E. coli* and source markers by site at Moore's Creek (frequently sampled sites)

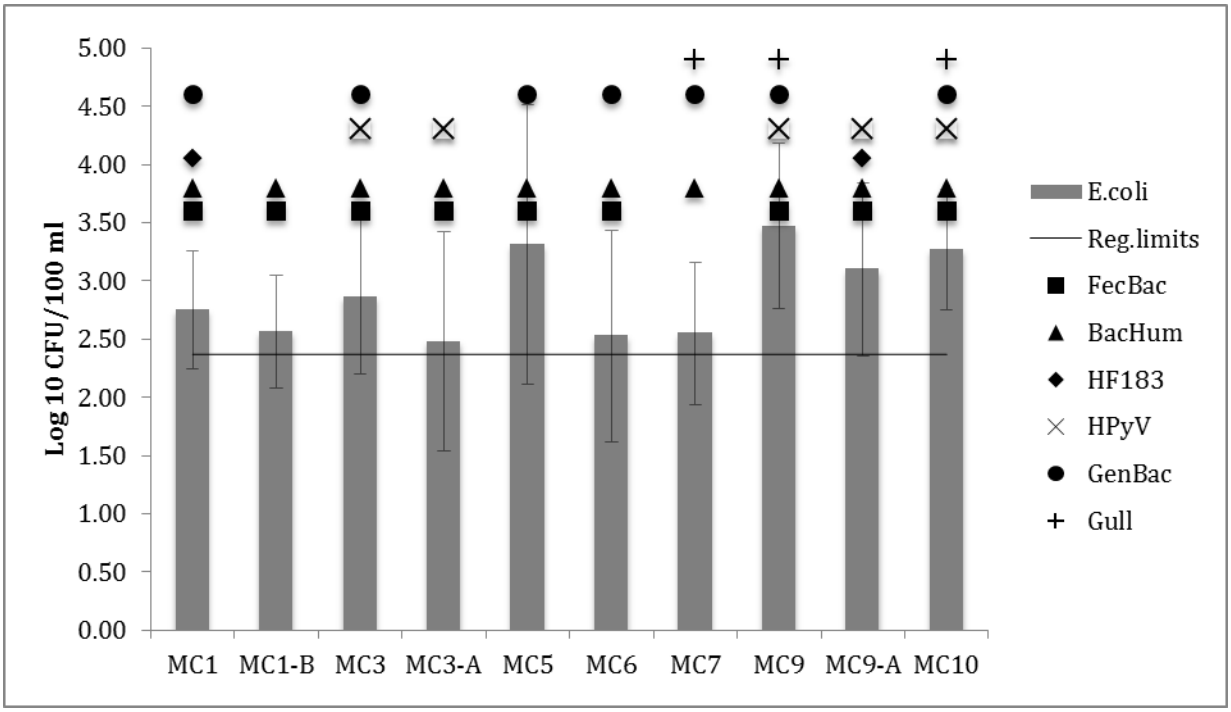

Figure 2.2 Results for *E. coli* and source markers by site at Moore's Creek (all sites)

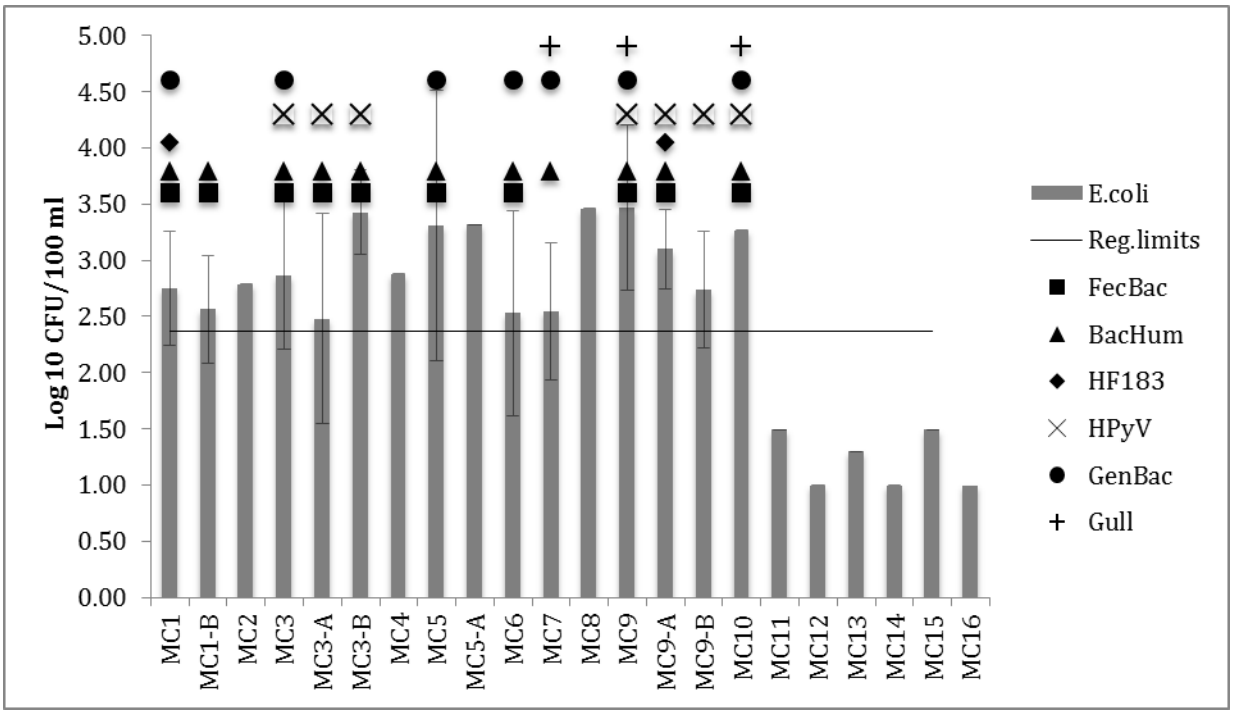

Figure 2.3 Results for *Enterococcus*, *E. coli*, rainfall, and source markers at Moore's Creek, Site MC3. The regulatory limit for freshwater is represented by the horizontal solid line for *E. coli*, and a dashed horizontal line for *Enterococcus*. Error bars are standard deviations and FIB units are $\log_{10}$ CFU/100 ml.

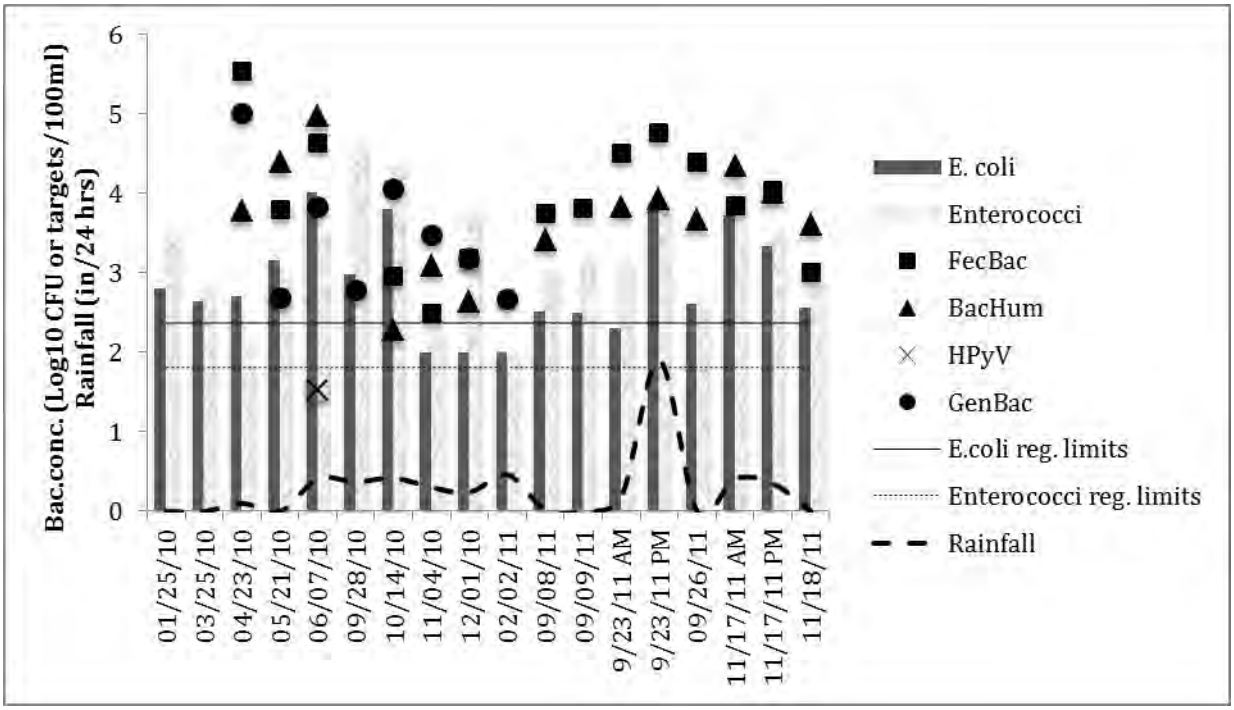

Table 3. Frequency of detection and quantitative range (gene copies/100 ml) of MST markers at Moore's Creek sites. MST analyses were not performed at sites MC11-MC16.

	Detection and Level of MST Markers											
Site	**GenBac**		**FecBac**		**BacHum**		**HF183**		**HPyVs**		**Gull**	
	% (n)[a]	Range[b]	% (n)	Range	% (n)	Range	% (n)	Range	% (n)	Range	% (n)	Range
MC1	100% (8)	1240-370000	87% (15)	0-2590318	67% (15)	0-73283	7% (15)	0-5146	0% (8)	0	0% (8)	0
MC1B	ND[c]	ND	100% (8)	0-13209	25% (8)	0-2944	0% (8)	0	ND	ND	ND	ND
MC2	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC3	100% (8)	467-103000	100% (14)	309-347348	93% (14)	0-96049	0% (11)	0	13% (8)	0-33	0% (8)	0
MC3A	ND	ND	80% (10)	0-136580	80% (10)	0-137205	0% (10)	0	ND	ND	ND	ND
MC3B	ND	ND	100% (2)	344174-461473	100% (2)	2031-2124	0% (2)	0	ND	ND	ND	ND
MC4	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC5	100% (8)	1000-71640	75% (4)	0-12420	100% (4)	225-1756030	0% (4)	0	0% (8)	0	0% (8)	0
MC5A	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC6	100% (5)	573-46672	100% (4)	168-6804030	75% (4)	0-20879	0% (4)	0	0% (5)	0	0% (5)	0
MC7	100% (4)	344-71640	0% (3)	0	100% (3)	9-45131	0% (3)	0	0% (4)	0	50% (4)	1880-11944
MC8	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
MC9	100% (8)	240-20960	77% (13)	0-195926	85% (13)	0-69298	0% (13)	0	13% (8)	0-33	13% (8)	0-6575
MC9A	ND	ND	90% (10)	0-779066	80% (10)	0-15933	10% (10)	0-6812	ND	ND	ND	ND
MC9B	ND	ND	50% (2)	0-774526	0% (2)	0	0% (2)	0	ND	ND	ND	ND
MC10	100% (6)	1350-6600	60% (5)	0-300940	80% (5)	0-84743	0% (5)	0	17% (6)	0-40	33% (6)	0-35693

Table 4. Sampling events and analysis performed at Moore's Creek sites. Abbreviations: Ec, *E. coli;* Ent, *Enterococcus;* FC, fecal coliforms; FBac, fecal *Bacteroides;* BacHum, *B. thetaiotiamicro;* HF183, human-specific *Bacteroide;* HpyVs, human polyomaviruses; GenBac, General *Bacteroidales;* Gull, *Catellicoccus marimammaliumm*

Site	Date	FIB Analyses	PCR Analyses
MC1	01/25/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	03/25/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/23/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	06/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	02/02/11	Ec, Ent	FBac, BacHum, HF183
	09/08/11	Ec, Ent	FBac, BacHum, HF183
	09/09/11	Ec, Ent	FBac, BacHum, HF183

Site	Date	FIB Analyses	PCR Analyses
	09/23/11 AM	Ec, Ent	FBac, BacHum, HF183
	09/23/11 PM	Ec, Ent	FBac, BacHum, HF183
	09/26/11	Ec, Ent	FBac, BacHum, HF183
	11/17/11 AM	Ec, Ent	FBac, BacHum, HF183
	11/17/11 PM	Ec, Ent	FBac, BacHum, HF183
	11/18/11	Ec, Ent	FBac, BacHum, HF183
MC1B	09/08/11	Ec, Ent	FBac, BacHum, HF183
	09/09/11	Ec, Ent	FBac, BacHum, HF183
	09/23/11 AM	Ec, Ent	FBac, BacHum, HF183
	09/23/11 PM	Ec, Ent	FBac, BacHum, HF183
	09/26/11	Ec, Ent	FBac, BacHum, HF183
	11/17/11 AM	Ec, Ent	FBac, BacHum, HF183
	11/17/11 PM	Ec, Ent	FBac, BacHum, HF183
	11/18/11	Ec, Ent	FBac, BacHum, HF183
MC2	01/25/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
MC3	01/25/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	03/25/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/23/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/14/10	Ec, Ent	FBac, BacHum,

Site	Date	FIB Analyses	PCR Analyses
			HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	06/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	02/02/11	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/08/11	Ec, Ent	FBac, BacHum, HF183
	09/09/11	Ec, Ent	FBac, BacHum, HF183
	09/23/11 AM	Ec, Ent	FBac, BacHum, HF183
	09/23/11 PM	Ec, Ent	FBac, BacHum, HF183
	09/26/11	Ec, Ent	FBac, BacHum, HF183
	11/17/11 AM	Ec, Ent	FBac, BacHum
	11/17/11 PM	Ec, Ent	FBac, BacHum
	11/18/11	Ec, Ent	FBac, BacHum
MC3A	09/08/11	Ec, Ent	FBac, BacHum, HF183
	09/09/11	Ec, Ent	FBac, BacHum, HF183
	09/23/11 AM	Ec, Ent	FBac, BacHum, HF183
	09/23/11 PM	Ec, Ent	FBac, BacHum, HF183
	09/26/11	Ec, Ent	FBac, BacHum, HF183
	11/17/11 AM	Ec, Ent	FBac, BacHum,

Site	Date	FIB Analyses	PCR Analyses
			HF183
	11/17/11 PM	Ec, Ent	FBac, BacHum, HF183
	11/18/11	Ec, Ent	FBac, BacHum, HF183
	01/23/12 AM	Ec, Ent	FBac, BacHum, HF183
	01/23/12 PM	Ec, Ent	FBac, BacHum, HF183
MC3B	01/23/12 AM	Ec, Ent	FBac, BacHum, HF183
	01/23/12 PM	Ec, Ent	FBac, BacHum, HF183
MC4	01/25/10	Ec, Ent	
MC5	01/25/10	Ec, Ent	
	03/25/10	Ec, Ent	
	04/23/10	Ec, Ent	
	04/30/10	Ec, Ent	
	05/07/10	Ec, Ent	
	05/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	
	06/07/10	Ec, Ent	
	09/28/10	Ec, Ent	HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	02/02/11	Ec, Ent	
	02/15/11	Ec, Ent	
	02/18/11		
MC5A	02/15/11	Ec, Ent	
MC6	01/25/10	Ec, Ent	
	03/25/10	Ec, Ent	
	04/23/10	Ec, Ent	
	04/30/10	Ec, Ent	
	05/07/10	Ec, Ent	
	05/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	

Site	Date	FIB Analyses	PCR Analyses
	06/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/28/10	Ec, Ent	HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
MC7	01/25/10	Ec, Ent	
	03/25/10	Ec, Ent	
	04/23/10	Ec, Ent	
	04/30/10	Ec, Ent	
	05/07/10	Ec, Ent	
	05/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	
	06/07/10	Ec, Ent	
	09/28/10	Ec, Ent	HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
MC8	01/25/10	Ec, Ent	
MC9	03/25/10	Ec, Ent	
	04/23/10	Ec, Ent	
	04/30/10	Ec, Ent	
	05/07/10	Ec, Ent	
	05/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	06/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/28/10	Ec, Ent	HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs,

Site	Date	FIB Analyses	PCR Analyses
			GenBac, Gull
	12/01/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	02/02/11	Ec, Ent	HPyVs, GenBac, Gull
	09/08/11	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/09/11	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/23/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/23/11 PM	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/26/11	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/17/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/17/11 PM	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/18/11	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
MC9A	09/28/10	Ec, Ent	FBac, BacHum, HF183
	10/14/10	Ec, Ent	FBac, BacHum, HF183
	11/04/10	Ec, Ent	FBac, BacHum, HF183
	12/01/10	Ec, Ent	FBac, BacHum, HF183
	02/02/11	Ec, Ent	FBac, BacHum, HF183
	09/08/11	Ec, Ent	FBac, BacHum, HF183
	09/09/11	Ec, Ent	FBac, BacHum, HF183
	09/23/11 AM	Ec, Ent	FBac, BacHum, HF183
	09/23/11 PM	Ec, Ent	FBac, BacHum, HF183

Site	Date	FIB Analyses	PCR Analyses
	09/26/11	Ec, Ent	FBac, BacHum, HF183
	11/17/11 AM	Ec, Ent	FBac, BacHum, HF183
	11/17/11 PM	Ec, Ent	FBac, BacHum, HF183
	11/18/11	Ec, Ent	FBac, BacHum, HF183
	01/23/12 AM	Ec, Ent	FBac, BacHum, HF183
	01/23/12 PM	Ec, Ent	FBac, BacHum, HF183
MC9B	01/23/12 AM	Ec, Ent	FBac, BacHum, HF183
	01/23/12 PM	Ec, Ent	FBac, BacHum, HF183
MC10	03/25/10	Ec, Ent	
	04/23/10	Ec, Ent	
	04/30/10	Ec, Ent	
	05/07/10	Ec, Ent	
	05/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/21/10	Ec, Ent	
	06/07/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	09/28/10	Ec, Ent	HPyVs, GenBac, Gull
	10/14/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	11/04/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
MC11	03/25/10	Ec, Ent	
MC12	03/25/10	Ec, Ent	
MC13	03/25/10	Ec, Ent	
MC14	03/25/10	Ec, Ent	
MC15	03/25/10	Ec, Ent	
MC16	03/25/10	Ec, Ent	

Figure 3.1 Results for *E. coli* and source markers by site at Shingle Creek (frequently sampled sites)

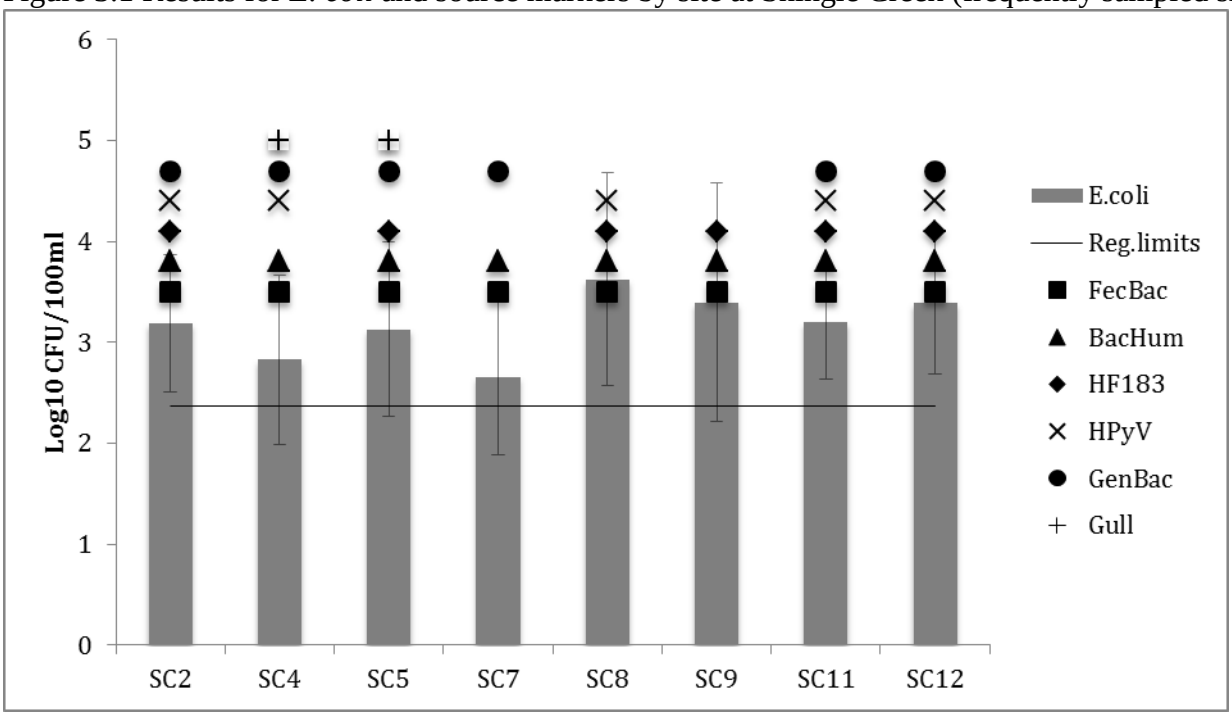

Figure 3.2 Results for *E. coli* and source markers by site at Shingle Creek (all sites)

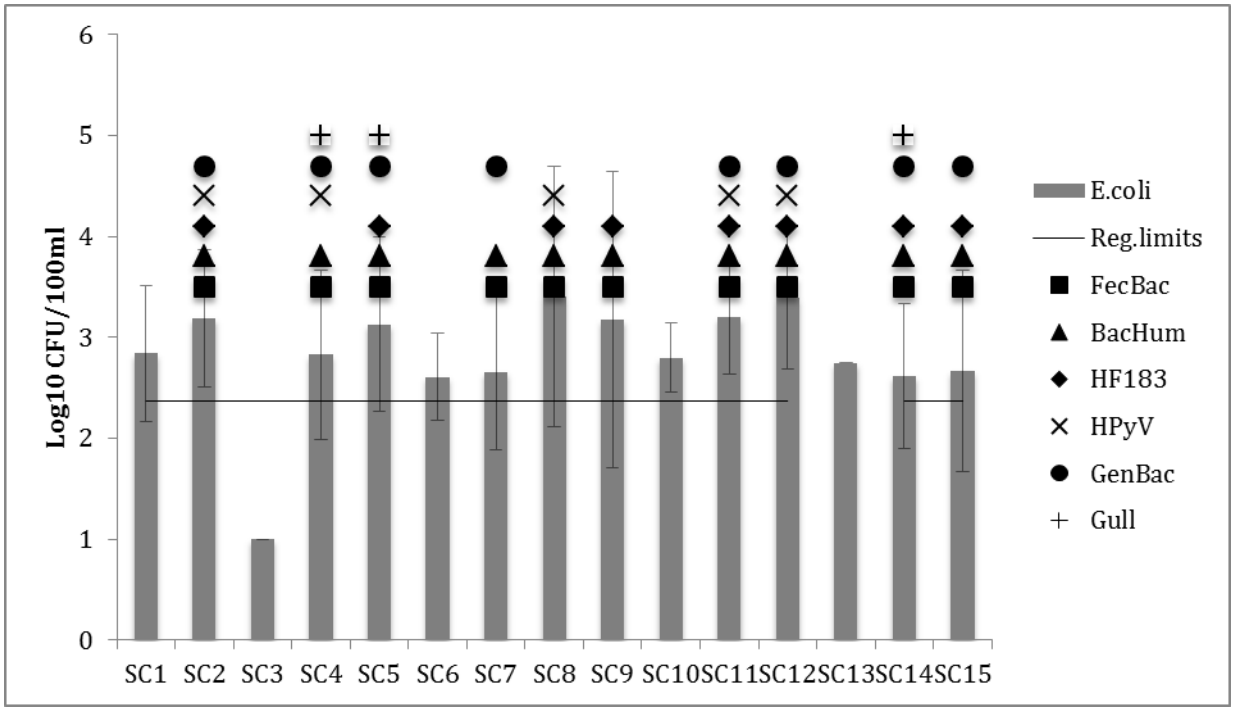

Figure 3.3 Results for *Enterococcus* and source markers by site at Shingle Creek (frequently sampled sites)

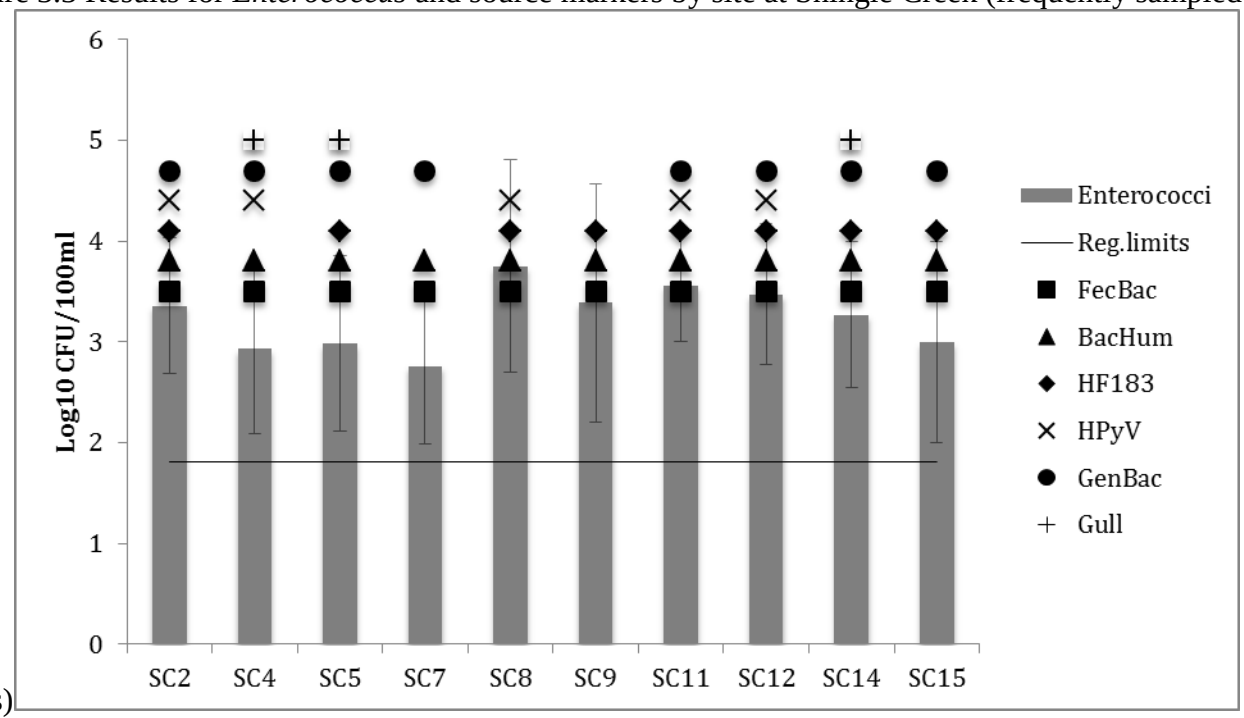

Figure 3.4 Results for *Enterococcus* and source markers by site at Shingle Creek (all sites)

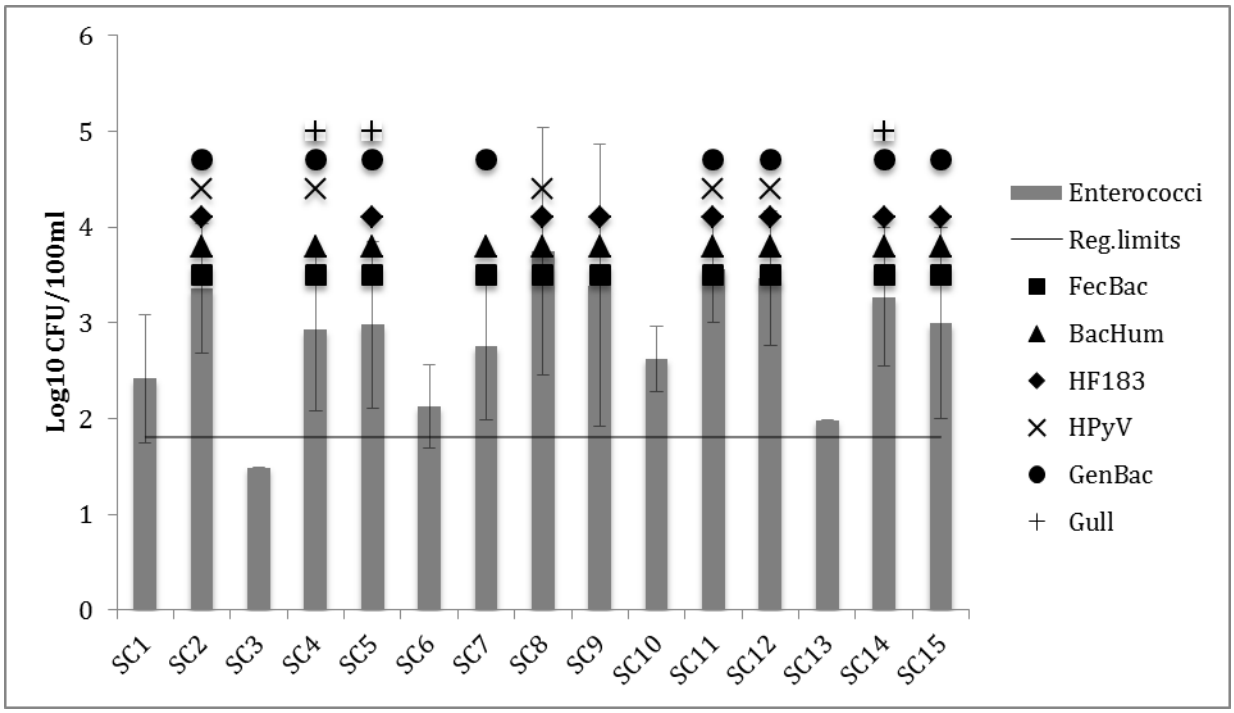

Figure 3.5 Results for *E. coli* and rainfall, all Shingle Creek

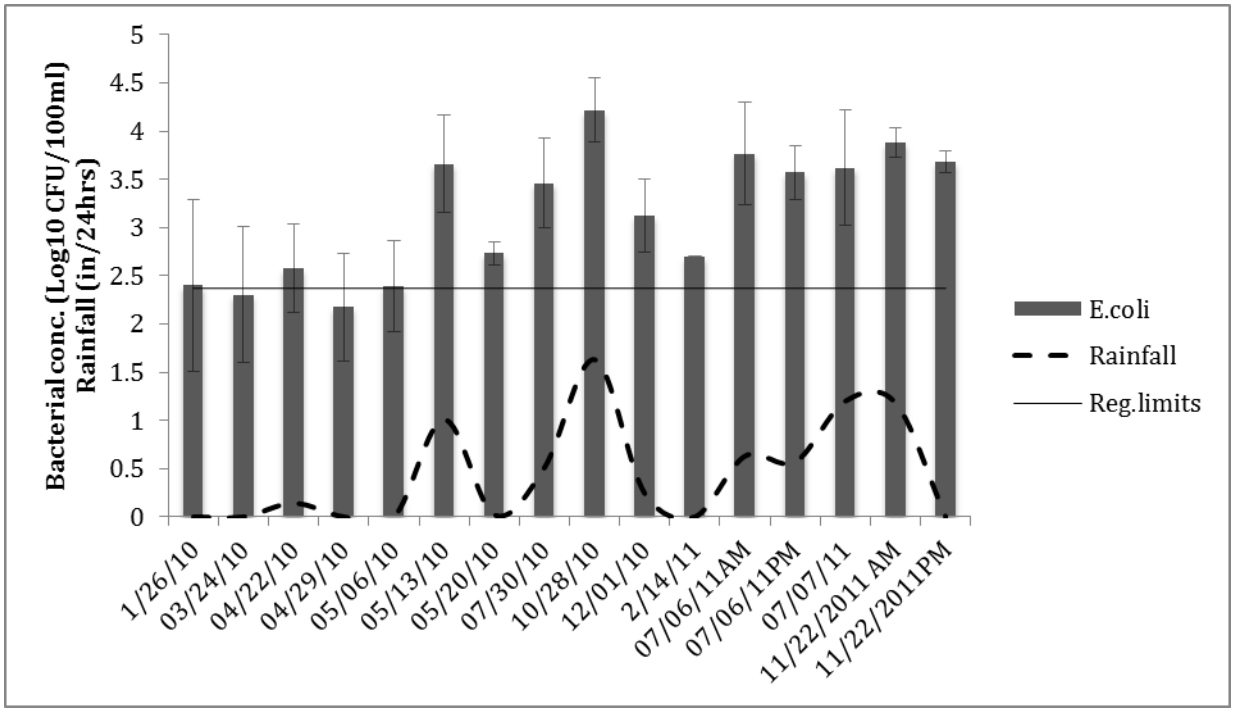

Table 5. Frequency of detection and quantitative range (gene copies/100 ml) of MST markers at Shingle Creek sites

	Detection and Level of MST Markers											
Site	**GenBac**		**FecBac**		**BacHum**		**HF183**		**HPyVs**		**Gull**	
	% (n)[a]	Range[b]	% (n)	Range	% (n)	Range	% (n)	Range	% (n)	Range	% (n)	Range
SC1	ND	ND	100% (1)	46645	100% (1)	37086	0% (1)	0	ND	ND	ND	ND
SC2	100% (6)	3860-1870000	100% (6)	335-12780956	83% (6)	0-23985	33% (6)	0-331833	17% (6)	0-60	0% (6)	0
SC3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SC4	100% (5)	13300-815000	100% (5)	311-144419867	80% (5)	0-53667	0% (5)	0	20% (5)	0-60	20% (5)	0-2200000000
SC5	100% (6)	7800-1686280	83% (6)	0-137467827	100% (6)	6439-128881	33% (6)	341091-16403325	0% (6)	0	17% (6)	0-1600000000
SC6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SC7	100% (5)	13900-53670	40% (5)	0-8447	80% (5)	0-50714	0% (5)	0	0% (5)	0	0% (5)	0
SC8	ND	ND	100% (3)	104968-139000	100% (3)	9132-185013	33% (3)	0-10541	67% (3)	0-188	ND	ND
SC9	ND	ND	100% (3)	15600-1007996	100% (3)	16622-112982	67% (3)	2070-12987	0% (3)	0	ND	ND
SC10	100% (1)	37700	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SC11	100% (9)	3470-5700000	100% (12)	4892-18797109	92% (12)	0-127787	42% (12)	335-23355	17% (12)	0-244	0% (9)	0
SC12	100% (8)	37280-2560000	100% (7)	466-11060464	71% (7)	0-178678	29% (7)	0-1275	25% (8)	0-450	0% (8)	0
SC13	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SC14	100% (8)	1190-2700000	100% (11)	3353-21238467	100% (11)	787-44174	9% (11)	0-26700050	0% (11)	0	13% (8)	0-450275200
SC15	100% (4)	3200-2300000	75% (4)	0-12628989	100% (4)	5282-34477	25% (4)	0-627	0% (4)	0	0% (4)	0

Table 6. Sampling events and analysis performed at Shingle Creek sites. Abbreviations: Ec, *E. coli;* Ent, *Enterococcus;* FC, fecal coliforms; FBac, fecal *Bacteroides;* BacHum, *B. thetaiotiamicro;* HF183, human-specific *Bacteroide;* HpyVs, human polyomaviruses; GenBac, General *Bacteroidales;* Gull, *Catellicoccus marimammalium*

Site	Date	FIB Analyses	PCR Analyses
SC1	01/26/10	Ec, Ent	
	03/24/10	Ec, Ent	
	12/01/10	Ec, Ent	FBac, BacHum, HF183
SC2	01/26/10	Ec, Ent	
	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	
	05/13/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	10/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/01	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
SC3	01/26/10	Ec, Ent	
SC4	01/26/10	Ec, Ent	
	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/13/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull

	10/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
SC5	01/26/10	Ec, Ent	
	03/24/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/22/10	Ec, Ent	
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/13/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	10/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/01	Ec, Ent	
SC6	01/26/10	Ec, Ent	
	03/24/10	Ec, Ent	
SC7	01/26/10	Ec, Ent	
	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	
	05/13/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	10/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/01	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
SC8	03/24/10	Ec, Ent	

	07/06/11 AM	Ec, Ent	
	07/06/11 AM	Ec, Ent	
	07/07/11	Ec, Ent	
	11/22/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/22/11 PM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/23/11		FBac, BacHum, HF183, HPyVs
SC9	03/24/10	Ec, Ent	
	07/06/11 AM	Ec, Ent	
	07/06/11 AM	Ec, Ent	
	07/07/11	Ec, Ent	
	11/22/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/22/11 PM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/23/11		FBac, BacHum, HF183, HPyVs
SC10	03/24/10	Ec, Ent	
	12/01/10	Ec, Ent	HPyVs, GenBac, Gull
SC11	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	
	05/13/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	10/28/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/10	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/06/11 AM	Ec, Ent	HPyVs, GenBac, Gull
	07/06/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs, GenBac, Gull
	07/07/11	Ec, Ent	FBac, BacHum, HF183, HPyVs,

			GenBac, Gull
	11/22/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/22/11 PM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/23/11	Ec, Ent	FBac, BacHum, HF183, HPyVs
SC12	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	05/13/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	10/28/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	12/01/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	02/14/11	Ec, Ent	HPyVs, GenBac, Gull
SC13	03/24/10	Ec, Ent	
SC14	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	
	05/13/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	
	10/28/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull

	07/06/11 AM	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	07/06/11 AM	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	07/07/11	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	11/22/11 AM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/22/11 PM	Ec, Ent	FBac, BacHum, HF183, HPyVs
	11/23/11	Ec, Ent	FBac, BacHum, HF183, HPyVs
SC15	03/24/10	Ec, Ent	
	04/22/10	Ec, Ent	
	04/29/10	Ec, Ent	
	05/06/10	Ec, Ent	
	05/13/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	05/20/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	07/30/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull
	10/28/10	Ec, Ent	Bac, BacHum, HF183, HPyVs, GenBac, Gull