Fecal BMAP Implementation: Source Identification Hillsborough River Watershed

Summary Report

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Executive Summary

The state of Florida has identified 226 tributary **W**ater**b**ody **I**dentifications (WBIDs) that do not meet state water quality standards for fecal coliform contamination and are listed as impaired. In 2006, PBS&J, in collaboration with the Tributary Assessment Team, developed and evaluated a methodology for assessing the source(s) of fecal pollution for WBIDs in Duval County that consistently exhibit high fecal coliform counts. The study used a decision-tree approach based on a "toolbox" of microbial source tracking (MST) methods, including several library-independent methods relying on the genetic typing of indicator organisms or host-specific DNA markers. MST was performed at the University of South Florida (USF). The results of the tests, together with an extensive collection and review of infrastructure mapping, historical monitoring, and land-use data, provided the information needed for a weight-of-evidence assessment of the contribution of potential sources to indicator organism concentrations in these impaired waters. The Tributary Pollution Assessment Manual that resulted from this project provided a blueprint for conducting site assessments to locate dominant sources of fecal contamination and identifying appropriate corrective actions to restore WBIDs to their designated use.

The effort in Duval County demonstrated that a detailed review of existing data, in conjunction with continual collaboration with local entities, is necessary to effectively guide the field reconnaissance and sampling stages of these studies. This "Initial Screening Process" was therefore further developed and incorporated as Phase I of the overall tiered approach to identifying sources of fecal pollution in Hillsborough River tributaries. The use of data such as GIS coverage and historical data, together with local knowledge of the systems, is especially important given the considerable costs associated with MST analyses. Phase II, Implementation, of the Hillsborough River project uses a modified form of the decision-tree approach described in the Tributary Pollution Assessment Manual.

Since a number of WBIDs within Florida will have a total maximum daily load (TMDL) for fecal coliform, the approach developed and documented in the lower Hillsborough River watershed offers a useful, cost-effective tool for guiding fecal Basin Management Action Plan (BMAP) implementation. This is especially important, given the continuing development and very limited lab capacity for MST analysis in the state. In addition, by actively involving local stakeholders in all aspects of the project, the conclusions drawn truly represent a consensus on the most probable sources.

Acknowledgements

Several local stakeholders and private parties who comprise the BMAP Steering Committee contributed time, effort, and supplemental funding toward the implementation of this study. The sampling and analysis that were used in Phase II were conducted with the assistance from the University of South Florida and the Florida Department of Environmental Protection Southwest District Office. In addition to advice and suggestions made during the duration of the project, team members also provided additional reconnaissance and investigative efforts to help better identify local sources of fecal coliform contamination.

Organizations comprising the BMAP Steering Committee and the corresponding primary contact for this project include:

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Environmental Protection Commission, Hillsborough County, Byron Bartlett
Florida Department of Agricultural and Consumer Services, Jessica McCoy
Florida Department of Environmental Protection, Chris Anastasiou and Chris Person
Florida Department of Transportation, Susan Moore
Hillsborough County Health Department, Michael Dreyer and Jim Watson
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Hillsborough County Water Resources Advisor, Scott Emery
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Acronym List

ANOVA	Analysis of Variance
AWQM	Ambient Water Quality Monitoring
BK	Baker Creek
BMAP	Basin Management Action Plan
BMP	Best Management Practice
BOD	Biological Oxygen Demand
BW	Blackwater Creek
CDOM	Colored Dissolved Organic Matter
CFU	Colony Forming Units
Chl A	Chlorophyll A
COT	City of Tampa
CR	County Road
CSS	Contaminant Source Survey
CWA	Clean Water Act
DACS	Department of Agriculture and Consumer Service
DNA	Deoxjribonucleic acid
DO	Dissolved Oxygen
ELAPP	Environmental Land Acquisition Protection Program
EPA	Environmental Protection Agency
EPCHC	Environmental Protection Commission of Hillsborough County
FDEP	Florida Department of Environmental Protection
FL	Flint Creek
FOG	Fats, Oils, and Grease
FOWA	Florida Onsite Wastewater Association
GIS	Geographic Information System
HC	Hillsborough County
HCHD	Hillsborough County Health Department
HPyV	Human Polyomavirus
HR	Hillsborough River
IO	Indicator Organism
ISAT	Impervious Surface Analysis Tool
MDS	Multidimensional Scaling
MST	Microbial Source Tracking
MWQA	Microbial Water Quality Assessment
NEMO	Nonpoint Education for Municipal Officials
NOAA	National Oceanic and Atmospheric Administration
NOx	Nitrogen Oxides
NR	New River
NRCS	Natural Resource Conservation Service
NWIS	National Water Information System
OB	Optical Brightener
OSTDS	Onsite Treatment and Disposal System
PCR	Polymerase Chain Reaction
RV	Recreational Vehicle
SB	Spartman Branch
SR	State Road

SSO	Sanitary Sewer Overflow
STORET	Storage and Retrieval
SWFWMD	Southwest Florida Water Management District
SWIM	Surface Water Improvement and Management
TBEP	Tampa Bay Estuary Program
UFL IFAS	University of Florida Institute of Food and Agricultural Sciences
USDA	United States Department of Agriculture
USF	University of South Florida
USGS	United States Geological Survey
UV	Ultraviolet
WBID	Water Body Identification
WWTF	Wastewater Treatment Facility

Section 1.0 Introduction

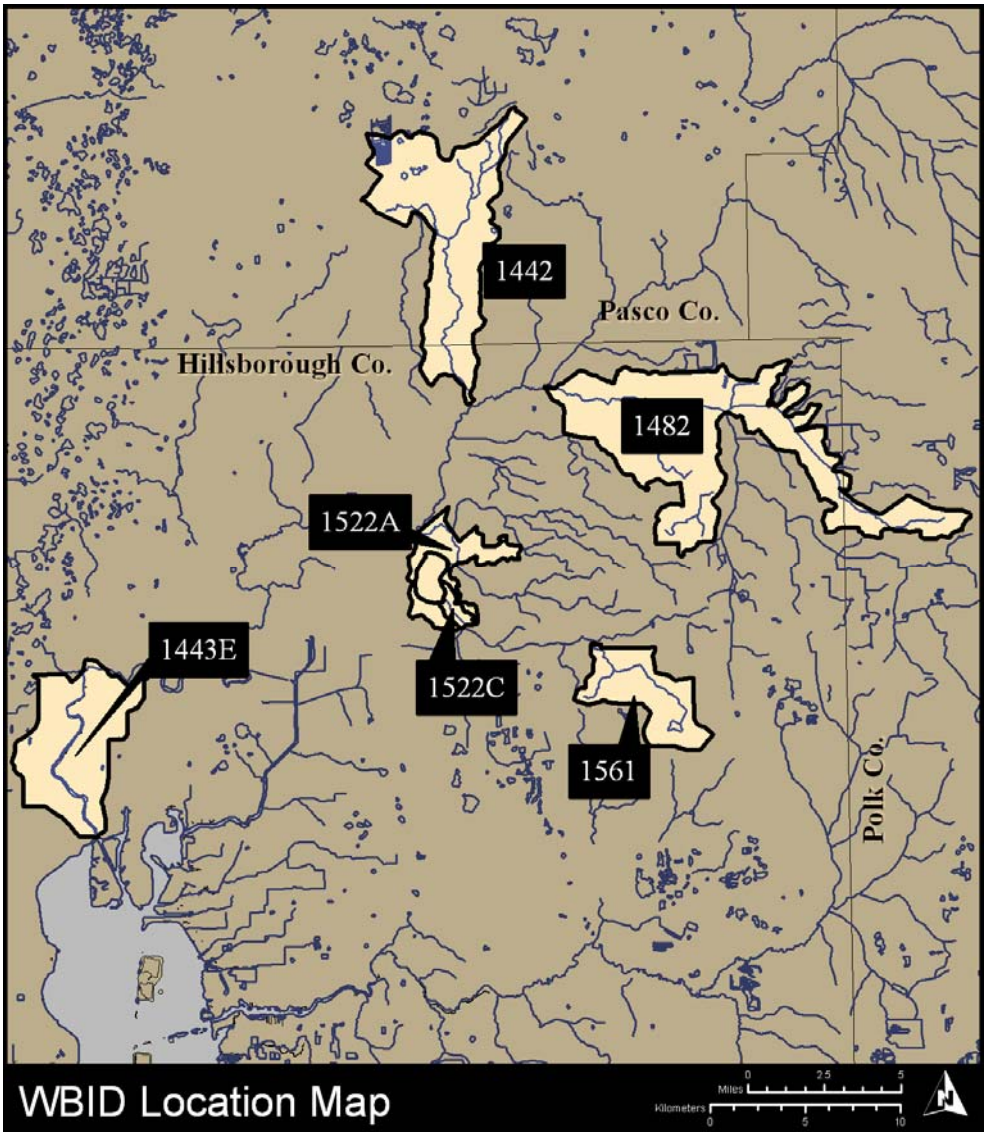
Under the federal Clean Water Act, states are required to identify impaired waters that do not meet water quality standards, submit to the U.S. Environmental Protection Agency (EPA) a list of these waters, and develop TMDLs for each of them. A TMDL is the maximum amount of a specific pollutant that a waterbody can assimilate and still meet its designated uses, such as swimming or fishing.

Florida's 52 river basins are divided into 29 groups that are distributed among the Florida Department of Environmental Protection's (FDEP) 6 districts. Using a rotating cycle in which 1 basin is assessed in each district every year, FDEP and local stakeholders have identified impaired waters requiring the development of TMDLs. The first 5-year cycle of assessments is now completed in all 29 basin groups, and Florida is moving forward with the implementation of management plans to address these impairments. By 2012, 1,262 TMDLs must be developed for 810 waters.

While nutrient TMDLs are complex and difficult to develop, bacteria TMDLs can be developed quickly and easily. To implement a bacteria TMDL, however, requires knowledge of sources that is not generally available. This knowledge allows management actions to be specifically targeted to individual sources, which can include human (wastewater and stormwater), animal, and natural sources. Based on this need, PBS&J was contracted by the FDEP to work in cooperation with the Basin Management Action Plan (BMAP) Steering Committee to develop a cost-effective tool for identifying and prioritizing bacteria sources so that management actions can be targeted to those sources. The tool has also proven very effective in securing agreement among affected stakeholders on the most likely sources of impairment. The BMAP Steering Committee is comprised of representatives from organizations including, but not limited to, the Tampa Bay Estuary Program (TBEP), the University of South Florida (USF), the Environmental Protection Commission of Hillsborough County (EPCHC), Hillsborough County (HC), the City of Tampa (COT), and the Hillsborough County Health Department (HCHD).

The objectives of the Fecal BMAP Implementation Project for the lower Hillsborough River watershed were to (1) build on the Tributary Pollution Assessment Manual developed and tested in Duval County (PBS&J 2006a) and further develop a useful, relatively inexpensive tool that FDEP can use to guide fecal BMAP implementation throughout the state; and (2) establish the accurate level and potential sources of impairment in six representative tributary WBIDs in the Hillsborough River watershed (Map 1). The six tributaries included:

Spartman Branch (1561);
Blackwater Creek (1482);
Flint Creek (1522A);
Baker Creek (1522C);
New River (1442); and
Hillsborough River (1443E).



Map 1. WBID location map.

The methodology was developed as a phased approach to address pollution sources in tributaries impaired by indicator organisms. Phase I included the compilation and synthesis of relevant documents and local knowledge and a detailed review of existing data to guide the field reconnaissance and sampling stages of the project (Figure 1). Phase II, Implementation, utilized a decision tree designed to build on the results of Phase I and continue screening for potential sources of fecal contamination by using lower-cost, more basic methods first, followed by higher-cost, more sophisticated methods to minimize cost and time.

The decision tree was designed to consider appropriate methods to define sources to a level at which remedial action can be defined and successfully implemented, while also considering cost. A weight-of-evidence approach, in which the results of the decision tree were used in conjunction with background knowledge of the watershed and land-use patterns, was used to document and assess the contribution of various potential sources to

waterbodies impaired by high levels of fecal coliform bacteria. Determining contributing sources is particularly important for fecal microbial exceedances, because the measured indicators often do not readily discriminate between human, livestock, and wildlife sources. Because pathogens from human sources present the highest potential for infection, identifying the type of source (human, livestock, or wildlife) also affects the evaluation of risk. As a result, source identification is critical to implementing action to improve water quality and protect human health.

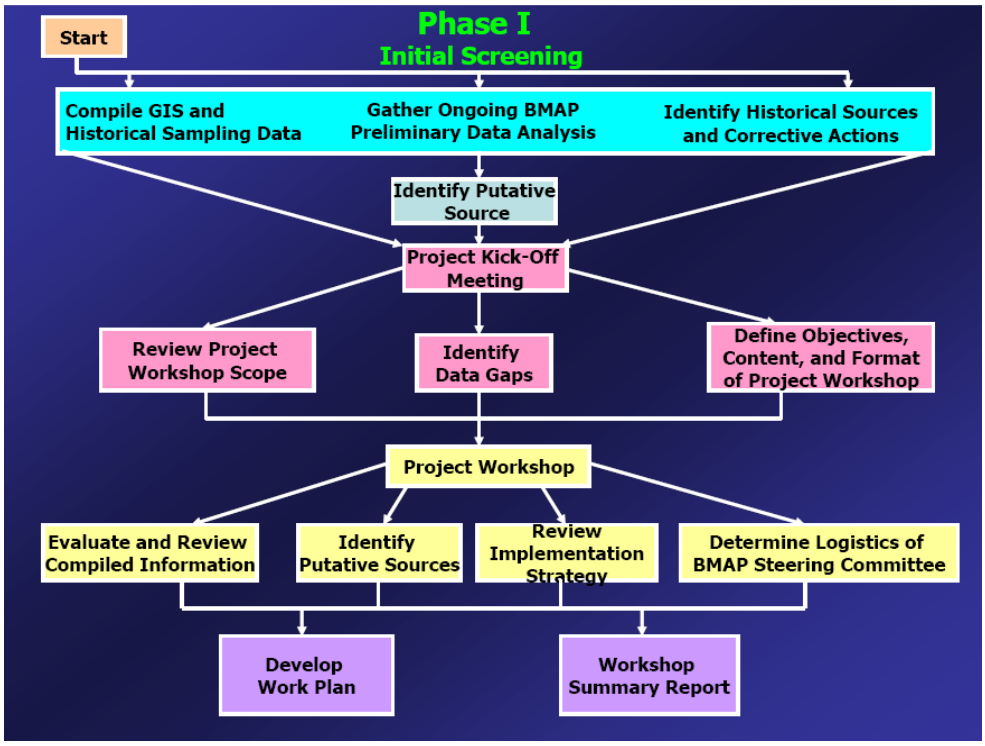


Figure 1. Phase I approach to identifying sources of fecal pollution in Hillsborough River tributaries.

Section 1.1 Fecal Contamination Thresholds

Water quality standards are based upon the use of a lake, stream or river (e.g., for drinking water, shellfishing, or recreation) and set specific water quality criteria to safely achieve that function. Under the Clean Water Act (CWA) Section 304(a), the Environmental Protection Agency (EPA) is required to publish water quality criteria that accurately reflect the latest scientific knowledge for the protection of human health and aquatic life. The EPA's Ambient Water Quality Criteria for Bacteria -1986 was developed for the protection of waters designated for recreational uses (EPA 1986). Epidemiological studies conducted by the EPA demonstrated that for fresh water, *E. coli* (a member of the fecal coliforms), and enterococci are best suited for predicting the presence of pathogens that cause illness, and that for marine waters, enterococci are most appropriate (EPA 2002). The FDEP, however, uses fecal coliforms for regulating water quality and has adopted Criteria for Surface Water Quality Classifications [Chapter 62-302.530, Florida Administrative Code (F.A.C.)] (Table 1).

Table 1. FDEP-adopted Criteria for Surface Water Quality Classifications [Chapter 62-302.530] *(http://www.dep.state.fl.us/legal/Rules/shared/62-302/62-302.pdf).*

Parameter	Units	Class I	Class II	Class III: Fresh	Class III: Marine	Class IV	Class V
(6) Bacteriological Quality (Fecal Coliform Bacteria)	Number per 100 ml (Most Probable Number (MPN) or Membrane Filter (MF))	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 5 samples taken over a 30 day period.	MPN shall not exceed a median value of 14 with not more than 10% of the samples exceeding 43, nor exceed 800 on any one day.	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.	MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.		

Section 1.2 Available Assessment Techniques

Assessment and direct identification of sources of fecal contaminants is confounded by many variables inherent in the use of indicator organisms for monitoring and by variables in the dynamics of microbial populations in various substrates and environmental conditions. As a result, there is no single method able to determine the extent, severity, and causes of pathogen contamination. This report uses a combination of methods to better understand the presence of fecal coliforms and practical control measures. These methods range from conducting an initial screening investigation to measuring indicator bacteria and looking for genetic "markers" of human and animal sources. Detailed descriptions of the MST techniques used in these reports are located in Appendix A and the Tributary Pollution Assessment Manual (PBS&J 2006a); those used in this report include:

1. Suite of indicators; and
2. Library independent presence/absence testing using polymerase chain reaction (PCR) with human- or ruminant-specific primers.

Section 2.0 Phase I - Initial Screening

Section 2.1 Data Compilation and Evaluation

Phase I of the Fecal BMAP Implementation Project for the lower Hillsborough River watershed had three main components:

1. Gather and compile relevant documents and local knowledge on land use, previous efforts at characterizing water quality within the 6 WBIDs, historical sampling results, and GIS data;
2. Evaluate the compiled information and build on ongoing BMAP and preliminary data analysis efforts for the 6 WBIDs;
3. Use the information collected to identify potential sources of fecal contamination and develop a Work Plan finalizing the Implementation Strategy for Phase II.

Over 100 GIS data files were collected from federal, state, and local agencies and governmental entities. In the cases where information was not geo-referenced, best efforts were made to include the data in the database as accurate shapefiles. A summary of the GIS data acquisition timeline is included as Appendix B.

Intensive interviews were also conducted with key local staff regarding water quality problems and potential sources of fecal contamination. Such meetings were held with Scott Emery and Carol Henry (HSW Engineering), Fred Nassar and Ed Sherwood (EPCHC), and Charles Kovach and Chris Anastasiou (FDEP). In addition to informational interviews, both PBS&J and USF personnel, as well as Scott Emery, conducted independent preliminary field investigations of each of the six project WBIDs and identified specific points of interest and potential sampling locations. Knowledge gained through the combination of these efforts is described in Sections 4-9.

Water quality data for the 6 WBIDs were also downloaded from the Hillsborough County Water Resources Atlas (*http://www.hillsborough.wateratlas.usf.edu/research*) on January 23, 2007, including a wide range of water quality and physical parameters originally recorded by 102 independent monitoring stations between 1990 and 2007. The datasets were then consolidated into a single Excel database and sorted for stations with fecal coliform data that were located within the target WBID boundaries; 22 stations were identified in this way (Figure 2).

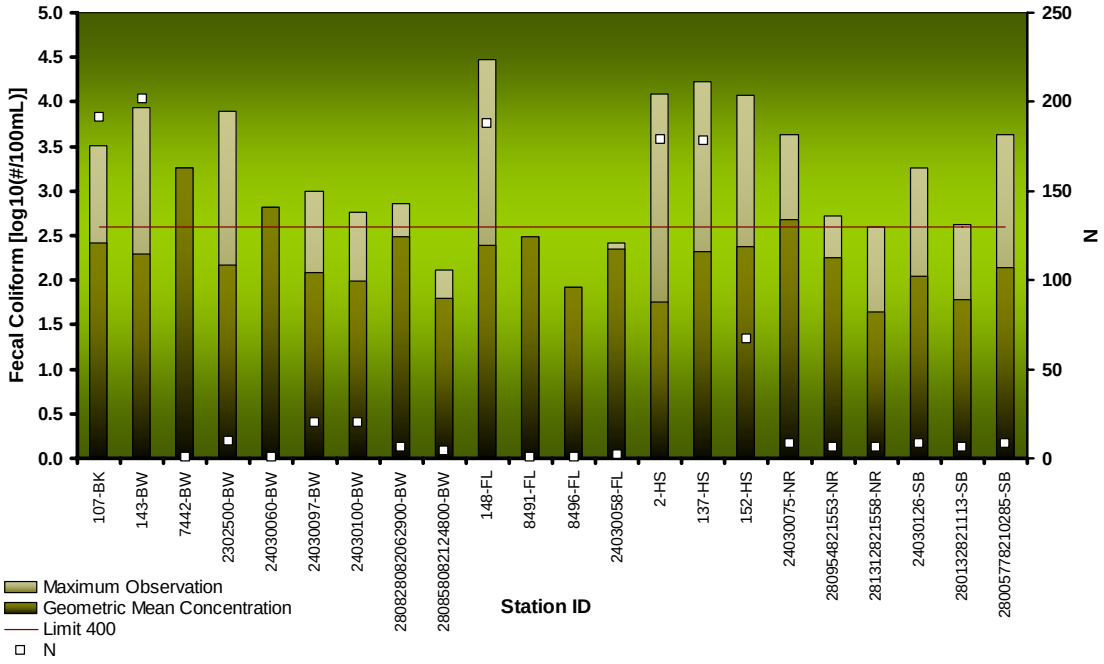


Figure 2. Geometric means, maximum recorded fecal coliform concentrations, and sample size (N) by sampling station for target WBIDs between 1990 and 2007. Station abbreviations: Baker Creek (BK), Blackwater Creek (BW), Flint Creek (FL), Hillsborough River (HR), New River (NR), and Spartman Branch (SB).

The initial analyses of fecal coliform concentration included the calculation of means, geometric means, standard errors, and minimum/maximum values over time. No clear temporal trends emerged for any single station or WBID; however, statistically robust datasets (i.e., N > 100) existed at only 5 of the 22 stations:

107 (Blackwater Creek),
143 (Blackwater Creek),
148 (Flint Creek),
2 (Hillsborough River), and
137 (Hillsborough River).

At these 5 stations, log-transformed fecal concentrations were then tested for possible correlations with all available water quality parameters—i.e., turbidity, conductivity, nitrogen oxides (NO_x), color, biological oxygen demand (BOD), dissolved oxygen (DO), and chlorophyll a (Chla). Available data identified particular stations where stormwater runoff may have played a dominant role in the transport of fecal populations. Although efforts such as these cannot definitively tie a specific fecal source to a particular problem area, the approach is valuable in guiding GIS data acquisition/analysis (i.e., stormwater infrastructure or sanitary sewer overflows) and future field investigation.

The data compilation effort culminated with the "Maps on the Table" exercise at the Project Workshop on February 13, 2007. Twenty-seven participants were randomly divided into two groups and were asked to combine the background information provided

in the workshop presentation with their specific local knowledge to develop a list of potential sources of fecal contamination and to spatially locate them on the maps. The maps provided to the groups included aerial images, infrastructure, land use, historical and potential new sampling locations, and soil data compiled by PBS&J for each WBID during the Initial Screening Process. For the lower Hillsborough River (WBID 1443E) a map of repetitive sanitary system overflows (SSOs) reported by the COT (2001-2007) was also provided. A summary table of points of interest and potential sampling locations identified by Cheryl Wapnick (PBS&J) and Valerie J. Harwood (USF) was also supplied to the participants for easy reference. Each group designated a recorder and a presenter and was given approximately 25 minutes to identify potential sources for each WBID. The results, later presented to the entire group, are included in Sections 4-9 of this report.

The Project Workshop also included a 20-minute presentation entitled "Effluent Source Tracking Using Optical Brightener Fluorescence" by Chris Anastasiou, FDEP. This presentation was designed to educate the participants regarding the potential for utilizing this method in the project WBIDs. In this presentation, Chris discussed the history of an Environmental Protection Agency (EPA)-funded project that began in 2006 with the goal of developing a method of using optical brighteners (OBs) as a proxy for effluent source tracking. This investigation was conducted in three Florida counties (Taylor, Citrus, and Sarasota) and demonstrated that the use of optical brighteners can be an effective screening tool and can provide guidance for discrete sampling. An added benefit is the ability to gather thousands of data points within a short time period and be able to view results in "real-time". Challenges associated with this method include: 1) possible interference of colored dissolved organic matter (CDOM) and salinity with the OB signal in estuarine waters; 2) the lack of understanding of temporal variability (e.g., effects of rainfall, laundry day, time of day); and 3) the need to link fluorometry results to bacteriological sampling results. Data analysis for this project is currently ongoing and preliminary results were presented in a report in June 2007 by PBS&J. This project, including additional deployments and the possibility of an expanded testing area, has been extended for one year.

Section 2.2 Identification of Potential Sources

The data acquisition and compilation assessment efforts summarized above were then used to identify potential sources of fecal coliform contamination in each of the six project WBIDs. Recognition of these potential sources and their possible geographic locations was a key element to designing the implementation portion of the project (e.g., determining sampling station locations and identifying appropriate and cost-effective source-tracking tests). Sections 4-9 provide details of these results; Table 2 presents a brief summary for each WBID.

Table 2. Summary of potential sources for each WBID.

WBID	Most Likely Sources	Additional Potential Sources	Suggested Number of Initial Sampling Stations
Hillsborough River (1443E)	Sewer (SSOs)	Stormwater Homeless populations Lowry Park Zoo (old source?) Live-aboard vessels Bird populations Dog track and parks Wildlife	10
Flint/Baker Creek (1522A/1522C)	OSTDS Livestock	Sewer (upstream of Baker Creek) Stormwater Wildlife	8
Blackwater Creek (1482)	OSTDS in eastern portion Livestock throughout Cone Ranch	Sewer (upstream of Blackwater Creek) Stormwater Chicken houses/manure spreading Wildlife	6
Spartman Branch (1561)	OSTDS Livestock	Sewer Stormwater Wildlife	7
New River (1442)	Livestock and/or sewer north of State Road (SR) 54 OSTDS south of SR54 Livestock in southern portion	Stormwater Wildlife	6

Section 2.3 Walk the WBIDs

The "Walk the WBIDs" exercise took place on May 7-10, 2007, and included participation from twelve individuals representing eight organizations: FDEP, EPCHC, COT, HCHD, University of Florida Institute of Food and Agricultural Sciences (UFL IFAS), Scott Emery (HC Water Resources Advisor), USF, and PBS&J. This effort had four main objectives:

1. confirm tributary characteristics and sampling locations;
2. verify land use and current ideas of potential sources in each watershed;
3. elicit any additional information from participants; and
4. improve the overall understanding of the characteristics of each WBID.

Information resulting from this exercise is included in Sections 4-9 of this report.

Section 2.4 Sampling Site Location Selection

Information collected in Phase I—including historical data, potential contamination sources, land-use types, hydrologic features, and site accessibility—was used to (1) confirm tributary characteristics and identify sampling locations, (2) verify land use and ideas about potential sources in each watershed, and (3) gain a better understanding of each WBID's characteristics. In addition, when historical data were available at consistent locations, the same sampling stations were used to help detect long-term trends and potential causes of declining (or improving) water quality.

A minimum of three "fixed" sampling stations should be used for any given tributary. Sampling stations were selected such that these sites would be sampled by USF and PBS&J and to provide consistency to the sampling program across seasons and would serve to characterize the upstream, midstream, and downstream areas of the WBID. If a potential source is identified, these stations should be located such that one is as close as possible to the potential source, one is downstream and one is upstream. The upstream sampling station would serve as a reference station.

In addition to the three "fixed" stations, "flexible" stations should be used for supplemental analysis and may need to be added during the duration of the sampling program in order to help focus the investigation toward areas containing more likely sources, e.g. an agricultural source such as a cow pasture or a pipe discharging to the tributary. Additional stations may also be utilized to further investigate potential illicit discharges or to help locate or confirm areas of failing infrastructure or septic tank seepage once a human source has been identified. If a potential source is not present, a minimum of three stations may be required to characterize the spatial extent of the watershed. If the water body branches at any point, it is also important, whenever possible, to sample each branch, especially if different land-uses are represented within the two (or more) watersheds. These "flexible" stations were then sampled by FDEP personnel.

Section 3.0 Phase II, Level I – Initial Testing Approach

Phase II, Implementation, utilized a decision tree-based approach (Figure 3) designed to build on the results of the Phase I effort and continue screening for potential sources of fecal contamination. To minimize cost and time, this approach uses lower-cost, more basic methods first, followed by higher-cost, more sophisticated methods. The approach follows the EPA (2005) recommendation of using some combination of MST methods coupled with chemical tracers where appropriate. Use of multiple MST methods increases the confidence in source identification because the error rates of individual procedures are affected by complex factors that are not fully explored due to the recent deployment of these methods (Stoeckel & Harwood 2007); the weight-of-evidence approach used in this study places more weight on the positive or negative result of multiple methods.

As indicated in the diagram (Figure 3), progression through the different levels is based on the results of the previous tests. The final step links the source identified from the testing process to the potential sources observed in the field in order to confirm results. Initially (e.g., for the first sample event), all of the advanced MST tests are conducted simultaneously in order to provide an overview of source-types that test present or absent throughout the watershed. As the sampling program progresses, it is expected that the use of the tests included in the decision-tree would become continually more site-specific. All tests are administered on an as-needed basis in order to provide the necessary weight-of-evidence to support the identification of a specific source. For example, a source or source-category identification is not made based solely on a single test result; test results are considered in conjunction with one another and/or are confirmed by background knowledge of the area or field observations. The expectation was that this endeavor will eliminate the need to continue the sampling effort in Level II for those WBIDs or sites that are impacted by more obvious sources.

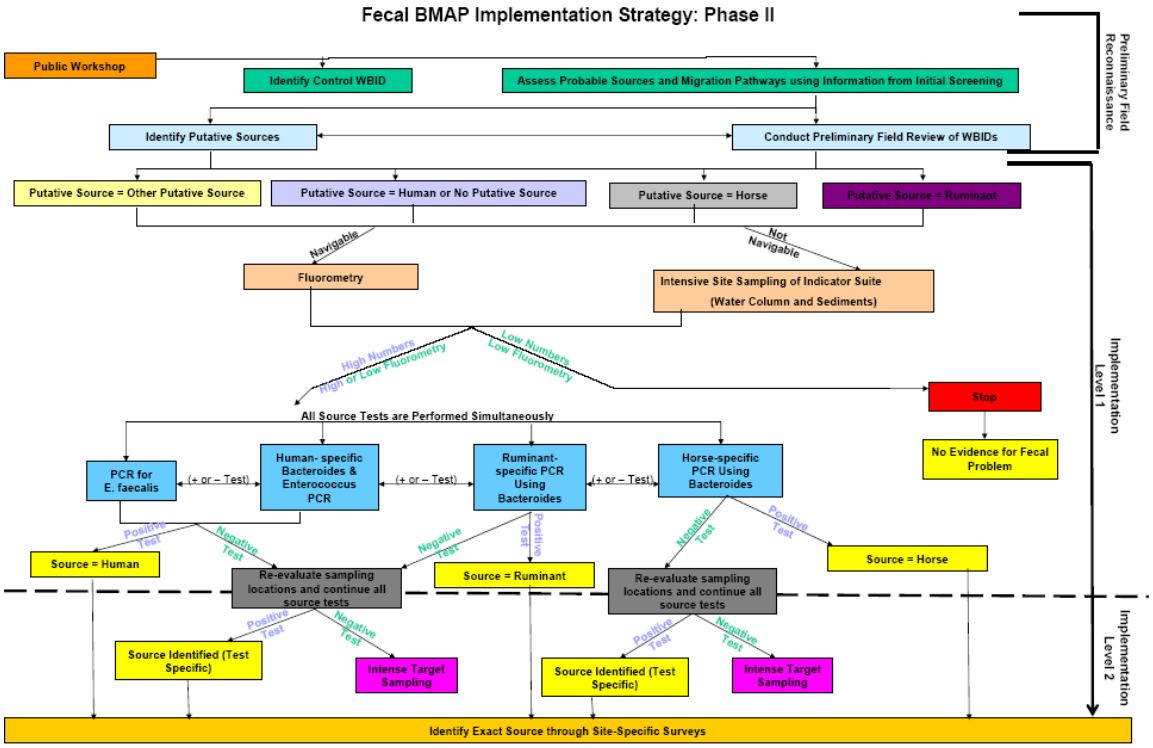


Figure 3. The decision-tree approach designed to use lower-cost, more basic methods first, followed by higher-cost, more sophisticated methods. This system, based on information gained through previous sampling results, is implemented each month to help plan for future sampling and analysis efforts.

Section 3.1 Bacteriological Assays

The relationship between different types of indicator organisms has for years been suggested as a means of discriminating among sources and relative age of fecal pollution. Versions of Standard methods for the Examination of Water and Wastewater before 1995 (APHA 1995) included using the ratio of fecal coliforms to fecal streptococci as a discriminator between human and animal pollution. *Clostridium perfringens* is a spore-forming organism that survives well in sediments for years, therefore some had suggested that its concentration in sediments might act as an indicator of older reservoirs of pollution (Hill et al. 1996). Over succeeding years, ample evidence has accumulated that the effect of environmental stressors on microbial survival is complex and does not affect all indicator bacteria groups equally. Furthermore, the relative density of the microbial population in animal feces and humans is unpredictable. The combined weight of these uncertainties has led to great caution when interpreting the difference between ratios of indicator bacteria types in environmental samples. With that caveat in mind, it has frequently been observed by the Harwood laboratory that waters recently contaminated by fecal pollution, whether from animals or humans, tends to contain high levels of fecal coliforms, *E. coli* and enterococci (all values are within 1 order of magnitude). When enterococci concentrations are high, but fecal coliform and *E. coli* levels are low (>1 order of magnitude lower than enterococci), host-specific markers indicating recent fecal contamination are rare. This situation frequently occurs under low-rainfall conditions when one would not anticipate contamination by run-off. It is hypothesized that the low *E. coli* – high enterococci suggests "aged" fecal pollution, in which the enterococci have

proliferated in surface waters/sediments, while the *E. coli* have not. The cases where fecal coliforms and enterococci are high, but *E. coli* are low, suggest a similar interpretation since some fecal coliform species seem to be quite persistent and may grow in Floridian freshwater systems. Again, these inferences must be made cautiously and with supporting data, but they may also aid in interpretation of data using a weight-of-evidence approach.

Section 3.1.1 Sampling Methodology

A minimum of 3 "fixed" sampling stations was used for each tributary. These 3 stations, sampled by USF and PBS&J staff, provided consistency to the sampling program across seasons and served to characterize the upstream, midstream, and downstream areas of the WBID. If a potential source was identified, these stations were located so that one was as close as possible to the potential source and two additional stations bracketed the area of interest, so that the upstream sampling location served as a reference station.

In addition to the 3 "fixed" stations, "flexible" stations were used for supplemental analysis and were added to help focus the investigation on areas containing more likely sources—e.g., an agricultural source such as a cow pasture. Additional stations were also used to further investigate potential illicit discharges or to help locate or confirm areas of failing infrastructure or septic tank seepage once a human source was identified. If a potential source was present, a minimum of three stations was required to characterize the spatial extent of the watershed. If the waterbody branched at any point, it was also important, whenever possible, to sample each branch, especially if different land uses were represented within the two (or more) watersheds. FDEP personnel sampled these "flexible" stations. Water samples were routinely analyzed for fecal coliforms at these locations; however, USF periodically conducted further analyses (e.g., indicator suite analysis of sediments, more advanced MST tests) at individual stations, as necessary. The number and location of "flexible" stations changed and generally increased over the duration of the project.

Monthly water samples were collected in each WBID from May to December 2006. The sampling time coincided with an outgoing tide for the Lower Hillsborough River, the only tidal waterbody. Sediment samples were also taken from each "fixed" site, and some "flexible" stations, at least once during the study period. To some extent, this allowed for a comparison of water and sediment bacterial concentrations, allowing inferences on whether (1) the sediment was acting as a reservoir for these bacteria due to previous pollution or (2) high concentrations were due to recent contamination. Additional field data and general observations were also collected to help determine contamination sources. MST methods were employed using the decision-tree approach for each site to identify specific sources (e.g., human, ruminant, or horse); each site was tested at least once, and sites showing consistent elevated bacterial concentrations or sporadic marker presence were tested more frequently.

Section 3.1.2 Enumeration of Indicator Bacteria

Water and sediment samples collected at "fixed" sampling locations were processed by membrane filtration (0.45µm pore-size, 47mm diameter) using a vacuum pump to enumerate a suite of indicator bacteria including *Escherichia coli*, fecal coliform, and

enterococci. These indicator organisms (IOs) were used to better characterize microbial pollution and distribution in the water column and sediments and to aid in identifying probable sources. Although these bacteria do not directly cause disease, large quantities suggest the presence of disease-causing agents. Appendix A provides details concerning USF laboratory methodologies.

Supplemental water samples collected from "flexible" stations were sampled and typically analyzed for fecal coliforms only, by FDEP personnel.

Section 3.1.3 Microbial Source Tracking

The MST methods summarized below were used to help identify sources of indicator bacteria pollution. These methods, including nonlibrary–based techniques, have not been adopted by regulatory agencies but have been incorporated into a draft EPA guidance document (2005). Consistent with other emerging fields in the environmental sciences, development, assessment, and validation of each of the methods is an adaptive process.

Polymerase chain reaction (PCR) is a process in which many copies of a specific DNA sequence can be synthesized from a small amount of starting material (the "template"). This process, called amplification, generates enough material to be visualized or manipulated for further investigation. The library independent presence/absence testing using PCR uses primers that target specific microbial DNA sequences unique to microbes from a specific source, such as humans and cattle. For example, *Bacteroides*, a fecal anaerobe genus present in both human and animal intestines, is used as a target for this type of source identification technique (Bernhard & Field 2000c, a).

Another target organism for this type of PCR technique is *Enterococcus faecium*, one of the dominant enterococci found in human feces. The virulence factor, entercoccal surface protein (*esp*), found in this species is linked to sewage originating from humans, not animals (Scott et al. 2004). The procedure and PCR conditions applied for *Bacteroides* assays, using primers for human- (Bernhard & Field 2000b), ruminant- (Bernhard & Field 2000b), and horse- (Dick et al. 2005a) associated *Bacteroides*, were changed during the sampling period. To increase the sensitivity of the assay and reduce PCR artifacts, PCR was performed on extracted DNA from water samples rather than whole-cell templates beginning in August, and the reamplification of PCR products was discontinued for all *Bacteroides* assays. Furthermore, the touchdown PCR program, used for all *Bacteroides* assays, was discontinued only for the horse-associated *Bacteroides* assay in October due to the production of spurious bands, and a standard cycle was implemented that resolved this issue. The presence of the human polyomavirus (HPyV), a nonpathogenic virus with a high carrier rate among humans, was also evaluated (McQuaig et al. 2006a).

Section 3.2 General Water Quality Parameters

Physical-chemical measurements in the water column included temperature, pH, conductivity, salinity, dissolved oxygen, and turbidity at each of the stations at the time of sampling. These measurements were taken by the entity responsible for collecting the bacteriological samples at each station. General observations, including weather

conditions and tributary characteristics, and data analysis exploring the possible relationships between these factors and bacteria levels were performed throughout Level I sampling to help identify potential sources.

Section 3.3 Fluorometry

Fluorescence is a characteristic of certain molecules that absorb specific wavelengths of light and consequently emit longer wavelengths of light. Optical brighteners (OBs), a common additive in laundry and dishwashing detergents, absorb light energy below 400 nm and emit light in a general range of 430 nm to 450 nm. Fluorometry can be used to detect and estimate quantities of OBs. Since laundry water is generally discharged to sanitary sewers or onsite treatment and disposal systems (OSTDS), OBs are typically present in most human waste treatment and conveyance systems. It should be noted that in rare cases, older residential structures may have drainage systems for laundry wastewater that are separate from either the septic tank drain field or a sewer line, although this is not a practice that is permitted or condoned by regulatory agencies in Florida. Releases of residential wastewater into water bodies may contain these compounds.

Fluorometry has been used successfully to detect plumes from OSTDS in surface waters (Grant 1998, TAMU 1999) and can be a cost effective and reliable technique for identifying domestic wastewater contamination when used in conjunction with fecal coliform bacteria (Waye 1999, Dixon et al. 2005); however, a methodology has not been approved by the EPA (Charlotte Harbor Environmental Center 2003). One of the primary advantages to this technique is that it can provide real-time results when a field instrument is employed. This technique may prove most beneficial when utilized as a flow-through instrument mounted on a vessel, providing continuous sampling output and patterns of fluorescence. This strategy would restrict the use of the method to navigable waterways.

The use of fluorometry in detecting human fecal contamination has yielded variable results likely due to the background fluorescence from organic compounds (Hartel et al. 2007a, Hartel et al. 2007b). In an estuarine environment where naturally-occurring substances, such as humic acids or tannins also fluoresce, the use of optical brighteners as an indicator of human wastewater may demand extensive post-processing to effectively separate the fluorescence signal of humics from that of the brighteners (Dixon et al. 2005). Another solution is to decrease this interference by utilizing excitation:emission filters that enhance the resolution of the instrument for certain wavelengths. The use of this more restricted wavelength filter reduces background fluorescence caused by organic matter by over 50% (Hartel et al. 2007b). Additional work has been done to discriminate OBs from other interfering organic molecules by comparing ultraviolet (UV)-degradation rates (Hartel et al. 2007a). OBs degrade rapidly when exposed to UV light, whereas organic tannins and humic acids break down more slowly; thus a large decrease in fluorescence after brief exposure to UV is in theory indicative of optical brighteners.

During this study, Dr. Harwood's laboratory and the FDEP Southwest District Office attempted to use fluorometry in the project WBIDs to help identify sources of fecal coliform contamination. The presence of organic compounds in Florida waters, however,

proved to interfere with the detection of OBs by fluorescing and giving a "false-positive" signal. USF utilized a hand-held fluorometer to conduct a series of experiments using various concentrations of "Tide" laundry detergent dissolved in purified water. The results suggest that this methodology, as currently utilized, is not as sensitive to gross fecal contamination as *E. coli* measurements and that it may be difficult to accurately discriminate sewage from naturally fluorescing compounds in Hillsborough River water. As a consequence of USF's preliminary fluorometry results, Dr. Harwood's laboratory focused on determining whether: 1) their instrument was sensitive enough to detect the OBs in environmentally relevant concentrations, and 2) if Dr. Hartel's method of exposing tannic waters to UV light and discriminating OBs from other molecules by comparing UV-degradation rates would work in Florida waters. Dr. Hartel's work in Georgia indicated that OBs degrade rapidly when exposed to UV light, whereas organic tannins and humic acids break down more slowly; thus a large decrease in fluorescence after brief exposure to UV is in theory indicative of optical brighteners. Dr. Harwood's laboratory results indicated that pure water and Hillsborough River water have similar degradation rates and that an adequate signal was not being obtained from sewage. Dr. Harwood's results from the field station samples also suggest that the levels of OBs at the different sites were all similar and could not be discriminated from one another, despite significant differences in fecal coliform levels.

Two fluorometry sampling strategies were also employed by the FDEP. The first involved the collection of grab samples at stations selected during the MST Level I Initial Testing Phase while the second involved the use of a real-time flow-through fluorescence system. The first methodology included the collection of three sets of OB samples at each site concurrently with the bacteriological and chemical samples. This technique was designed to discriminate optical brighteners from other compounds by utilizing a dual-fluorometer system for analysis in the laboratory. This methodology allowed for the calculation of relative fluorescence using a ratio between fluorescence resulting from CDOM and that by optical brighteners. The second technique implemented by the FDEP utilized an in-water probe that could be used in systems that are navigable by boat (e.g., Lower Hillsborough River, Lake Thonotosassa). Both of these methodologies are limited in their utility by many factors, including when people do their laundry and how long it takes that water to move through the system.

The FDEP conducted an initial field test using the flow-through system in Lake Thonotosassa. Most of their effort was focused in the northern portion of the lake. Preliminary results indicated that higher levels of OBs were identified, without a corresponding increase in CDOM, in the area around the outflow to Flint Creek. The benefits of using an *in situ*, real-time instrument that allows the investigation to be more focused in areas where higher levels of OBs are observed were evident. The lower Hillsborough River, Lake Thonotosassa, and Walden Lake would be good environments to continue testing this technique because they allow for continuous measurement.

Currently, results from both the USF and FDEP approaches have been largely inconclusive; however, direct comparisons of the various OB-fluorescence methods as well as results from the microbial tests are expected to continue in future months. Preliminary fluorometry results using raw optical brightener fluorescence in the lower Hillsborough River suggest a reverse relationship with salinity, as expected.

Section 4.0 Analysis of Phase II, Level I Water Quality Data

Upon completion of Phase II, Level I sampling, the *Hillsborough River BMAP Fecal Database* contained 241 unique entries consisting of the indicator suite (fecal coliform, *E. coli*, and enterococci) bacterial concentrations (i.e., log abundance), MST test results, and a host of abiotic parameters (e.g., pH, temperature, conductivity, salinity, turbidity) recorded during 8 sampling events (May-December 2007) at 55 stations throughout 6 WBIDs in west-central Florida. Stations falling within five miles of a daily rainfall gauge (three operated by COT, one by USGS; Map 2) were appended with time-lagged rainfall data (1-day, 2-day, 4-day, 7-day, and 14-day totals) matching the periods of record. All database entries were vetted for missing, duplicate, or erroneous values prior to further analysis. A general discussion of the variety of statistical results performed using this database is included below; a more detailed description of each analysis and the associated findings are included in Appendix C.

In general, each of the 6 watersheds sampled had sites with indicator bacteria concentrations that regularly exceeded the state standard for fecal coliform (*http://www.dep.state.fl.us/legal/Rules/shared/62-302/62-302.pdf*) designated at 400 CFU/10mL, as well as the EPA standard for enterococci, designated as 33 CFU/100mL (*http://www.epa.gov/waterscience/criteria/library/ambientwqc/bacteria1986.pdf*).
Geometric means of indicator organisms from sites for each WBID also showed elevated concentrations of indicator bacteria in water and sediments that exceeded this standard (Figures 4 and 5). Concentrations of each indicator bacteria in sediment showed significant positive correlations ($p \leq 0.0006$) with concentrations in the water column with r^2 values of 0.49 for fecal coliform, 0.47 for *E. coli*, and 0.27 for enterococci (Appendix C, Table 1).

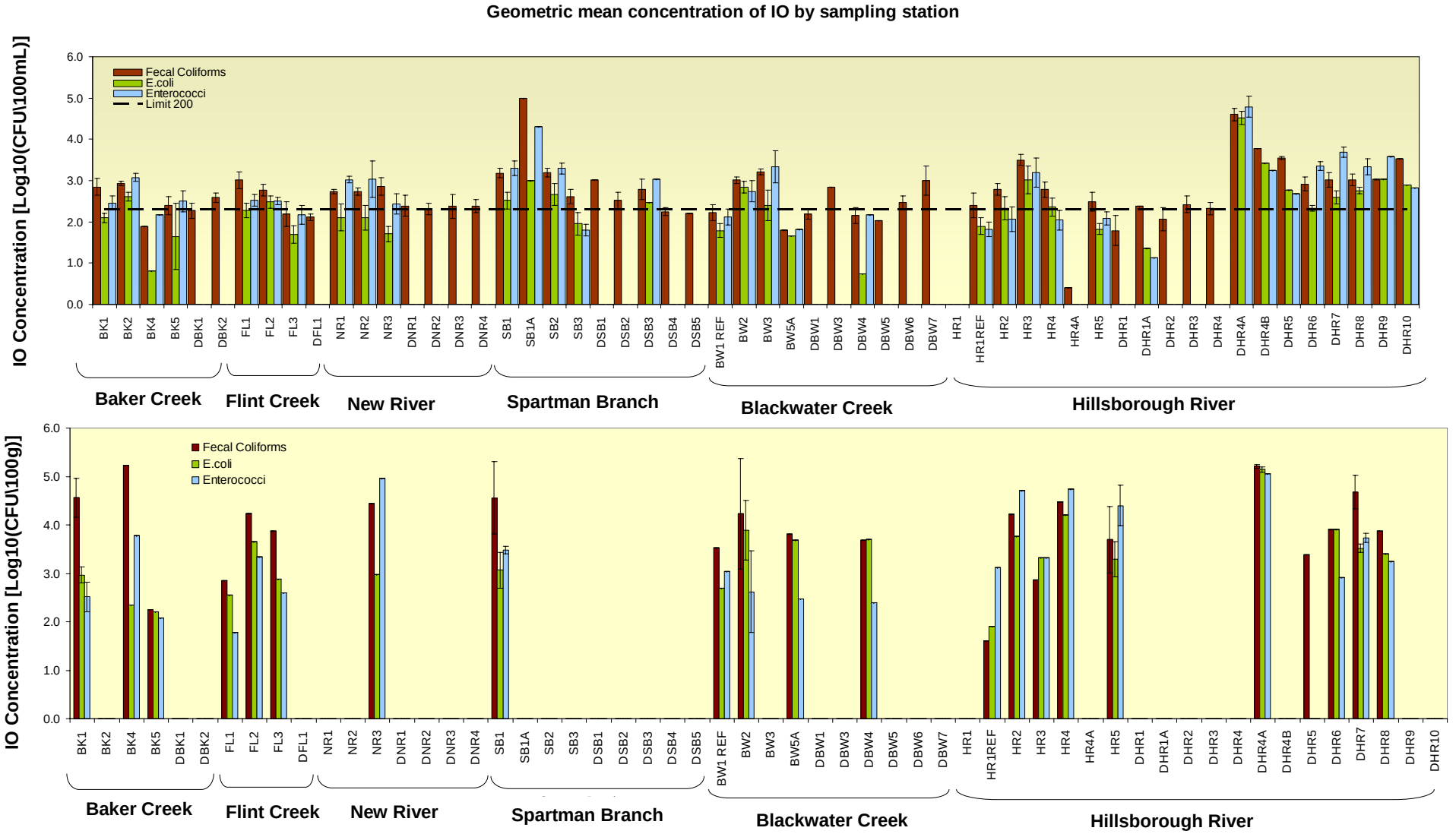


Figure 4. Geometric mean concentration of indicator organisms (IOs) by sampling station in water samples (top graph) and sediment samples (bottom graph). Calculations were performed on all available data collected under flowing conditions.

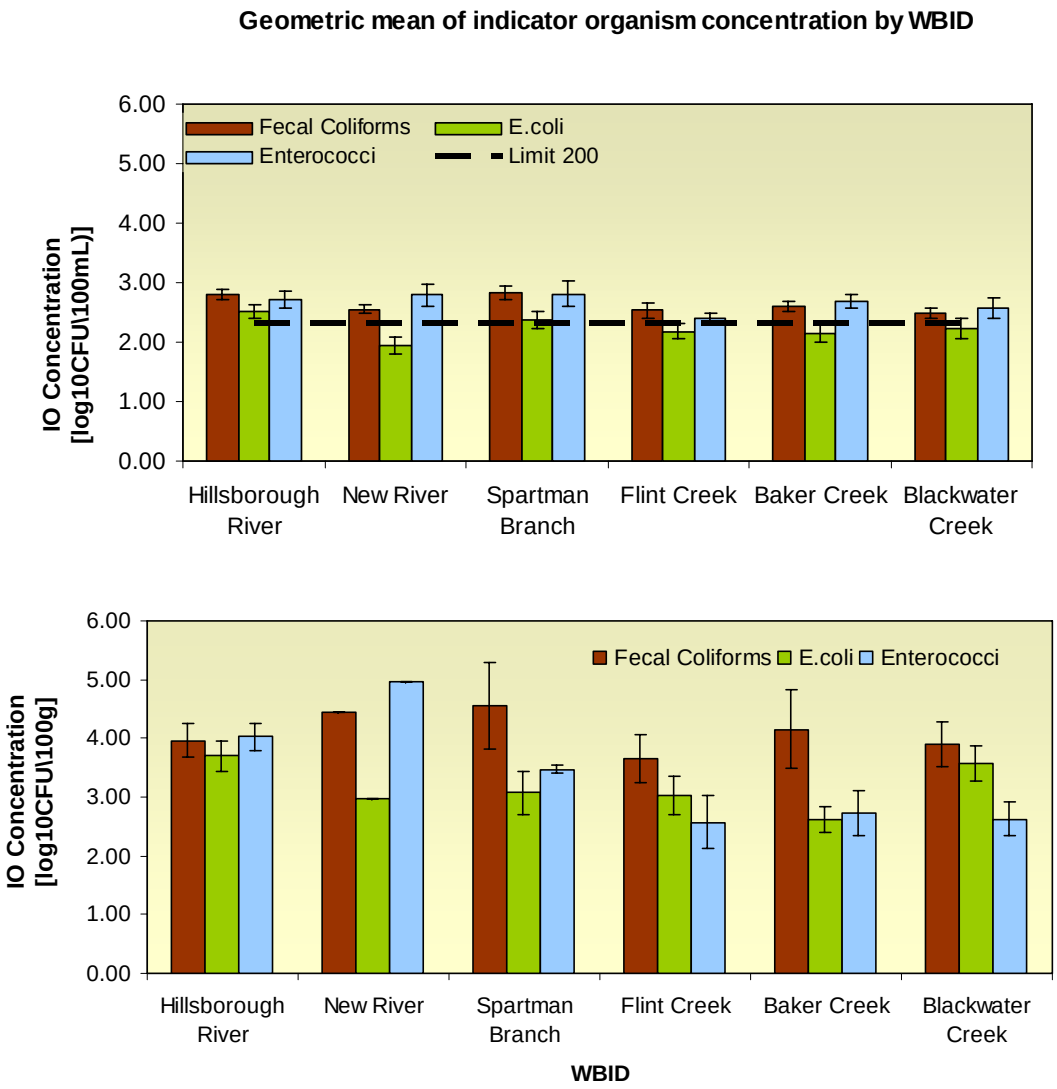


Figure 5. Geometric mean concentration of indicator organisms (IOs) by WBID in water samples (top graph) and sediment samples (bottom graph). Calculations were performed on all available data collected under flowing conditions.

Indicator suite organism (hereafter, 'IO Suite') abundance values were tested for correlations with available water quality parameters; i.e., turbidity, salinity, pH and temperature. Significant linear relationships were found for fecal coliform and *E. coli* abundance with pH at various stations; however, because the correlation itself and the direction of the correlation are not consistent, the detection of significance may merely be the result of chance after numerous tests were performed or the presence of an interacting factor. As expected, fecal coliforms were generally negatively correlated with salinity at several sites within the Lower Hillsborough basin. This is likely due to the high inactivation ("die-off") rate of both fecal coliforms and *E. coli* in saline waters (Anderson et al. 1979, Solic & Krstulovic 1992, Bordalo et al. 2002, Anderson et al. 2005). It should be noted that a small subset of abiotic parameter values were excluded from analysis due to apparent instrument error.

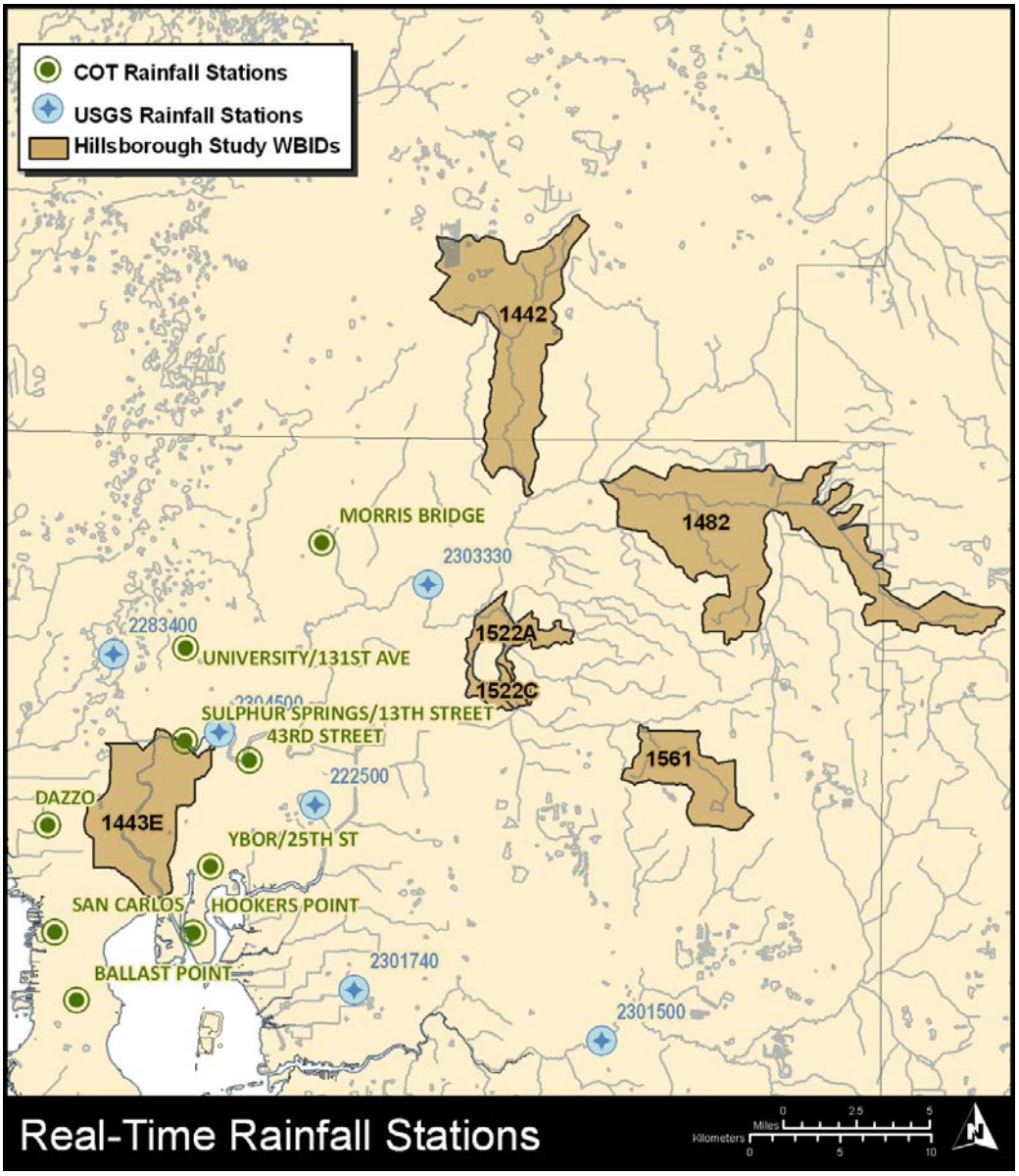
Comparisons among IO concentrations are important as the persistence, as well as the potential for multiplication, especially in warm subtropical waters, may differ among species. For example, higher concentrations of enterococccci may be indicative of an older source of pollution (e.g., stormwater runoff) as opposed to a more recent source (e.g., SSO). Although it has not yet been scientifically documented, enterococci appear to demonstrate greater persistence and growth under certain circumstances, such as within enclosures and underground stormwater storage units (personal communication, Dr. Valerie J. Harwood, October 3, 2007). In all cases other than New River, bacterial species were found to be positively correlated with each other (Table 3). This may indicate that the IOs all exhibited similar behavior, in terms of potential persistence and/or growth, under the circumstances present in all of the basins except the New River, over the duration of the project.

Table 3. Summary of results for the correlative analysis among IO Suite organism concentrations within each WBID. Significance was assessed at an alpha of 0.05. Reported values are Pearson correlation coefficients.

WBID	IO	Fecal Coliforms	E.coli
BK	Fecal Coliforms		
	E.coli	**0.715**	
	Enterococci	-	X (r=0.661,p=0.007)
BW	Fecal Coliforms		
	E.coli	**0.681**	
	Enterococci	**0.745**	**0.622**
FL	Fecal Coliforms		
	E.coli	**0.666**	
	Enterococci	**0.576**	**0.596**
HR	Fecal Coliforms		
	E.coli	**0.903**	
	Enterococci	**0.807**	**0.853**
NR	Fecal Coliforms		
	E.coli	-	
	Enterococci	-	-
SB	Fecal Coliforms		
	E.coli	X	
	Enterococci	X (r=0.747,p=0.001)	**0.743**

' - ' = Not significant
X = Not normally distributed

Analysis of bacteria levels with relation to rainfall was conducted for all sampling locations within five miles of an actively-reported rain gauge (Map 2). Significant correlations were only observed in the 7-day and 14-day totals for stations within the Lower Hillsborough River and these trends were always positive in nature. Most commonly, they reflected fecal coliform and enterococci concentrations, suggestive of the presence of a stormwater-related source; however, station HR2 yielded a strong correlation with 14-day rainfall and all three IOs possibly suggesting the presence of more recent contributions.



Map 2. USGS and COT rainfall stations and Lower Hillsborough River MST project WBIDs.

MST results did not always correlate with indicator concentrations. Exceptions include the human-specific *Bacteroides* marker (hereafter, 'human *Bacteroides*') at 'all WBIDs' and the Lower Hillsborough River and the ruminant-specific *Bacteroides* (hereafter, 'ruminant *Bacteroides*') at Blackwater and Baker Creeks, though these relationships, were relatively weak. In addition, it was determined that *E. coli* levels were positively

correlated with the detection of the ruminant marker. This implies that ruminant sources may be somewhat enriched with *E. coli* as compared to other potential sources. If this is the case, use of *E. coli* to fecal coliform ratios may be able to be utilized to discriminate ruminant sources from other contributors, though additional data is needed to draw a more definitive conclusion. Additional analyses used to better investigate the relationship between fecal coliform concentrations, particularly levels exceeding 400 CFU/100mL, and the presence or absence of MST markers, demonstrated that correlations existed for human-specific markers only and only in particular WBIDs. These results suggest that the use of the 400 CFU/100mL as a standard may be somewhat helpful in discriminating human-specific sources from other potential contributors.

The use of several MST markers for human contamination is one means of increasing the confidence in results. Because the markers are different in terms of sensitivity, specificity, and fate in the environment (e.g. *esp* does not survive well in septic systems; viruses are smaller and more mobile in surbsurface flow than bacteria), their results in the same sample frequently differ. The confidence with which one can conclude that "microorganisms from human sources are present" (or are likely to be absent) at a given site is greatly increased when multiple markers are observed across more than one sample event.

Human *Bacteroides* were detected significantly more frequently than other markers ($p < 0.0001$), possibly due to higher concentrations in water than either *esp* or HPyV. Although a complete understanding of the performance of the individual methods in the variety of complex environmental scenarios encountered in this study is still required, the relatively high level of detection of the human *Bacteroides* marker is also possibly due to a greater level of sensitivity of this assay as compared to the other human-specific markers (the HPyV marker is likely the least sensitive of the assays utilized in this study). It is also important to note that some of the lowest levels of co-occurrence were found between *esp* and the other human-specific markers. Although the *esp* gene is commonly found in human sewage resulting from sanitary sewer systems, it less frequently survives through the storage tanks and associated drainfields of septic systems [personal communication, Dr. Valerie J. Harwood, September 24, 2007; (Whitman et al. 2007)]. This disparity has not been observed for human *Bacteroides*. The lack of positive *esp* indicators, together with the presence of human *Bacteroides* and HPyV markers, lends support to the idea that OSTDS are a probable source. As a result, the low level of co-occurrence between *esp* and the other human-specific markers would be expected if some of the sources identified in the project WBIDs include OSTDS.

The horse-specific *Bacteroides* marker produced some false-positive results at the beginning of the study (notably at HR sites) due to the formation of PCR artifacts. Further testing showed that the spurious PCR products were due to the formation of primer dimers. The protocol was altered to lower the primer concentration and remove the "touchdown" component of the cycle. Subsequent testing against target and non-target feces and water samples showed the reaction to be sensitive and specific for horse feces. The new protocol was used from the October sampling event on, and produced no results that appeared to be false-positives (all positives were consistent with land use).

Section 5.0 New River – WBID 1442

Section 5.1 Background

The New River headwaters are located approximately four miles north of State Road (SR) 54 in Pasco County, just west of the City of Zephyrhills, Florida. The New River flows south into the northern portion of Hillsborough County (Map 3). The majority of the New River is contained in a single channel, with the exception of the portion just north of SR-54. The northern section includes one main bifurcation, forming the "eastern" and "western" branches. The eastern branch is the larger of the two and has two additional, yet smaller, divisions. This portion of the tributary originates in pasturelands northeast of County Road (CR) 579 (Handcart Road). The western branch consists of a single channel that originates in the Aberdeen and New River housing subdivisions. The river, in its entirety, is characterized by a relatively small water volume and low flow throughout; flow may be non-existent at times in many areas of the WBID. Analysis of impervious surface, using coefficients derived from those provided in the Impervious Surface Analysis Tool (ISAT) developed by the National Oceanic and Atmospheric Administration (NOAA) Coastal Services Center in partnership with the University of Connecticut, Nonpoint Education for Municipal Officials (NEMO) Project, indicates that the New River WBID contains less than 10% impervious surface; however, the area is currently undergoing rapid development. A soils survey [United States Department of Agriculture/Natural Resource Conservation Service (USDA/NRCS), provided by SWFWMD 2000] indicates that the majority of the New River WBID contains soils with slow to very slow infiltration rates. A few areas, primarily located in the residential area south of SR-54 and east of New River, contain soils with hydrologic classifications indicating high infiltration rates, which could compromise the effectiveness of OSTDSs.

Section 5.2 Preliminary Assessment

Data received for WBID 1442 from EPCHC AWQM, STORET, Legacy STORET, and USGS NWIS Stations between 1990 and 2006 indicate only modest levels of fecal impairment (geometric mean = 173 colony forming units (CFU)/100mL; maximum = 4,300 CFU/100mL; n = 20).

The results of the initial screening process indicate that the potential sources of fecal contamination in the New River differ with location within the WBID. North of SR-54, probable sources of fecal contamination include livestock (cattle and horses), a lower probability of human wastewater influence, and the potential for stormwater and wildlife impacts. The western branch of the river north of SR-54 is characterized by relatively new residential subdivisions that are serviced by public water and sewer (Appendix D, Photograph 1), and a growing commercial corridor along SR-54. Prior to the development of these subdivisions, the primary land use in this area was pastureland. The eastern branch is dominated by pasturelands with cattle, horses, occasional goats and other small domestic animals and woodlands (Appendix D, Photographs 2 and 3) mixed with low-density residential areas, including mobile homes, serviced by OSTDS; these

homes are generally not located in close proximity to the stream channel. Swales are present on both sides of Morris Bridge Road and act as a conveyance system for stormwater to reach the river. In addition, there is a new stormwater retention pond with an associated weir servicing the Oak Creek subdivision (established in 2004) that eventually discharges into the river.

The Southeast Regional Pasco County wastewater/reuse plant is located just west of the junction of Handcart Road and Eiland Boulevard, just east of the New River. An on-site consulting engineer for this wastewater treatment facility (WWTF) indicated on February 3, 2007, that the plant did not discharge to the river, even during wet weather events, and that 100% of the effluent was reclaimed; however, this facility was named in the FDEP consent order with Pasco County in 2006 as having a variety of wastewater violations which were primarily overflows and discharges from Handcart Road pond and associated storage tanks.

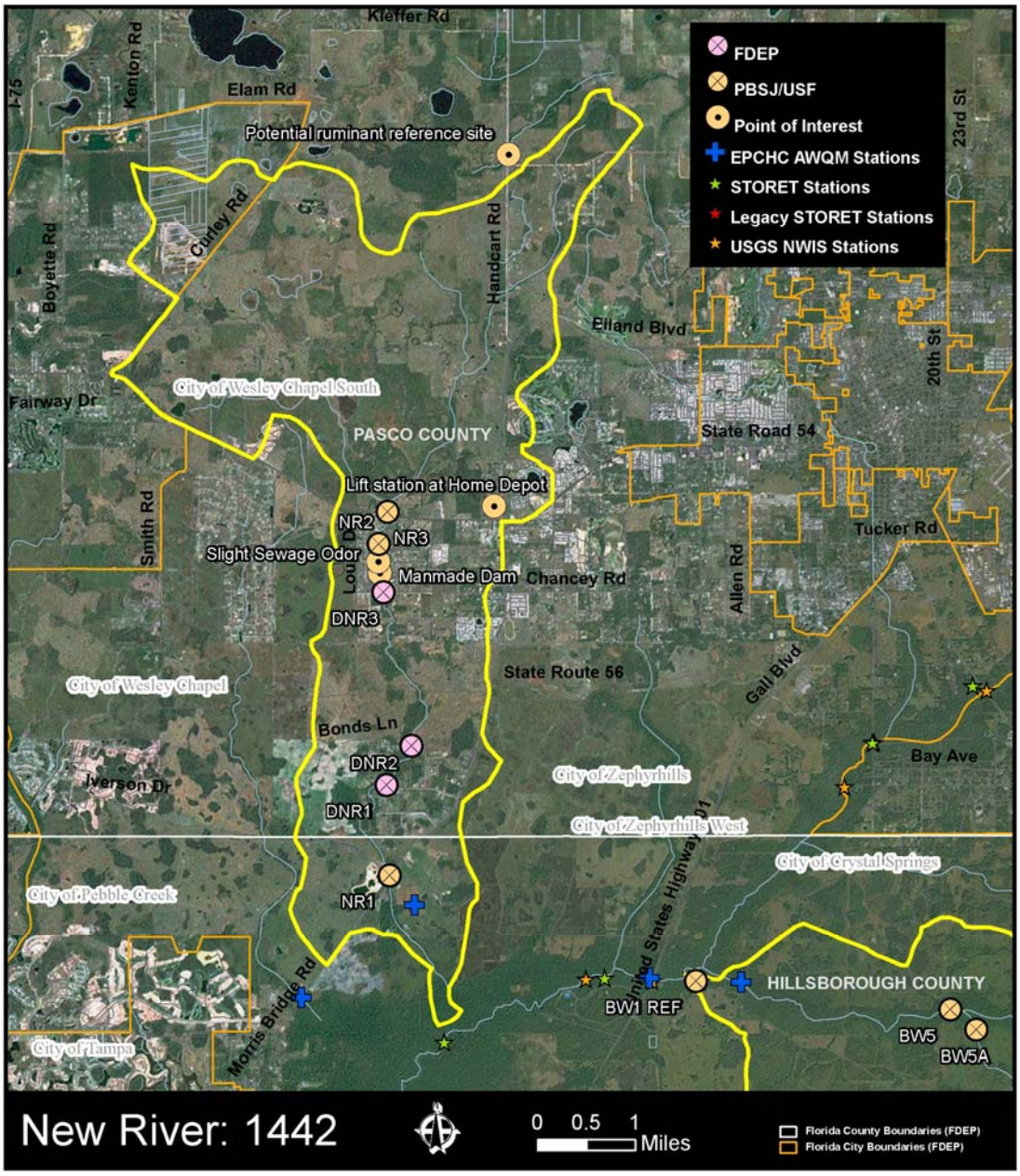
Likely sources of fecal contamination transition from probable animal sources north of SR-54 to primarily human sources (i.e., septic systems) south of Chancey Road. This section of the tributary is characterized by mobile home/recreational vehicle (RV) parks and rural homes (some of which are located on unpaved roads), which are in close proximity to surface waters and are serviced by OSTDS. Many homes lack mounded drain fields, although the area is generally low-lying. One RV park, located immediately south of SR-54 and east of the stream channel, contains a large septic tank mound within approximately 20 meters of the New River (Appendix D, Photographs 4 and 5). As noted above, soils in this area of the New River include those with high infiltration rates. The combination of these soil types and unmounded septic systems indicates a high likelihood that OSTDS account for some portion of local sources of fecal pollution. Field observations made on May 8, 2007, did not reveal any straight-pipe illicit discharges from OSTDS to the New River; however, an abundance of trash and a sewage odor was detected in this area (Map 3; Appendix D, Photographs 6 and 7). In addition, a wetland located between a manmade dam just south of Brisk Drive and Chancey Road (see Map 3; Appendix D, Photograph 8) may assist in remediating any potential upstream human sources of fecal pollution. Although a potable water line, presumed to carry potable water to nearby residents, was observed along Lado Drive during the preliminary site reconnaissance efforts, information received from Pasco County indicates a lack of sewer and water infrastructure in this area. Other sources of fecal contamination in this area may include animals such as cattle, horses, dogs, goats, chickens, and ducks (Appendix D, Photograph 9).

Further downstream, potential sources of fecal pollution include both human (i.e., septic systems) and non-human (e.g., livestock) related impacts. Areas with rural and mobile homes on dirt roads serviced by septic systems are distributed between pastures and undeveloped lands. One portion of the stream, located under Creek Road, is lined with cypress trees, indicating predominantly wet conditions. This alternation between possible sources continues south into the northern reaches of Hillsborough County. Three field reconnaissance efforts (January, February and May 2007) indicated that there was no flow in many areas of the New River, especially south of Chancey Road (Appendix D, Photograph 10).

There are no septic system "hot spot" areas identified by the HCHD (which uses an eight-point selection criteria) within the New River WBID boundaries or immediate contributing waters within Hillsborough County.

Section 5.3 Suggested Monitoring Stations

As described in Section 5.2, the initial screening process revealed that the potential sources of fecal contamination in the New River are specific to the different regions of the tributary. Upon completion of this phase of the project, it was suggested that six initial sampling stations (including three "fixed" stations and three "flexible" stations) be utilized to better assess these sources (Map 3; Table 4). These station locations were investigated and confirmed during the "Walk the WBIDs" portion of Phase II.



Map 3. Map of the New River upon completion of Phase I, including identified sampling locations for Phase II, historical sampling locations, and general points of interest.

Table 4. A summary of confirmed sampling locations within the New River WBID for Phase II, Level I analysis. Stations NR1, NR2, and NR3 were identified for sampling by USF and PBS&J staff and stations DNR1, DNR2, and DNR3 were identified for sampling by FDEP personnel. N/A = Not Applicable

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Comments
NR3	Livestock	SR-54	STORET 281312821558	Fixed	May need to add stations to account for East and West branches to north if see positive impacts for humans and livestock
NR2	Human (OSTDS)	Brisk Drive	N/A	Fixed	Livestock and domesticated animals may also contribute to fecal pollution in this area
DNR3	Human (OSTDS)	Chancey Road	STORET 280954821553	Flexible	Potential human impacts from upstream may be mediated by wetland between Brisk Drive and Chancey Road
DNR2	Livestock	Betts Drive	N/A	Flexible	Human impacts (OSTDS) may also contribute to fecal pollution in this area
DNR1	Human (OSTDS)	Creek Road	N/A	Flexible	
NR1	Livestock	Morris Bridge Road	EPCHC 523	Fixed	

Section 5.4 Phase II, Level I Sampling Results

Level I sampling was greatly restricted throughout the majority of the study due to a lack of ambient surface water in the New River basin (Table 5). Although all stations with adequate water were sampled, the results should be read with the understanding that flow was nonexistent at times. For example, the consequences of sampling surface waters that are not flowing may include results that represent the re-growth, or conversely, the die-off of bacteria due to exposure to environmental stressors such as ultraviolet radiation. Alternatively, sampling of non-flowing water may be beneficial as it could be representative of a groundwater source that may not otherwise be discerned due to dilution by surface waters. Although the interpretation of data from non-flowing water may prove useful in research investigations (e.g., source identification), these data should not be used in the establishment or implementation of regulatory standards. Water was flowing at all stations during the August, September, and October sampling events, with

the exception of station NR2 in August. Table 5 summarizes the New River sampling stations and their surface water status over the sampling period.

Table 5. A summary of the New River sampling stations and their surface water status over the sampling period (May – December). N/A = Not Applicable. All stations are identified on Map 4.

Station	Month Added	Months Sampled	Dry	No Flow
NR3	May	July - October	May, June, November, December	N/A
NR2	May	June - December	May	June, July, August, December
DNR4	October	October - December	N/A	N/A
DNR3	May	June, August - October	May, July, November, December	June
DNR2	May	August - October	May, June, July, November, December	N/A
DNR1	May	August - October	May, June, July, November, December	N/A
NR1	May	July - December	May, June	July, November, December

Results indicate that the New River, when flowing, is only mildly impaired according to the water quality criterion for fecal coliform concentration in Class III water bodies (Chapter 62-302 of the F.A.C.; Table 6, Figure 6). During flow conditions, fecal coliform levels in water samples collected at all monitoring stations exceeded 800 CFU/100mL on only one occasion (station NR3 on July 25, 2007 = 2,800 CFU/100mL). Although fecal coliform concentrations oftentimes fell below 400 CFU/100mL, this criterion was commonly exceeded during the September 2007 sampling event. Despite the lack of observed correlations amongst IOs within the New River basin (Table 3), enterococci concentrations frequently exceeded 800 CFU/100mL reaching levels of 1,040 CFU/100mL at station NR3 in July, 8,000 CFU/100mL at station NR2 in September, and 850 CFU/100mL, 900 CFU/100mL, and 1,550 CFU/100mL at station NR1 in August, September, and October, respectively.

As noted above, on one occasion (July 25, 2007), levels of fecal coliforms in the water column at station NR3 reached 2,800 CFU/100mL. Although evaluation of the potential impact of rainfall on bacterial numbers could not be conducted for the New River basin due to lack of proximity (>5 miles) to an actively-reported rain gauge, it should be noted that, according to USGS rain gauge 2303330 (Map 2), it had rained in the area for nine days prior to this sampling event, totaling 3.25 inches of rainfall. In contrast, each of the other sampling events, when indicator bacteria levels were considerably lower, was preceded by at least two days with little to no rain. This pattern suggests that the source

of pollution identified at station NR3 was stormwater-related and likely originated in upstream cattle pastures north of SR-54.

Although one sediment sample was taken at each of the "fixed" sites, only one sample, collected at station NR3, was taken within the New River watershed under flowing conditions. At stations NR1 and NR3, the IO Suite abundance was higher in the sediments than the water samples collected on the same date. In contrast, fecal coliform concentrations were substantially higher in the water column than the associated sediments at station NR2 in June under non-flowing conditions (water = 78,000 CFU/100mL, sediment = 29,500 CFU/100g; Figure 6). The extremely elevated counts of indicator bacteria in the sediments at stations NR3, NR2 and NR1 (fecal coliforms, *E. coli*, enterococci in CFU/100g; = 27,500, 950, 90,500; 29,500, 16,500, 3,000; 102,000, 1,000, 5,750 respectively; Figure 6), suggest either recent and/or periodic inoculation or extended persistence of indicator bacteria in the sediments (Davies et al. 1995, Anderson et al. 2005). Periodic re-inoculation of the water column from this reservoir is a likely explanation for the chronic and slightly elevated levels at these sites; however, higher levels of fecal coliforms in the water column relative to the sediments at station NR2 may suggest the presence of a more recent source.

Table 6. Geometric mean concentration of indicator organisms (IOs) in water and sediment samples collected in the New River basin. Calculations were performed on all available data collected under flowing conditions.

WBID	Fecal Coliforms				*E. coli*				Enterococci			
	GeoMean	N	SE	Max	GeoMean	N	SE	Max	GeoMean	N	SE	Max
New River (water)	2.56	22	0.08	3.45	1.94	10	0.15	2.62	2.79	10	0.18	3.90
New River (sediment)	4.44	1	N/A	4.44	2.98	1	N/A	2.98	4.96	1	N/A	4.96

The lack of flowing water throughout the New River watershed on a regular basis prevented analysis of IO Suite abundance in relation to the abiotic environment as well as the multivariate analysis of IO Suite abundance and MST results. Despite the inability to perform these investigations, a basic evaluation of the presence and absence of specific MST markers at various stations throughout the basin can provide useful information regarding contributing sources. For example, unsurprisingly, all human-specific MST tests conducted at stations NR3 and NR1 were negative while two of those performed at station NR2, coinciding with elevated levels of IOs, yielded positive results for human *Bacteroides* and HPyV (Figure 6; Table 7). Although the *esp* gene is commonly found in human sewage resulting from sanitary sewer systems, it less frequently survives through the storage tanks and associated drainfields of septic systems [personal communication, Dr. Valerie J. Harwood, September 24, 2007; (Whitman et al. 2007)]. This disparity has not been observed for human *Bacteroides*. The lack of positive *esp* indicators, together with the presence of human *Bacteroides* and HPyV markers, lends support to the idea that OSTDS are a probable source of the fecal contamination at station NR2. The occurrence of the human *Bacteroides* marker identified in non-flowing water with fecal coliform concentrations reaching 6,800 CFU/100mL adds support to the contention that the source of contamination at station NR2 is transported through groundwater and not surface flow, thereby further suggesting OSTDS as a likely source. Station DNR4 was

added during the October-December sampling events in the vicinity of the sewage odor detected during the "Walk the WBIDs" effort to help determine the spatial extent of the fecal coliform contamination downstream of station NR2 (Map 4). During these months, both station NR2 and DNR4 demonstrated only minor, if any, exceedances of fecal coliforms (605 CFU/100mL and 500 CFU/100mL = maximum levels at NR2 and DNR4). It should be noted that station DNR4 is located upstream of a wetland that may help to mitigate impacts before the pollution reaches station DNR3.

As predicted, ruminant *Bacteroides* was detected at stations NR1 and NR3 under flowing conditions (Figure 6; Table 7), indicating that animals such as cattle and deer (most likely cattle from upstream pastures) are responsible for the fecal contamination present at these locations. It must be noted that detection of the ruminant-specific marker at these locations coincided with low-to-moderate IO concentrations. The ruminant-specific marker was also observed at NR2 where, on one occasion, it coincided with low levels of all IOs and may be explained by the livestock north of SR-54, the "back-yard" farms scattered throughout this neighborhood, or from a more local deer population. Given the presence of human-specific markers at this location as well as the nature of the site, it is more likely that OSTDS are the primary contributors of fecal coliform pollution in this area.

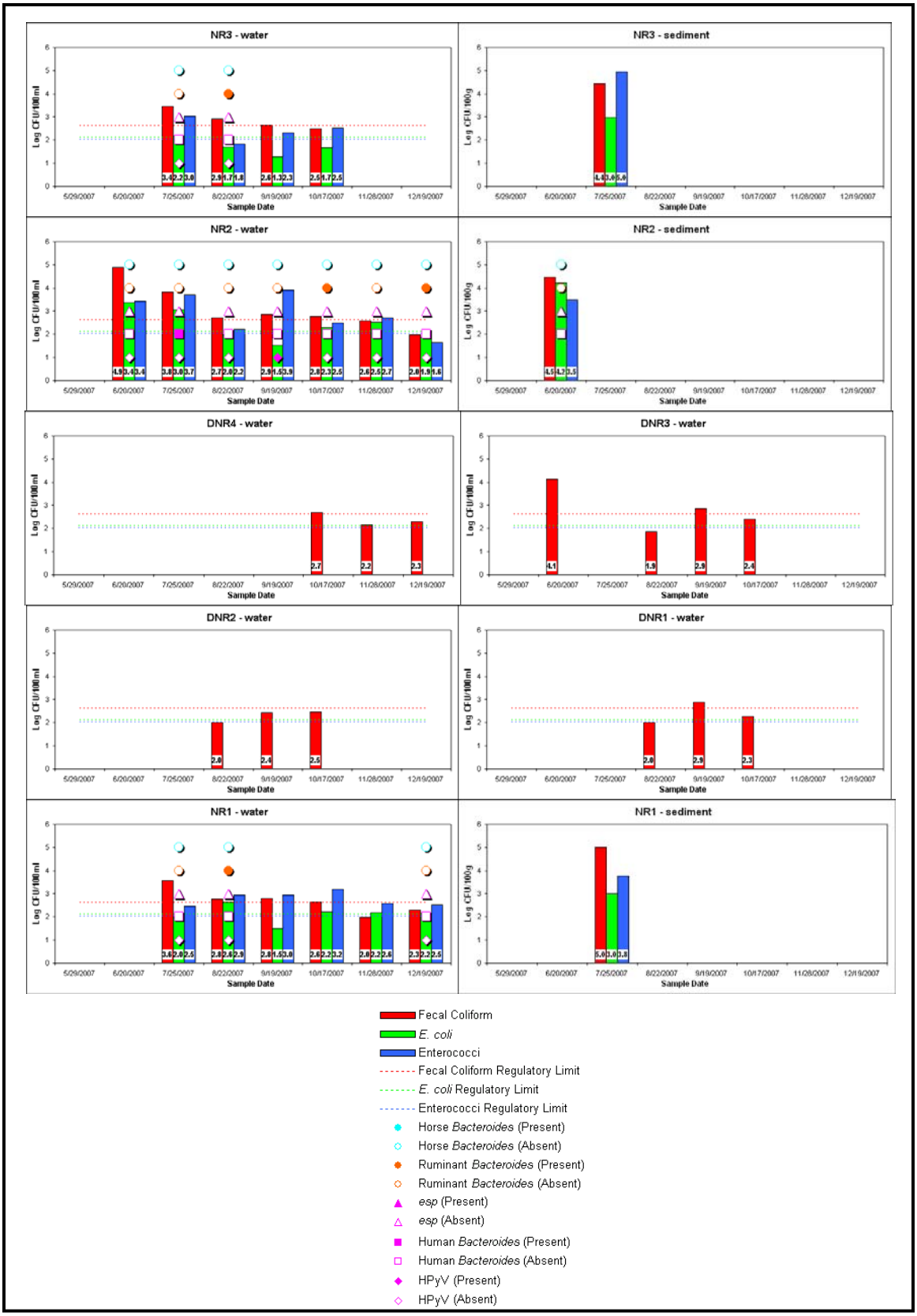


Figure 6. Indicator bacteria results at New River from the May through December 2007 sampling events. Typically only water samples were collected and analyzed for fecal coliforms at the "flexible" stations, including DNR1, DNR2, DNR3 and DNR4.

Table 7. Results of the source-specific microbial tracking tests utilized in this project as of December 2007. Results are reported as the number of positive results/total number of tests performed. For example, 0/1 = 0 positive tests out of 1 total test conducted. Unless otherwise indicated, analyses were conducted on water samples. Yellow shading indicates positive results.

Station	Human Bacteroides	*Enterococcus* (*esp*)	Human Polyomavirus	Ruminant Bacteroides	Horse Bacteroides
NR1	0/3	0/3	0/3	1/3	0/3
NR2	1/7	0/7	1/7	2/7	0/7
NR2 (sediment)	0/1	0/1	0/0	0/1	0/1
NR3	0/2	0/2	0/2	1/2	0/2

Section 5.5 Fecal Coliform Source Assessment Summary

This evaluation indicates that the probable sources of fecal contamination in the New River differ with location within the WBID. North of SR-54, cattle are the most likely source of fecal contamination, as detected at station NR3; however, it must be noted that the ruminant-specific marker was only detected once at this location, at which time it coincided with low levels of *E. coli* and enterococci and only moderately-elevated fecal coliform concentrations (fecal coliforms = 800 CFU/100mL). Further downstream, south of SR-54 near station NR2, the most likely source of contamination appears to be human-related. The presence of human-specific markers, with the exception of *esp*, under both flowing and non-flowing conditions suggest OSTDS as a primary contributor. Elevated levels of IOs, coincident with the detection of human-specific markers, as well as the presence of residential communities in this relatively low-lying area with un-mounded septic system drainfields, further supports OSTDS as a local source of fecal coliform pollution. Additional sources, including upstream livestock north of SR-54, the "back-yard" farms scattered throughout this neighborhood, or a more local deer population, may also contribute to the contamination detected in this area. Under flowing conditions, the pollution identified in the upstream portion of the WBID does not appear to be regularly transported downstream, as indicated by the results at stations DNR4, DNR3, DNR2, and DNR1. This is likely due to the presence of a wetland area just south of station DNR4 as well as the ephemeral nature of the waterbody.

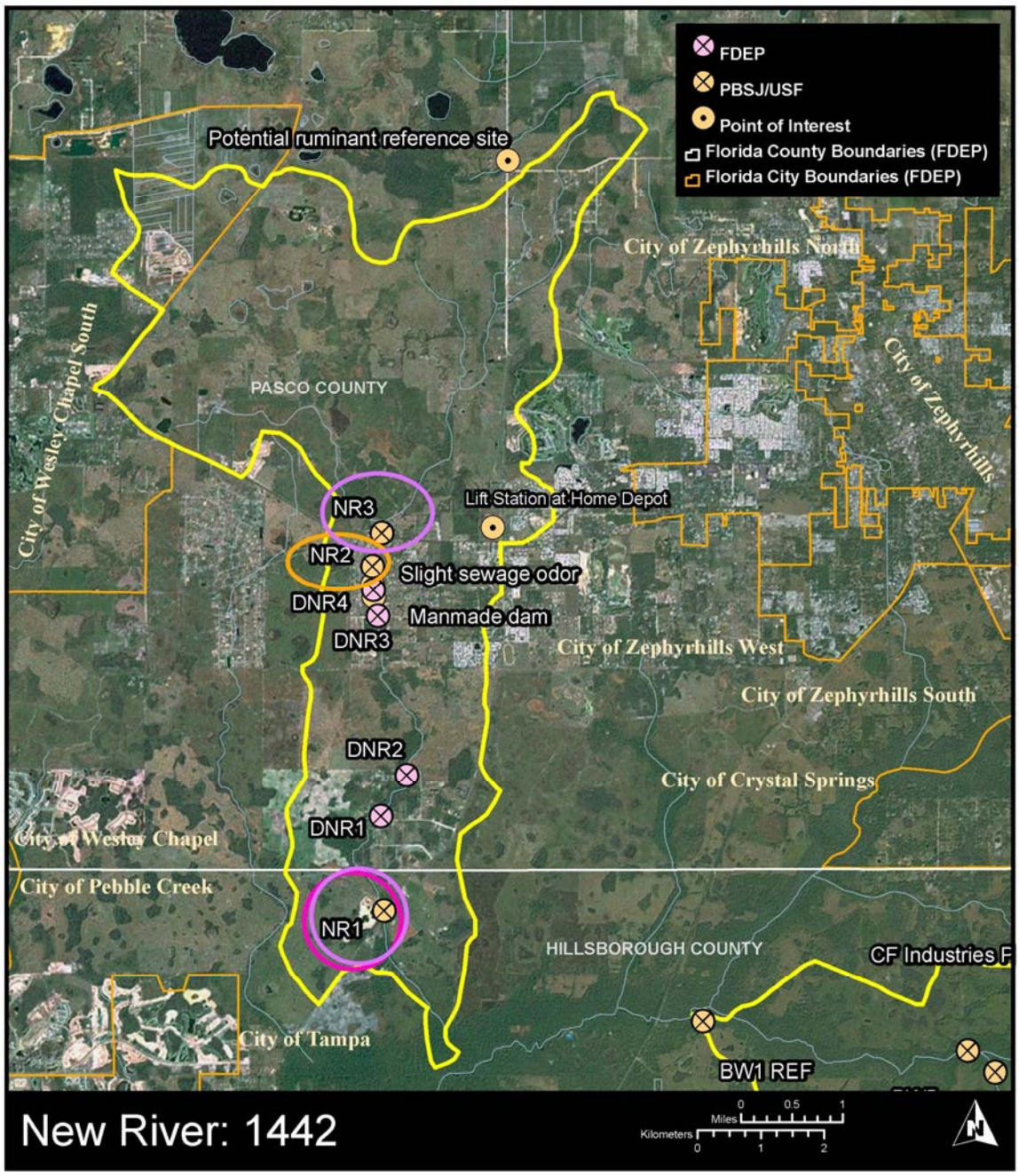
The potential source of fecal pollution changes once again in the most downstream segment of the New River, as indicated by the results at station NR1. As predicted, human-specific markers were not detected at this site despite regular exceedances of IOs under both flowing and non-flowing conditions. Ruminant *Bacteroides*, however, was detected on one occasion, simultaneous with low- to moderately-elevated levels of all IOs. These results, together with the primary local land use, indicate that animals such as cattle and deer (most likely cattle from upstream pastures), are the primary contributors of fecal coliform contamination in this area. Table 8 identifies the most likely sources of fecal contamination for each area of the New River.

Suggested corrective actions, specifically to address likely OSTDS-related sources in the vicinity of station NR2 and potential livestock- and wildlife-associated contributions upstream of station NR1, are included in Sections 11.1 and 11.3.

Table 8. Summary of most probable sources of fecal contamination contributing to the New River sampling locations (listed from upstream to downstream). N/A = Not Applicable.

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments and Other Potential Sources
NR3	Cattle	SR-54	STORET 281312821558	Fixed	Ruminant-specific MST Presence of upstream cattle pastures Highest IO levels after July rainfall event suggests transport of source via stormwater from upstream or overland flow	Relatively new residential subdivisions serviced by public sewer are located north of SR-54 Elevated bacterial levels in sediments (especially enterococci and fecal coliforms relative to *E. coli*) may suggest older source and may cause re-inoculation of surface waters
NR2	Human (OSTDS)	Brisk Drive	N/A	Fixed	Human-specific MST, except *esp* marker Human-specific markers detected under flowing and non-flowing conditions suggests local source, possibly transported via groundwater Low-lying area Older residential communities with un-mounded OSTDS and soils with high infiltration rates	Livestock and domesticated animals may also contribute to fecal pollution in this area Elevated bacterial levels in sediments (especially *E. coli* and fecal coliforms relative to enterococci) may suggest recent source and may cause re-inoculation of surface waters Higher bacteria concentrations in surface waters than associated sediments may suggest recent source

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments and Other Potential Sources
DNR4	N/A	South of Brisk Drive	N/A	Flexible	N/A	Minor exceedances, possibly due to growth of bacteria in stagnant water Heavily shaded Slight sewage odor detected during "Walk the WBIDs" effort Upstream of man-made dam and small wetland
DNR3	N/A	Chancey Road	STORET 280954821553	Flexible	N/A	Potential human impacts from upstream may be mediated by upstream wetland between Brisk Drive and Chancey Road Exceedances likely due to growth of bacteria in stagnant water Often dry-limited data (n=4)
DNR2	N/A	Betts Drive	N/A	Flexible	N/A	No observed exceedances Often dry-limited data (n=3)
DNR1	N/A	Creek Road	N/A	Flexible	N/A	One observed exceedance may be due to upstream OSTDS Often dry-limited data (n=3)
NR1	Cattle Wildlife	Morris Bridge Road	EPCHC 523	Fixed	**Cattle** Ruminant-specific MST Presence of upstream cattle pastures **Wildlife** Only one MST marker detected Land use amenable to wildlife	Low to moderate bacteria exceedances under flowing conditions Exceedances likely influenced by growth of bacteria in stagnant water Elevated bacterial levels in sediments may cause re-inoculation of surface waters



Map 4. Map of the New River upon completion of Phase II, including identified sampling locations for Phase II, historical sampling locations, and general points of interest. Results of the May through December 2007 sampling events suggest the presence of "hot spots" for fecal contamination in the vicinity of NR2 and NR1. The most likely sources in general areas are denoted by circles of different colors: orange = septic systems; purple = cattle; and pink = wildlife.

Section 6.0 Flint Creek and Baker Creek – WBIDs 1522A and 1522C

Section 6.1 Background

Flint and Baker Creeks are closely associated (both spatially and hydrologically) and will therefore be discussed collectively in this report. The primary headwater of Baker Creek is Pemberton Creek, which in turn, drains Mill Creek and Spartman Branch and, ultimately, a large portion of the City of Plant City. A description of Spartman Branch is included in Section 7 below. Baker Creek itself runs in a northwest direction for approximately 3 miles before emptying into Lake Thonotosassa [a hypereutrophic lake and one of SWFWMD's Surface Water Improvement and Management (SWIM) Priority Water Bodies]. Prior to reaching the lake, two contributing tributaries (one from the west and one from the south; with a confluence just southeast of Collins Ranch Road) merge with the mainstem of Baker Creek to the west of Pemberton Creek Drive. The outfall for Lake Thonotosassa is the headwater for Flint Creek (Map 5; Appendix D, Photograph 11). Flint Creek flows eastward for less than one-quarter mile, turns northward and intersects with waters from Campbell Branch before continuing as a single channel to the northwest where it connects with the Hillsborough River.

Both Flint Creek and Baker Creek generally contain flowing water. Analysis of impervious surface (see Section 5.1) indicates that the entire area within the Flint and Baker Creek WBID boundaries contains less than 10% impervious surface. Here too, residential development is occurring. A soils survey (USDA/NRCS, provided by SWFWMD 2000) shows that the majority of area around Lake Thonotosassa contains soils with high infiltration rates; however, the soils immediately surrounding Baker Creek and in the region northeast of the junction between Flint Creek and Campbell Branch are typically those with slow to very slow infiltration rates.

Section 6.2 Preliminary Assessment

Data received for WBIDs 1522A and 1522C from EPCHC AWQM, STORET, Legacy STORET, and USGS NWIS Stations between 1990 and 2006 indicate typically moderate levels of fecal impairment with evidence of episodic events with highly elevated levels (1522A geometric mean = 245 CFU/100mL; 1522A maximum = 30,000 CFU/100mL; 1522A n = 192; 1522C geometric mean = 263 CFU/100mL; 1522C maximum = 3,200 CFU/100mL; 1522C n = 191).

Findings from the initial screening process suggest that the potential sources of fecal contamination in Flint and Baker Creeks primarily include a mixture of human- (e.g., septic systems and upstream sewer sources) and non-human- (e.g., livestock, wildlife) related impacts. A large portion of the land south of Lake Thonotosassa is occupied by tree crops, mainly orange groves, while land to the west and east is largely comprised of new housing developments (Appendix D, Photograph 12) that utilize OSTDS but have a minimum 200-foot setback from the lake. As a result, these homes are unlikely sources of fecal contamination; however, this new development has led to a recent diversion of

flow in tributaries such as Campbell Creek which run into Flint Creek (personal communication with local ranch owner, May 2007). Older homes in the area, especially around Flint Creek, may only have a 75-foot setback from the tributary and are generally located in the areas with high infiltration rate soils. As a result, these areas are more likely sources of fecal pollution originating from OSTDS.

Unlike the area immediately surrounding Lake Thonotosassa, the Flint and Baker Creek watersheds are characterized by a semi-rural landscape with pockets of small ranches and medium-density residential areas. Some of the local ranches are located in relatively low-lying areas and livestock were observed standing alongside creek beds and associated wetlands during the January and May preliminary field reconnaissance efforts (Appendix D, Photographs 13 and 14). It was also common to see numerous cow feces ("cow pies") scattered throughout pastures adjacent to the tributaries as well as in the creek bed itself. It should also be noted that portions of Baker Creek and contributing waters are periodically dredged for sediment and vegetation removal; this was observed during the May 8, 2007 field visit. It is likely that bacterial blooms occur as a result of colonization of dead and dying vegetation.

There are no septic system "hot spot" areas identified by the HCHD within the Flint Creek and Baker Creek WBID boundaries or immediate contributing waters within Hillsborough County. Field observations made on May 8, 2007 did not identify any straight-pipe illicit discharges from septic systems into Flint or Baker Creeks.

Section 6.3 Suggested Monitoring Stations

Sampling locations were determined using the same criteria as described in Section 2.4. The results of the initial screening process, as illustrated in Section 6.2, indicated that the potential sources of fecal pollution in Flint and Baker Creeks include a mixture of human and animal impacts. Although these may be easily discerned in specific locations, the majority of the WBIDs contain multiple potential sources (Table 9). Upon completion of Phase I of this project, it was suggested that eight initial sampling stations (including six "fixed" stations and two "flexible" stations) be utilized to better assess these sources (Map 5; Table 9). These station locations were investigated and confirmed during the "Walk the WBIDs" portion of Phase II.

Map 5. Map of Flint and Baker Creeks upon completion of Phase I, including identified sampling locations for Phase II, historical sampling locations, and general points of interest.

Table 9. A summary of confirmed sampling locations within the Flint Creek and Baker Creek WBIDs for Phase II, Level I analysis. Stations FL1, FL2, FL3, BK1, BK2, and BK3 were identified for sampling by USF and PBS&J staff and stations DFL1 and DBK1 were identified for sampling by FDEP personnel. N/A = Not Applicable.

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Comments
FL3	Unknown	Kelso Road	EPCHC 118	Fixed	Highly eutrophic headwaters of Flint Creek, account for any potential sources leaving Lake Thonotosassa
DFL1	Livestock	Campbell Creek	N/A	Flexible	Sample to account for contributing waters to Flint Creek; human impacts (OSTDS) may also contribute to fecal pollution in this area
FL2	Human (OSTDS)	Knights Griffin Road	STORET 8496	Fixed	Livestock and stormwater may also contribute to fecal pollution in this area
FL1	Unknown	US 301	EPCHC 148	Fixed	Wildlife may contribute to fecal pollution in this area
BK3	Livestock	Kingsway Road	N/A	Fixed	Discarded trash is commonly observed at this site (Appendix D, Photograph 15)
DBK1	Livestock	Muck Pond Road	EPCHC 168	Flexible	SWFWMD station
BK2	Human (OSTDS)	Pemberton Creek Drive	EPCHC 529	Fixed	
BK1	Unknown	Thonotosassa Road	EPCHC 107	Fixed	Thonotosassa Park Boat ramp, account for any potential sources entering Lake Thonotosassa

Section 6.4 Phase II, Level I Sampling Results

Section 6.4.1 Baker Creek

Level I sampling was somewhat restricted at Baker Creek throughout the sampling period due to low water levels (Table 10). Although all stations with adequate water were sampled, the results should be read with the understanding that flow was nonexistent at times (see Section 5.4 for discussion). Surface flow was not observed at station BK3 during any month; however, it must be noted that the headwaters of Baker Creek (located in the vicinity of BK3) are groundwater-fed. As a result, flow may be difficult to discern. Water samples were collected at this site during each sampling event through October. In September-December, additional sampling stations (BK4 and BK5) were added further downstream to better identify potential local contributing sources. A summary of the Baker Creek sampling stations and their surface water status over the sampling period is provided in Table 10.

Table 10. A summary of the Baker Creek sampling stations and their surface water status over the sampling period (May – December). N/A = Not Applicable. All stations are identified on Map 6.

Station	Month Added	Months Sampled	Dry	No Flow
BK3	May	May - October	N/A	Always
BK4	September	September	N/A	September
BK5	November	November - December	N/A	N/A
DBK1	May	May - December	N/A	June
DBK2	July	July - August	N/A	N/A
BK2	May	May - December	N/A	May, June
BK1	May	May - December	N/A	May, June

Results reveal that the waters of Baker Creek commonly exceeded state standards for indicator bacteria at stations BK1 and BK2, as well as BK3 under both flowing and non-flowing conditions (Table 11, Figure 7); maximum fecal coliform concentrations in surface waters never exceeded 3,300 CFU/100mL under non-flowing conditions and 3,050 CFU/100mL under flowing conditions (observed at stations BK3 and BK1, respectively on July 25, 2007). Enterococci levels rarely exceeded 800 CFU/100mL at all stations except BK2 where concentrations reached a maximum of 3,400 CFU/100mL on October 17, 2007. Maximum *E. coli* values were 940 CFU/100mL (recorded at station BK2 on May 29, 2007). Comparisons of IO concentrations within the waters of Baker Creek, under flowing conditions, demonstrate a significant positive correlation between fecal coliforms and *E. coli* ($r = 0.715$; $p<0.05$; Table 3). Although data used to compare levels of enterococci and *E. coli* were not normally distributed, a similar trend was apparent ($r = 0.661$; $p=0.007$). These results suggest that the individual IOs demonstrated similar levels of persistence and growth under the circumstances present throughout the Baker Creek basin over the duration of the project (see Section 4.0).

Indicator bacteria levels at stations DBK1 and DBK2, accounting for potential sources from the southern tributary and the upstream portion of the mainstem of Baker Creek, respectively, remained below 470 CFU/100mL with the exception of DBK1 during the June sampling event, when fecal coliforms reached 1,691 CFU/100mL. It should be noted that the June sampling event was the only time that DBK1 was sampled under non-flowing conditions. These results may be explained by the potential for persistence and growth of bacteria in stagnant, highly vegetated waters (Ksoll et al. 2007) (Appendix D, Photograph 16) or the presence of a localized and episodic source. As expected, due to the lack of flow observed at station DBK1 in June, the high levels of bacteria identified at this time did not appear to have been transported downstream to station BK1. This observation suggests that the routinely elevated bacterial levels detected a station BK1 are not originating from this portion of the watershed. Although station DBK2 was only sampled on two occasions (July and August), there is no evidence that contributions in this area accounted for the high bacteria levels identified further downstream at station BK2. This implies the presence of a contributing source between stations DBK2 and BK2 on the mainstem of Baker Creek.

It should be noted that the EPCHC currently has an enforcement action for effluent disposal against the Sun Tampa East RV Park and McIntosh Utilities, both located upstream of station DBK1 (personal communication, EPCHC, March 13, 2008; Map 6). The associated WWTF serves two parks located on US-92 just west of McIntosh Road. A study to determine the capabilities of their disposal system and what will be needed for compliance is currently being performed. Surface water samples collected on March 19, 2008 near these locations and analyzed by Dr. Harwood's laboratory indicate elevated levels of indicator bacteria, most notably at the northside stormpipe, as well as the presence of multiple human-specific markers, most commonly *esp* and *Methanobrevibacter smithii*. While fecal coliform and enterococci concentrations in water samples collected at station DBK1 the same day were below 300 CFU/100mL (samples were not tested for *E. coli*), the *esp* marker was detected, suggesting the possibility for transport of pollution from upstream.

As noted above, on one occasion (July 25, 2007), concentrations of fecal coliforms in the water column at stations BK1 and BK3 reached maximum levels (>3,000 CFU/100mL at each site). The evaluation of the potential impact of rainfall on bacterial numbers could not be conducted for any station within Baker Creek, with the exception of station BK1, due to lack of proximity (>5 miles) to an actively-reported rain gauge (Map 2). There were no identified correlations between IO concentration and rainfall at this site (Appendix C, Table 4). Despite the lack of statistical analysis at station BK3 and the lack of detected correlations at station BK3, it should be noted that, according to USGS rain gauge 230330 (Map 2), it had rained in the area for nine days prior to the July sampling event, totaling 3.25 inches of rainfall. Although peak concentrations were observed in July, fecal coliform levels reached 1,750 CFU/100mL at BK1 and 1,290 CFU/100mL at BK2 during the September sampling event which was preceded by two days without rainfall. This pattern suggests that rainfall plays a limited role, if any, in the bacterial loadings of Baker Creek.

A total of seven sediment samples were analyzed for IOs from the Baker Creek watershed, three of which were collected under flowing conditions. Bacteria concentrations in the sediments analyzed at all stations, with the exception of station BK5, were higher than those in the water column sampled on the same date; however, all IO concentrations within the water column at station BK5 at this time were under 565 CFU/100g. Sediments collected at station BK1 were highly variable and may be related to water flow and recent dredging activities. For example, fecal coliform levels ranged from 250 CFU/100g in May (no flow) to 91,500 CFU/100g in September (flow) and 14,500 CFU/100g in October (flow) (Figure 7). According to Hillsborough County, all vegetation associated with the May dredging effort was removed from Baker Creek between the July and August sampling events; however, observations made during a field visit on August 22, 2007 indicated that at least some of the dredge spoil had been piled next to the creek alongside the adjacent dirt road suggesting the potential for recontamination of bacteria-laden sediments. Although the variation in contamination levels in the sediments at this location may merely be reflective of the patchy nature of bacteria, it is more likely that the bacterial loadings at this location have been contributed by a more recent source. The extremely elevated levels of IOs in the sediments at stations BK3 and BK4 [fecal coliforms = 55,000 CFU/100g (July) and 172,000 CFU/100g (September)], respectively, may also suggest recent and/or periodic inoculation of bacteria since both corresponding *E. coli* and enterococci values were significantly lower (did not exceed 6,000 CFU/100g) at these locations; however, the extremely low concentrations of *E. coli* (<500 CFU/100g) relative to both fecal coliforms and enterococci may be more indicative of extended persistence and growth of the other IOs (Davies et al. 1995, Anderson et al. 2005) and an older source at these locations. Interpretations regarding bacterial concentrations in sediments must be viewed with a great deal of caution, as few sediment samples have been analyzed among the Florida sites. Since portions of Baker Creek and contributing waters are periodically dredged for sediment removal (observed during the May 8, 2007 field visit), it is possible that a bacteria bloom, resulting from colonization of dead and dying vegetation, accounted for the elevated levels at BK1 during the July 25, 2007 sampling event; however, several MST markers were identified at this time indicating a more recent source of pollution.

Table 11. Geometric mean concentration of indicator organisms (IOs) in water and sediment samples collected in the Baker Creek basin. Calculations were performed on all available data collected under flowing conditions.

WBID	Fecal Coliforms				*E. coli*				Enterococci			
	GeoMean	N	SE	Max	GeoMean	N	SE	Max	GeoMean	N	SE	Max
Baker Creek (water)	2.60	24	0.10	3.48	2.15	15	0.16	2.95	2.69	15	0.12	3.53
Baker Creek (sediment)	4.15	4	0.67	5.24	2.62	4	0.21	3.13	2.72	4	0.39	3.78

IO Suite abundance values from three stations within the Baker Creek watershed (BK1, BK2, and DBK1) were tested for correlations with available water quality parameters as described in Appendix C, Section 1.2. A significant positive relationship was identified between pH and *E. coli* concentration at station BK2 ($r = 0.841$, $p<0.05$; Appendix C,

Table 2). In contrast, a marginally significant, negative correlation between pH and fecal coliform abundance was observed at station BK1 ($r = -0.808$, $p = 0.052$; Appendix C, Table 2). The inconsistency in the direction of this correlation suggests that the detection of significance may merely be the result of chance after numerous tests were performed or the presence of an interacting factor.

Human- and ruminant-specific MST markers were common at stations BK1 and BK2 while only human-specific markers were detected at stations BK3, BK4, and BK5 (Figure 7, Table 12). Each human marker was detected at station BK1 and co-occurrence of human *Bacteroides* and *esp,* or HPyV and *esp,* was noted in half of the samples in which any human marker was detected (two of four). These data constitute strong evidence for a human source of contamination at this site. Similar results were observed at station BK2 where co-occurrence of human *Bacteroides* and HPyV was detected. BK3 was only sampled twice for MST markers, since it was determined after several months that it is a groundwater-fed site with little contribution from upstream.

It is important to remember that only limited sampling was performed at stations BK4 and BK5, as they were added later in the study. Although the *esp* gene is commonly found in human sewage resulting from sanitary sewer systems, it rarely survives through the storage tanks and associated drainfields of septic systems [personal communication, Dr. Valerie J. Harwood, September 24, 2007; (Whitman et al. 2007)] This disparity has not been observed for human *Bacteroides*. The prevalence of the *Bacteroides* (44% detection rate) and HPyV markers (28% detection rate), as compared to the *esp* gene (17% detection rate), implies the presence of an OSTDS source within the Baker Creek watershed. There is no obvious connection between the presence of MST markers and rainfall; however, the occurrence of all three human-specific markers identified at least once at a minimum of one station during a period of no rain and in non-flowing water, further supports the contention that the human-related source of contamination in Baker Creek is transported through groundwater and not surface flow and is indicative of OSTDS as a likely source. It is also interesting to note that 29% of all HPyV markers detected throughout this project were within the Baker Creek watershed, providing strong evidence for a human source contribution to this basin.

Ruminant *Bacteroides* was detected twice at station BK1 and four times at BK2 (Figure 7; Table 12), indicating that animals such as cattle (most likely those observed in the creek just downstream of station DBK1; Appendix D, Photograph 16) and either cattle or deer, respectively, are responsible for some of the fecal contamination present at these locations. Given the preponderance and regularity of detection of human-specific markers in this basin as well as the presence of upstream OSTDS in a groundwater-fed system, it is more likely that OSTDS are the primary contributors of fecal coliform pollution in this area. There were no horse-specific markers detected in Baker Creek.

Multivariate analyses of bacterial community data showed a significant effect of MST results (presence or absence of MST markers) on per-sample bacterial communities for ruminant *Bacteroides* in Baker Creek, though this relationship was relatively weak (Global $R = 0.354$, $p<0.05$; Appendix C, Table 5; Appendix C Figures 3 and 4). SIMPER

identified *E. coli* concentration as a major driver of 45.54% of group dissimilarity whereby higher bacteria abundance was correlated with the detection of the ruminant marker (see Section 4.0). A chi-squared goodness of fit test was used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for the individual MST markers); however, for each marker, greater than 20% of expected values were under 5 and were subsequently removed from the analysis.

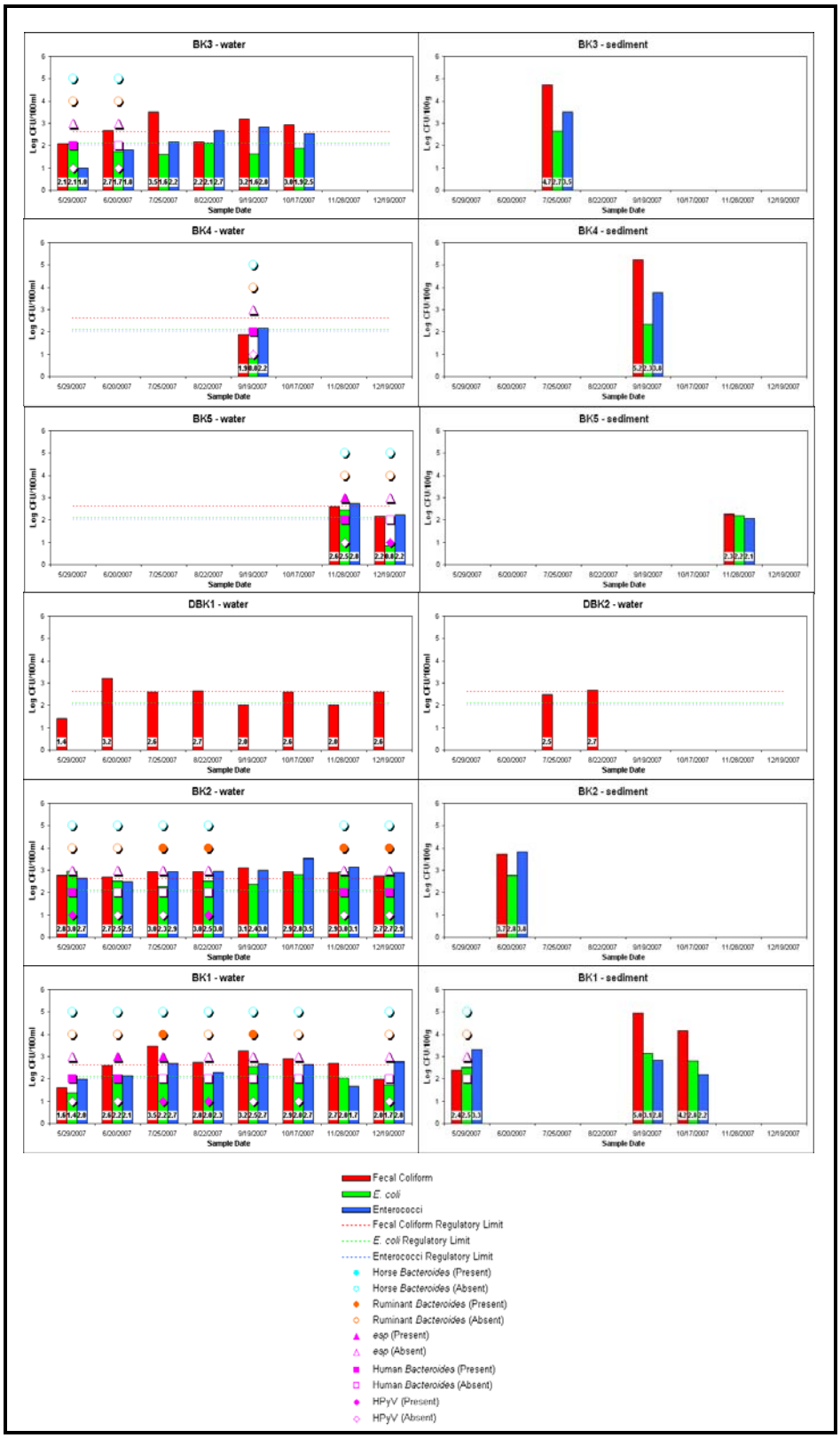


Figure 7. Indicator bacteria results at Baker Creek from the May through December 2007 sampling events. Typically only water samples were collected and analyzed for fecal coliforms at the "flexible" stations, including DBK1 and DBK2.

Table 12. Results of the source-specific microbial tracking tests utilized in this project as of December 2007. Results are reported as the number of positive results/total number of tests performed. For example, 0/1 = 0 positive tests out of 1 total test conducted. Unless otherwise indicated, analyses were conducted on water samples. Yellow shading indicates positive results.

Station	Human Bacteroides	*Enterococcus* (*esp*)	Human Polyomavirus	Ruminant Bacteroides	Horse Bacteroides
BK1	2/7	2/7	2/7	2/7	0/6
BK1 (sediment)	0/1	0/1	0/0	0/1	0/1
BK2	3/6	0/6	2/6	4/6	0/6
BK3	1/2	0/2	0/2	0/2	0/2
BK4	1/1	0/1	0/1	0/1	0/1
BK5	1/2	1/2	1/2	0/2	0/2

Section 6.4.2 Flint Creek

Sampling limitations due to low water levels were encountered at Flint Creek during the May through August sampling events (Table 13). Although all stations with adequate water were sampled, the results should be read with the understanding that flow was nonexistent at times (see Section 5.4 for discussion). For example, Campbell Creek, represented by station DFL1, was dry in May and June and although there was sufficient water for sampling, there was no flow at station FL1 in May, June, and August. A summary of the Flint Creek sampling stations and their surface water status over the sampling period is provided in Table 13.

Table 13. A summary of the Flint Creek sampling stations and their surface water status over the sampling period (May – December). N/A = Not Applicable. All stations are identified on Map 6.

Station	Month Added	Months Sampled	Dry	No Flow
DFL1	May	July - October	May, June	N/A
FL3	May	May – December	N/A	N/A
FL2	May	May - December	N/A	N/A
FL1	May	May - December	N/A	May, June, August

Results indicate that the waters of Flint Creek often exceeded state standards for indicator species at station FL2 and, to a lesser degree, at station FL1 (Table 14; Figure 8). Under flowing conditions, maximum fecal coliform concentrations in surface waters reached 3,750 CFU/100mL (observed at station FL1 on July 25, 2007); fecal coliform levels never exceeded 800 CFU/100mL under non-flowing conditions. Enterococci concentrations remained below 800 CFU/100mL at all stations within Flint Creek while levels of *E. coli* reached or exceeded 800 CFU/100mL at stations FL1 and FL2 on November 28, 2007 (800 CFU/100mL and 950 CFU/mL, respectively). Despite these observations, statistical comparison of IO concentrations within Flint Creek surface waters, under flowing conditions, revealed significant positive correlations between all

three IOs; fecal coliforms and *E. coli* ($r = 0.666$, $p<0.05$), fecal coliforms and enterococci ($r = 0.576$, $p<0.05$), and *E. coli* and enterococci ($r = 0.596$, $p<0.05$; Table 3). These results indicate that the indicator organisms all exhibited similar behavior, in terms of potential persistence and/or growth, under the circumstances present throughout the Flint Creek watershed over the duration of the project (see Section 4.0).

The only fecal coliform exceedance at station FL3 (3,100 CFU/100mL) occurred during the June sampling event, at which time *E. coli* and enterococci levels (245 CFU/100mL and 665 CFU/100mL, respectively) were relatively low (Figure 8). High fecal coliform concentrations observed during this event are not likely the result of water entering Flint Creek from Lake Thonotosassa as water was only observed coming over the control structure at station FL3 during the July, August and September sampling events (at which times, there were minimal-to-no IO exceedances detected at station FL3). The lack of significant exceedances during these times may be due to a dilution effect from significant volume contributions from the lake; however, similar concentrations were also observed when flow over the control structure was not observed.

It is important to note that the high concentrations observed at station FL3 in June coincided with the maximum fecal coliform levels downstream at station FL2. Although there is a slight discernable relationship between elevated levels among the three "fixed" sampling stations that may be suggestive of transport from upstream to downstream sites, it is not consistent among IOs or over time. For example, both *E. coli* and enterococci did not follow the same pattern as fecal coliforms from station FL3 to station FL2 in June. In addition, during the August sampling event, indicator bacteria levels were under 100 CFU/100mL at station FL3 and moderately exceeded standards at station FL2. Similar inconsistencies can be seen between stations FL2 and FL1 (Figure 8); however, it is interesting that the only detection of a human-specific marker (*esp*) at station FL1 occurred under flowing conditions and simultaneously with the detection of the same marker at FL2. The occasional discernable relationship between stations does not otherwise appear to be driven by flow patterns as flow was always observed at stations FL3 and FL2.

Analyses at station DFL1 were performed from July-October under flowing conditions and were restricted to fecal coliforms only. Concentrations remained below 210 CFU/100mL, indicating that bacteria loadings were not entering Flint Creek via Campbell Creek.

Similar to both the New River and Baker Creek, maximum concentrations of fecal coliforms (3,750 CFU/100mL) in the surface waters of Flint Creek were observed on July 25, 2007, at station FL1, after a nine-day rainfall total of 3.25 inches. Despite this apparent relationship, there were no identified correlations between IO concentration and rainfall at either station FL1 or FL3 (Appendix C, Table 4); rainfall analysis could not be conducted for the other sites due to lack of proximity (>5 miles) from an actively-reported rain gauge (Map 2). These data suggest that rainfall plays a limited role, if any, in the bacterial loadings of Flint Creek at station FL1.

Four sediment samples were collected from the Flint Creek watershed and analyzed for IOs over the duration of this study, three of which were obtained under flowing conditions. With the exception of those samples collected at station FL1, bacteria concentrations in the sediments analyzed from stations FL2 and FL3 were higher than those in the associated surface waters sampled on the same day. In addition IO levels were considerably higher in the sediments at stations FL2 and FL3 than at station FL1. For example, fecal coliform concentrations were 17,000 CFU/100g and 7,500 CFU/100g at stations FL2 and FL3, respectively and only reached 700 CFU/100g at station FL1. *E. coli* and enterococci levels demonstrated a similar trend. The observed differences among indicator species in the sediments, specifically the higher concentrations of fecal coliforms and *E. coli* over enterococci, may indicate a relatively recent source of pollution. However, this interpretation must be viewed with a great deal of caution, as few sediment samples have been analyzed among the Florida sites. Periodic re-inoculation of the water column from this reservoir at stations FL2 and FL3 is a likely contributor to the elevated levels of surface water contamination. Flow did not appear to play a role in the abundance of bacteria observed in the sediments.

Table 14. Geometric mean concentration of indicator organisms (IOs) in water and sediment samples collected in the Flint Creek basin. Calculations were performed on all available data collected under flowing conditions.

WBID	Fecal Coliforms				*E. coli*				Enterococci			
	GeoMean	N	SE	Max	GeoMean	N	SE	Max	GeoMean	N	SE	Max
Flint Creek (water)	2.54	24	0.13	3.57	2.18	19	0.13	2.98	2.40	19	0.09	2.89
Flint Creek (sediment)	3.65	3	0.42	4.23	3.03	3	0.33	3.65	2.57	3	0.45	3.34

IO Suite abundance values from two stations within the Flint Creek watershed (FL2 and FL3) were tested for correlations with available water quality parameters as described in Appendix C, Section 1.2. As observed in Baker Creek (station BK1), a significant negative relationship was identified between pH and fecal coliform concentration at FL2 ($r = -0.892$, $p<0.05$; Appendix C, Table 2); however, the inconsistency in the direction of this correlation across WBIDs and individual stations suggests that the detection of significance may merely be the result of chance after numerous tests were performed or the presence of an interacting factor.

Human-specific MST markers, specifically *esp* and HPyV, were commonly detected throughout Flint Creek (Figure 8; Table 15), although human *Bacteroides* was observed on only one occasion (at station FL2). The inconsistency among markers, in particular the presence of *esp* and HPyV (18% detection rates), and not human *Bacteroides* (6% detection rate), may suggest that OSTDS is not a primary source of pollution (see explanation in Section 4.0), although the fact that the majority of homes are on OSTDS in the area argues against this hypothesis.

The strongest evidence for human contamination in Flint Creek was at station FL2, where human *Bacteroides* and HPyV were detected on separate dates, and HPyV and *esp* co-occurred once out of six sample events. Station FL3 was positive for *esp* and HPyV each

on one occasion, giving confidence that sporadic or low-level human contamination occurs at the upstream site. Station FL1 was positive for one human marker (*esp*) only once, suggesting transport of this marker from the upstream sites.

It should also be noted, that the detection of human-related pollution did not always coincide with elevated levels of indicator species. Multivariate analyses of IO Suite concentration data did not detect a relationship between the MST results (presence or absence of MST markers) and per-sample bacterial communities for any marker within Flint Creek. A chi-squared goodness of fit test, used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for the individual MST markers), did however, indicate a relationship between the 400 CFU/100mL limit and the detection of HPyV and *esp* in the Flint Creek basin (X^2=2.79, df=1, P=0.094 and X^2=2.65, df=1, P=0.105, respectively).

Other findings have been published (Whitlock et al. 2002, McQuaig et al. 2006b) which also demonstrate that indicator bacteria levels are frequently not correlated with the finding of human contamination. This disconnect is no doubt due in part to the fact that elevated levels of indicator bacteria are frequently not directly related to a human source, but may be entering the water from stormwater or other sources, or the markers have a "patchy" distribution and are not always detected in the water column. In addition, wet weather runoff containing bacteria from many nonhuman sources may have the effect of masking a persistent human signal that is detectable when surface water levels are low and the human contamination is entering via groundwater. The presence of a major stormwater conveyance system that connects into Flint Creek from the east just upstream of FL2 may be a contributing factor. Interestingly, however, despite the lack of statistical correlation with rainfall, according to USGS rain gauge 02303330 (Map 2), the only sampling effort that occurred after a rain event was in July when nearly half of the human-specific markers were detected and IO exceedances occurred at each "fixed" sampling location. This further suggests that the human-related source of contamination in Flint Creek is through surface flow, and not groundwater.

There were no ruminant- or horse-specific markers detected in Flint Creek; however, other animal sources (e.g., wildlife) may be potential contributors. Given the land use around stations FL3 (Lake Thonotosassa) and FL1 (highly wooded area), it is likely that wildlife is a source in these areas. This potential contribution of wildlife may be relatively greater at station FL3 since human-specific markers were only detected when IO concentrations were relatively low, as opposed to station FL1 where *esp* was identified simultaneously with elevated IOs

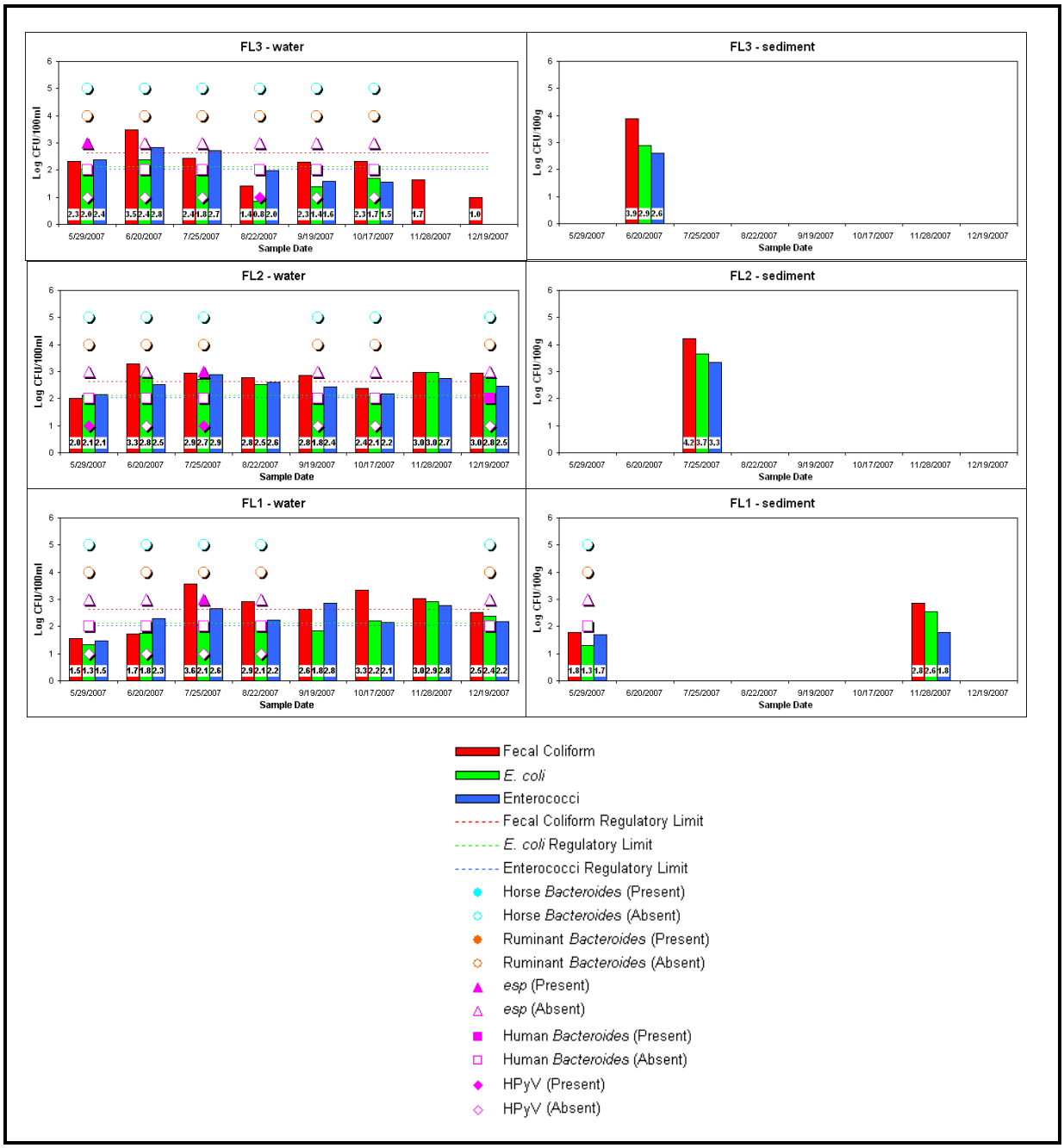


Figure 8. Indicator bacteria results at Flint Creek from the May through December 2007 sampling events. Typically only water samples were collected and analyzed for fecal coliforms at the "flexible" stations, including DFL1.

Table 15. Results of the source-specific microbial tracking tests utilized in this project as of December 2007. Results are reported as the number of positive results/total number of tests performed. For example, 0/1 = 0 positive tests out of 1 total test conducted. Unless otherwise indicated, analyses were conducted on water samples. Yellow shading indicates positive results.

Station	Human Bacteroides	*Enterococcus* (*esp*)	Human Polyomavirus	Ruminant Bacteroides	Horse Bacteroides
FL1	0/5	1/5	0/5	0/5	0/5
FL1 (sediment)	0/1	0/1	0/0	0/1	0/1
FL2	1/6	1/6	2/6	0/6	0/6
FL3	0/6	1/6	1/6	0/6	0/6

Section 6.5 Fecal Coliform Source Assessment Summary

Section 6.5.1 Baker Creek

The results described above suggest that the most probable sources of fecal contamination vary throughout the Baker Creek watershed. The main sources of pollution originate: 1) along the western tributary contributing to Baker Creek (represented by stations BK3 and limited sampling efforts at stations BK4 and BK5); 2) on the main stem of Baker Creek between stations DBK2 and BK2; and 3) possibly downstream of station DBK1 before the confluence with the western tributary.

It is most probable that the primary sources of human-related contamination are OSTDS-related, as indicated by: 1) the relative lack of *esp* markers compared to the more prevalent detection of human *Bacteroides* and HPyV; and 2) the presence of residential communities dispersed throughout some low-lying areas with both mounded and un-mounded septic system drainfields. In addition, portions of the Baker Creek watershed are groundwater-fed (e.g., the western tributary) thereby decreasing the relative potential for regular and significant loadings transported via surface flow. This pollution appears to originate between stations BK3 and BK5 and between stations DBK2 and BK2. The contamination from both contributing tributaries is likely carried downstream where additive effects are detected at station BK1. The possibility of ruminant-specific pollution is evident downstream of station DBK1 (cattle observed standing in creek, Appendix D, Photograph 16) as well as between stations DBK2 and BK2. This pollution likely explains the presence of ruminant markers at downstream station, BK1. Table 16 identifies the most likely sources of fecal contamination for each area of Baker Creek.

Suggested corrective actions, specifically to address potential OSTDS-related sources in the vicinity of stations BK2, BK3, BK4, and BK5 and likely livestock-associated contributions downstream of station DBK1 and between stations DBK2 and BK2, are included in Sections 11.1 and 11.3.

Table 16. Summary of most probable sources of fecal contamination contributing to the Baker Creek sampling locations (generally listed from upstream to downstream). N/A = Not Applicable.

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of Evidence for Source Identification	Comments and Other Potential Sources
BK3	Human (OSTDS)	Kingsway Road	N/A	Fixed	Human-specific MST Groundwater-fed system Presence of OSTDS in area with history of failure	Livestock may also contribute to fecal pollution in this area (observed close to creek in low-lying areas) Surface flow never observed Rainfall plays limited role, if any, in bacterial loadings Extremely elevated bacterial levels in sediments may cause re-inoculation of surface waters Trash commonly observed in water
BK4	Human (OSTDS)	East of Kingsway Road, northwest of McCormick Water Ski and Wakeboard School	N/A	Flexible	Human-specific MST OSTDS in surrounding, low-lying area	Limited data (n=1) Presence of marker coincided with low IO concentrations Extremely elevated bacterial levels in sediments may cause re-inoculation of surface waters Livestock may also contribute to fecal pollution in this area (observed close to creek in low-lying areas upstream)
BK5	Human (OSTDS)	East of Kingsway Road, northeast of McCormick Water Ski and Wakeboard School	N/A	Fixed	Human-specific MST OSTDS in surrounding, low-lying area	Limited data (n=2) Presence of markers coincided with low IO concentrations Livestock may also contribute to fecal pollution in this area (observed close to creek in low-lying areas upstream)

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of Evidence for Source Identification	Comments and Other Potential Sources
DBK1	N/A	Muck Pond Road	EPCHC 168	Flexible	N/A	Only one fecal coliform exceedance (observed under non-flowing conditions) suggests persistence and growth of bacteria in stagnant, highly vegetated waters SWFMD station EPCHC enforcement action with RV parks upstream
DBK2	N/A	Mcintosh Road	N/A	Flexible	N/A	Limited data (n=2) and only one slight exceedance
BK2	Human (OSTDS) Cattle/Deer	Pemberton Creek Drive	EPCHC 529	Fixed	**OSTDS** Human-specific MST, except *esp* marker Co-occurrence of human markers is strong evidence for human contamination Human-specific markers detected under flowing and non-flowing conditions, may suggest local source and transport via groundwater Upstream residential communities on OSTDS **Cattle/Deer** Ruminant-specific MST	

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of Evidence for Source Identification	Comments and Other Potential Sources
BK1	Human (OSTDS) Cattle	Thonotosassa Road	EPCHC 107	Fixed	**OSTDS** Human-specific MST Co-occurrence of human markers is strong evidence for human contamination Human-specific markers detected under flowing and non-flowing conditions, suggests possible transport via groundwater, likely from western branch **Cattle** Ruminant-specific MST likely transported from upstream of station BK2 and southern branch downstream of station DBK1 (observed cattle standing in water) Relationship identified between higher *E. coli* concentrations and the detection of ruminant *Bacteroides*	Contamination likely originates upstream Rainfall plays limited role, if any, in bacterial loadings Highly variable sediment loads over time and flow conditions may indicate recent source Thonotosassa Park Boat ramp (no visible restroom facilities)

Section 6.5.2 Flint Creek

This assessment suggests that human-related pollution is a probable source of fecal contamination identified within the Flint Creek watershed. Although the detection rate of all human-specific markers was relatively low all through the basin (18% and under), each of the three markers was identified at station FL2 throughout the duration of the project, lending significant support to the presence of a chronic human source in this area. This contention is further supported by consistently high IO concentrations at this location. In contrast, the upstream and downstream sites appear to have more episodic contributions. Although there is a slight discernable relationship between elevated bacteria levels among the three "fixed" sampling stations that may be suggestive of transport, the lack of consistency in this pattern may be more indicative of multiple distinct sources.

Despite the loading abundance, the relative proportion of the different human-specific markers detected may indicate that the human source is not related to OSTDS. Additionally, despite the lack of statistical correlation between the bacterial community and rainfall, the detection of human-specific markers together with elevated IO concentrations during the July sampling event, following a nine-day rainfall, may lend further support to the presence of a source contributed via surface flow, not groundwater. Care must be taken in drawing this conclusion, however, considering the low detection rate of all markers all through this watershed as well as the presence of OSTDS distributed throughout the basin.

Although there were no ruminant- or horse-specific markers detected within Flint Creek, it may be possible, given the surrounding land use (especially in the vicinity of stations FL3 and FL1), that wildlife is a contributing source. As noted above, the potential role of wildlife may be relatively greater at station FL3 as compared to FL1 since human-specific markers were only detected at the prior when IO concentrations were relatively low, as opposed to station FL1 where *esp* was identified simultaneously with elevated IOs. The possibility of a wildlife contribution to Flint Creek is also supported by the lack of relationship identified by the multivariate analysis between MST results (i.e., presence or absence of individual human-specific MST markers) and per-sample bacterial communities. This result should be taken cautiously as a chi-squared goodness of fit test, used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for the individual MST markers), did indicate a relationship between the 400 CFU/100mL limit and the detection of HPyV and *esp* in the Flint Creek basin (X^2=2.79, df=1, P=0.094 and X^2=2.65, df=1, P=0.105, respectively). Given the evidence, it is likely that there is a combination of human-specific and animal sources contributing to this watershed.

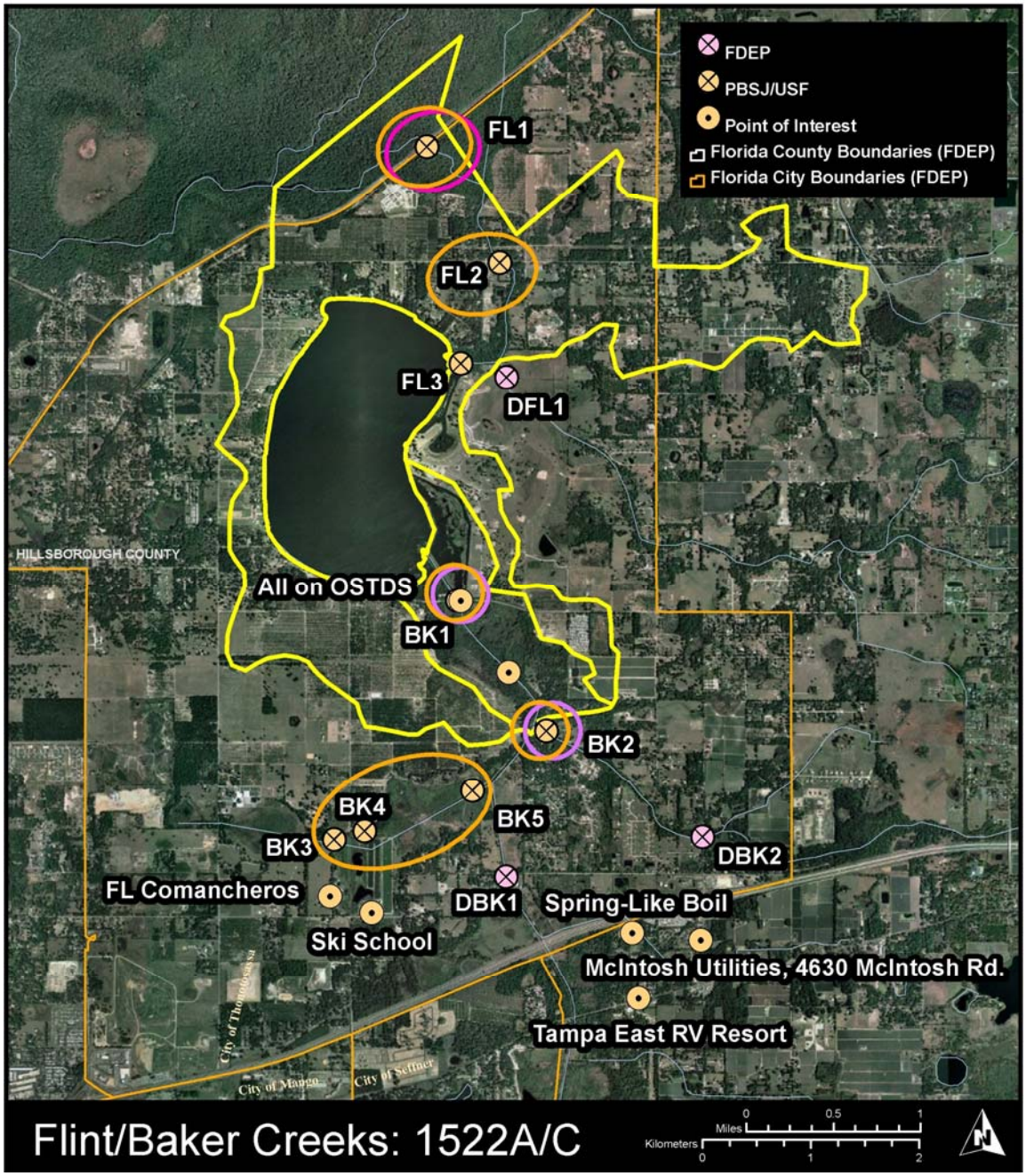
As expected, due to the large size and volume of water associated with Lake Thonotosassa, there does not appear to be any correlation between elevated levels recorded at station BK1, entering the lake, and those observed at FL3, leaving the lake. In addition, according to the results at station FL3, Lake Thonotosassa is not responsible for contributing significant bacteria loadings to Flint Creek. Table 17 identifies the most likely sources of fecal contamination for each area of Flint Creek.

Suggested corrective actions, specifically to address possible OSTDS-related sources in the vicinity of station FL2 and potential wildlife-associated contributions near stations FL1 and FL3, are included in Sections 11.1 and 11.3.

Table 17. Summary of most probable sources of fecal contamination contributing to the Flint Creek sampling locations (generally listed from upstream to downstream). N/A = Not Applicable.

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments and Other Potential Sources
FL3	Human Wildlife	Kelso Road	EPCHC 118	Fixed	**Human** Human-specific MST Detection of markers did not coincide with elevated fecal coliform concentrations Only one fecal coliform exceedance Sporadic or low-level human contamination **Wildlife** Only two MST markers identified throughout study and they did not coincide with highest bacteria concentrations Land use suggests potential for wildlife source	Highly eutrophic headwaters (green) of Flint Creek, account for any potential sources leaving Lake Thonotosassa Only fecal coliform exceedance did not coincide with water flowing over control structure from lake Inactive fish often observed at surface of water Rainfall plays limited role, if any, in bacterial loadings Elevated bacterial levels in sediments may cause re-inoculation of surface waters
DFL1	N/A	Campbell Creek	N/A	Flexible	N/A	Sample to account for contributing waters to Flint Creek No observed exceedances
FL2	Human (OSTDS)	Knights Griffin Road	STORET 8496	Fixed	Human-specific MST Majority of markers and most consistent exceedances in basin Detection of markers typically coincides with elevated IO concentrations Relationship identified between higher fecal coliform concentrations and the detection of HPyV and *esp* Majority of surrounding residences on OSTDS	Stormwater may also contribute to fecal pollution in this area Rainfall plays limited role, if any, in bacterial loadings Elevated bacterial levels in sediments may cause re-inoculation of surface waters Bridge construction completed in August 2007

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments and Other Potential Sources
FL1	Human Wildlife	US 301	EPCHC 148	Fixed	**Human** Human-specific MST Likely transported from upstream (marker and majority of bacteria exceedances detected under flow conditions) Relationship identified between higher fecal coliform concentrations and the detection of HPyV and *esp* Bacteria concentrations in water column exceeded those in associated sediments, also suggestive of transport from upstream source **Wildlife** Only one MST marker identified throughout study and they did not coincide with highest bacteria concentrations Land use suggests potential for wildlife source	Rainfall plays limited role, if any, in bacterial loadings



Map 6. Map of the Flint and Baker Creeks upon completion of Phase II, including identified sampling locations for Phase II, historical sampling locations, and general points of interest. Results of the May through December 2007 sampling events suggest the presence of "hot spots" for fecal contamination between BK2 and BK3, downstream of DBK1, between DBK2 and BK2, and between FL2 and FL1. The most likely sources in general areas are denoted by circles of different colors: orange = septic systems; purple = cattle; and pink = wildlife.

Section 7.0 Spartman Branch – WBID 1561

Section 7.1 Background

Walden Lake, located in the Walden Lake residential golf community, is the headwater for Spartman Branch (Appendix D, Photograph 17). Spartman Branch flows to the northwest for approximately three miles before joining with a smaller branch (that originates in the vicinity of Tanner Road between Turkey Creek Road and Branch Forbes Road near the western WBID boundary) just south of U.S. 92 (Hillsborough Avenue). A single channel continues to the northwest for another 1-2 miles before connecting with Mill Creek to become Pemberton Creek (north of I-4) and eventually Baker Creek (see Section 6).

The tributary consists of several land use types. The area around the headwater is characterized by high-density, relatively new residential areas which transition quickly into a highly industrial region between Sydney Road northwest to SR-574 (Dr. Martin Luther King Boulevard West). These areas are predominantly serviced by central sewer. The area northwest of SR-574 to I-4 primarily includes various-density residential areas (which utilize a mixture of central sewer and OSTDS) and pasturelands. It should be noted that although there was no flow out of Walden Lake during the May 10, 2007, site visit, there were small volumes of water and low flow throughout many areas of the tributary; flow may be non-existent at times in many areas of the WBID.

Analysis of impervious surface (see Section 5.1) indicates that the Spartman Branch WBID averages 10-25% impervious surface. A soils survey (USDA/NRCS, provided by SWFWMD 2000) shows that the majority of areas directly adjacent to the Spartman Branch channel contain soils with slow to very slow infiltration rates; however, parts of the northern reaches of the channel, where residences are using OSTDS, enter small regions with classifications indicating high infiltration rates. As a result, the effectiveness of septic tank drainfields in these areas may be comprised.

Section 7.2 Preliminary Assessment

Data received for WBID 1561 from EPCHC AWQM, STORET, Legacy STORET, and USGS NWIS Stations between 1990 and 2006 indicate only modest levels of fecal coliforms, although the water body is designated as impaired (geometric mean = 102 CFU/100mL; maximum = 4,300 CFU/100mL; n = 22).

The initial screening process results indicate that the potential sources of fecal contamination in the Spartman Branch WBID generally differ with location within the WBID but may overlap in certain locations. Due to the fact that the residential areas in close proximity to the headwater are relatively new and are serviced by central sewer, there is a low likelihood of a human source originating in this area. The more industrial portion of Spartman Branch includes a small municipal airport which is on central sewer serviced by the City of Plant City (Appendix D, Photographs 18 and 19). During the site visit on May 10, 2007, a discolored stormwater pond was identified in this area, near the

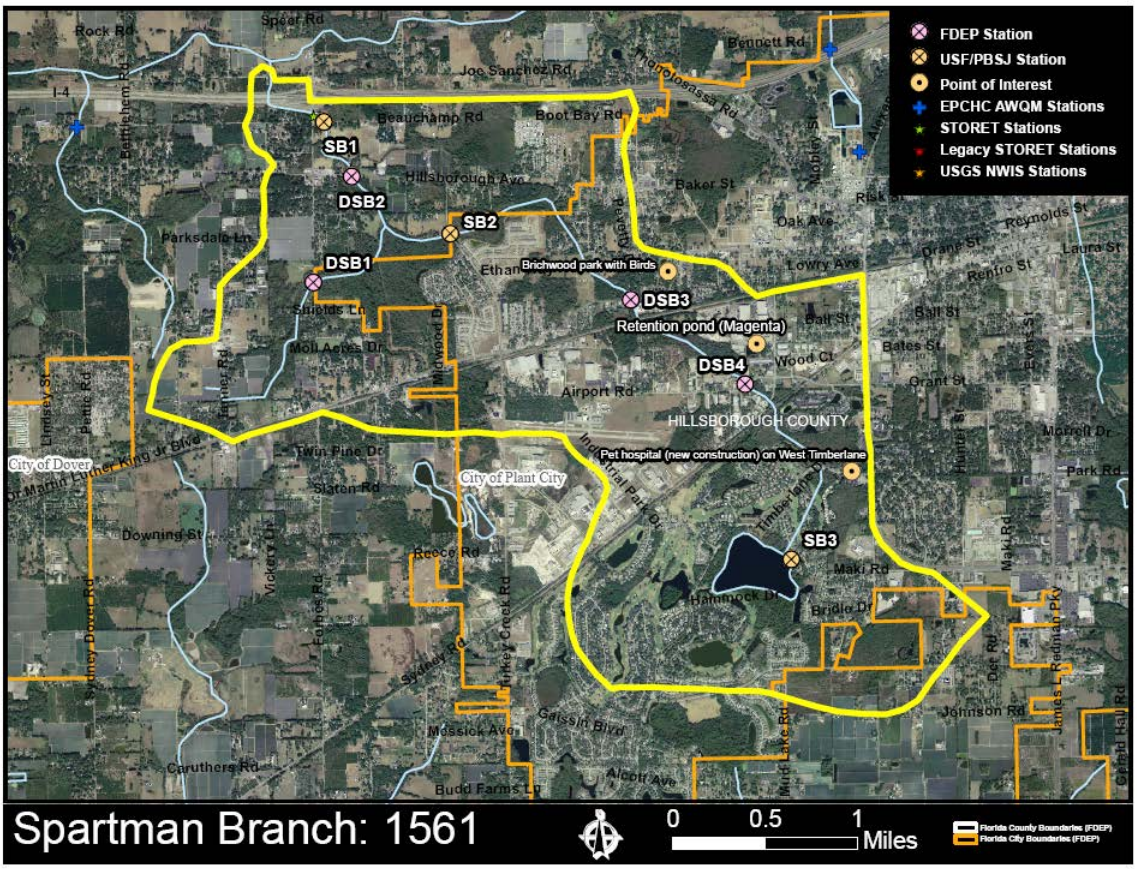
junction of Sammonds Road and Woodrow Wilson Street (Appendix D, Photograph 20) which spurred an immediate investigation by the EPCHC, FDEP and City of Plant City. Albert Miller, Environmental Coordinator, City of Plant City, reported that discoloration of this pond was also identified in April 2006 at which time "the City inspected the area surrounding the ponds and found no evidence of dumping or illicit connections/illicit discharges." The investigation in May 2007 also showed "no evidence of spills, or illicit connections/illicit discharges." Potential sources of fecal contamination in this area are primarily human (sewer) and stormwater-related.

The possibility of human inputs of fecal contamination continue throughout the area where Spartman Branch crosses North Turkey Creek Road; however, this region represents a transitional area between potential human, wildlife, and livestock sources. For example, a cattle pasture abuts a fixed sampling station (SB2) off Turkey Creek Rd. Although high-density residential areas such as Birchwood and Sugar Creek developments that utilize septic systems are present along with pasturelands, natural landscapes are also represented and may help to alleviate potential fecal pollution impacts (Appendix D, Photographs 21 and 22). Natural settings along the southern tributary of Spartman Branch may have similar influences. The likelihood of livestock impacts increases downstream closer to I-4 (Appendix D, Photograph 23).

There are no septic system "hot spot" areas identified by the HCHD within the Spartman Branch WBID boundaries or immediate contributing waters within Hillsborough County. Field observations made on May 10, 2007, did not reveal any straight-pipe illicit discharges from OSTDS to Spartman Branch.

Section 7.3 Suggested Monitoring Stations

Sampling locations were determined using the same criteria as described in Section 2.4. The results of the initial screening process, as described in Section 7.2, indicate that although the potential sources of fecal contamination in Spartman Branch are sometimes discernible by area, there are multiple potential source types in certain locations (Table 18). Upon completion of Phase I, it was suggested that seven initial sampling stations (including three "fixed" stations and four "flexible" stations) be utilized to better assess these sources (Map 7; Table 18). These station locations were investigated and confirmed during the "Walk the WBIDs" portion of Phase II.



Map 7. Map of Spartman Branch upon completion of Phase I, including identified sampling locations for Phase II, historical sampling locations, and general points of interest.

Table 18. A summary of confirmed sampling locations within the Spartman Branch WBID for Phase II, Level I analysis. Stations SB1, SB2 and SB3 were identified for sampling by the USF and PBS&J staff and stations DSB1, DSB2, DSB3 and DSB4 were identified for sampling by FDEP personnel. N/A = Not Applicable.

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Comments
SB3	Unknown	Golfview Drive	N/A	Fixed	Headwater of Spartman Branch; account for any potential sources leaving Walden Lake
DSB4	Human (sewer)	Airport Road	N/A	Flexible	Stormwater may also contribute to fecal pollution in this area
DSB3	Human (OSTDS)	SR 574 (Dr. Martin Luther King Jr. Hwy)	N/A	Flexible	Stormwater may also contribute to fecal pollution in this area
SB2	Human and Livestock	Turkey Creek Road	STORET 28005778210285	Fixed	Wildlife may contribute to fecal pollution in this area
DSB2	Human and Livestock	U.S. 92 (West Baker Street)	STORET 24030126	Flexible	Wildlife may contribute to fecal pollution in this area
SB1	Livestock	Beauchamp Road	EPCHC 533	Fixed	Human impacts (OSTDS) may contribute to fecal pollution in this area
DSB1	Human (OSTDS)	Branch Forbes Road	N/A	Flexible	Wildlife may contribute to fecal pollution in this area; account for smaller branch of tributary

Section 7.4 Phase II, Level I Sampling Results

Sampling was greatly restricted at Spartman Branch due to a lack of water or flow at nearly all stations at some point over the project duration (Table 19). Although all stations with adequate water were sampled, the results should be read with the understanding that flow was nonexistent at times (see Section 5.4 for discussion). For instance, station DSB1, representative of the branch of the tributary that originates near Tanner Road, only had sufficient water (and flow) for sampling in July. As a result, it is unlikely that waters from this branch are contributing the contamination observed in the main stem of Spartman Branch. A summary of the Spartman Branch sampling stations and their surface water status over the sampling period is provided in Table 19.

Table 19. A summary of the Spartman Branch sampling stations and their surface water status over the sampling period (May – December). N/A = Not Applicable. All stations are identified on Map 8.

Station	Month Added	Months Sampled	Dry	No Flow
SB3	May	May - December	N/A	May, June
DSB5	August	August	N/A	N/A
DSB4	May	June - August	May	N/A
DSB3	May	June – September, November	May	N/A
SB2	May	June - December	May	June, September, December
DSB2	May	July - December	May, June	N/A
SB1	May	July - December	May, June	N/A
DSB1	May	July	May, June	August, September, October

Results demonstrate that the surface waters of Spartman Branch frequently exceeded state standards for indicator species, with both chronic and episodic sources apparent (Table 20; Figure 9). For example, stations SB1, SB2 exceeded regulatory standards every month while other stations (e.g., station DSB2) had less frequent exceedances. Given that station DSB2 did not consistently exceed regulatory standards when station SB2 (upstream) and SB1 (downstream) did, suggests the presence of two, relatively local sources of pollution and limited transport of microorganisms.

Under flowing conditions, maximum levels of fecal coliforms in Spartman Branch reached 2,950 CFU/100mL at station SB1 (on July 25, 2007). This was coincident with the discovery of a trailer with a leaking pipe in the fruit stand parking lot near station SB1. Despite the leaking sewage sampled from the pipe (station SB1A; fecal coliforms = 100,000 CFU/100mL), no evidence of a direct connection to the surface waters was observed. It should be noted, however, that it had been raining for several days at the time of sampling and the discharge may have been conveyed to the creek via runoff. This situation was reported to the EPCHC, who notified the owner regarding the need for repair on August 22, 2007. Fecal coliform concentrations remained high at station SB1 in September and October, reaching levels of 2,550 CFU/100mL in October, but dropped

considerably (<650 CFU/100mL) in November and December, at which time the HPyV marker was detected once. The highest fecal coliform concentrations (3,750 CFU/100mL) in Spartman Branch were identified at station SB2 under non-flowing conditions on June 20, 2007.

Enterococci levels were regularly over 1,000 CFU/100mL in the mid- to downstream segments of Spartman Branch (i.e., stations SB1 and SB2), peaking at 6,950 CFU/100mL at station SB1 in October. *E. coli* concentrations followed a similar pattern with typically higher levels observed at the more downstream sites and maximum concentrations reaching 1,850 CFU/100mL during the November sampling event. The relationship between enterococci and *E. coli* concentrations was further revealed by correlative analysis that demonstrated a significant positive result ($r = 0.743$, $p<0.05$; Table 3). Although data used to compare concentrations of enterococci and fecal coliforms were not normally distributed, a similar relationship was also identified ($r = 0.747$, $p=0.001$). These results suggest that similar levels of persistence and/or growth under the circumstances present throughout the Spartman Branch watershed for the duration of the project (see Section 4.0).

The lack of pollution transport demonstrated between stations SB2, DSB2, and SB1, referred to above, was also exhibited between stations SB3 and DSB4 during the July sampling event, after a nine-day rainfall event totaling 3.25 inches. In this case, the fecal coliform concentration was moderately elevated (1,360 CFU/100mL) at station SB3 but remained below regulatory limits at DSB4 (as it did each time it was measured; Figure 9). Indicator bacteria levels at station SB3 were relatively low compared to many of the other study sites; although several exceedences of the fecal coliform standard for recreational waters were observed intermittently. Exceptions, when station SB3 demonstrated moderately-elevated concentrations, may be related to the lack of flow of water entering Spartman Branch from Walden Lake resulting in stagnant conditions. Flow from the lake was observed during the July-September and December sampling events. Although fecal coliform concentrations ranged from 150-1,360 CFU/100mL during these events, enterococci and *E. coli* levels remained below 102 CFU/100mL and 120 CFU/100mL, respectively. When flow was not observed here, fecal coliform and enterococci concentrations reached 1,650 CFU/100mL and 2,750 CFU/100mL (both in June) and *E. coli* levels peaked at 1,200 CFU/100mL in November. These results either suggest that an episodic source is contributing fecal contamination to station SB3 or, more likely, that a more chronic source is responsible for the pollution that is then intermittently diluted by lake inputs. Regardless of the original source, the bacteria concentrations in this area appear to be affected by stagnant conditions. Station DSB5, located at a retention pond connected to Spartman Branch near station SB3, was added and analyzed for fecal coliforms during the August sampling event (under flowing conditions) to help determine the source of the contamination. IO concentrations at both stations at this time did not exceed regulatory standards (Figure 9); however, additional samples are necessary to draw more definitive conclusions.

Although fecal coliform concentrations at station DSB3 consistently exceeded regulatory standards (555-2,250 CFU/100mL) during the June-September sampling events, low levels (73 CFU/100mL) were observed in November. Despite the anomaly at this location, the bacteria population at downstream station SB2 remained moderately-

elevated (fecal coliforms = 1,010 CFU/100mL). Similar to other areas throughout the basin, this is indicative of a lack of pollution transport throughout the basin.

Evaluation of the potential impact of rainfall on bacterial concentrations could not be conducted for the Spartman Branch watershed due to lack of proximity (>5 miles) to an actively-reported rain gauge (Map 2). Although, as noted above, maximum levels of fecal coliforms in Spartman Branch, under flowing conditions, were achieved on July 25, 2007 at station SB1 after a significant rainfall event (as indicated by USGS rain gauge 2303330; Map 2), similar concentrations were observed after multiple days with little to no rain. These results suggest that the sources of pollution within Spartman Branch are primarily not stormwater-related.

At least one sediment sample was analyzed from each of the "fixed" sampling locations, though only two of these (both from station SB1) were collected under flowing conditions. Regardless of the similar flow circumstances, the IO concentrations in the sediments at station SB1 varied considerably from the July to the August sampling event (e.g., fecal coliforms = 201,500 CFU/100g and 6,500 CFU/100g, respectively). This may be reflective of the patchy nature of bacteria in sediments, though it may also indicate contributions by a more recent source in July. Since bacteria abundances in the water column were relatively consistent, and lower than the associated sediments, during these months (e.g., fecal coliforms = 2,950 CFU/100mL and 1,100 CFU/100mL, respectively), a recent source would only be likely if it had already been flushed from the sampled sediments and related surface waters. Of course, this is possible considering the recent large-scale rain event in the area. Both *E. coli* and enterococci concentrations in the sediments remained below 3,600 CFU/100g in July and August. Interestingly, under non-flowing conditions, enterococci and fecal coliform (to a lesser degree) levels in the sediments at station SB2 were extremely elevated (160,500 CFU/100g, 16,500 CFU/100g, respectively) as compared to the observed *E. coli* concentration (1,280 CFU/100g). This pattern may suggest "aged" fecal pollution, in which the enterococci have proliferated in surface waters and sediments, while the *E. coli* have not. Periodic re-inoculation of the water column from the sediment reservoir at stations SB1 and SB2 is a potential contributor to the more chronically-elevated levels of surface water contamination. In contrast to the more downstream locations, the IO levels in the sediments at station SB3 were only moderately elevated (none exceeded 760 CFU/100g).

Table 20. Geometric mean concentration of indicator organisms (IOs) in water and sediment samples collected in the Spartman Branch basin. Calculations were performed on all available data collected under flowing conditions.

WBID	Fecal Coliforms				*E. coli*				Enterococci			
	GeoMean	N	SE	Max	GeoMean	N	SE	Max	GeoMean	N	SE	Max
Spartman Branch (water)	2.82	32	0.11	5.00	2.38	17	0.14	3.27	2.81	17	0.21	4.30
Spartman Branch (sediment)	4.56	2	0.75	5.30	3.07	2	0.37	3.44	3.48	2	0.08	3.56

IO Suite abundance values from two stations within the Spartman Branch basin (SB3 and DSB2) were tested for correlations with available water quality parameters as described

in Appendix C, Section 1.2. In contrast to what was observed in both Flint and Baker Creeks (stations FL2 and BK1), a marginally significant, positive correlation between pH and fecal coliform abundance was evident at station SB3 ($r = 0.804$, $p = 0.054$; Appendix C, Table 2). The inconsistency in the direction of this correlation across WBIDs and individual stations suggests that the detection of significance may merely be the result of chance after numerous tests were performed or the presence of an interacting factor.

Of all the WBIDs tested, Spartman Branch had the least evidence of human source contamination as detection of a human MST marker was not detected more than once at each site. Only five source-specific MST markers were identified in Spartman Branch, in addition to the *esp* marker detected in the trailer discharge (Figure 9; Table 21). On one occasion, a human-specific marker, HPyV detected at SB3 during the August sampling event, did not coincide with elevated levels of indicator bacteria. The ruminant-specific *Bacteroides* marker was detected at DSB3 in September and the horse-specific *Bacteroides* marker was identified at SB1 in August. In both cases, indicator bacteria levels exceeded regulatory standards and human-specific markers were not detected. Light agriculture, including horses, are present upstream of station SB1, therefore detection of the horse-specific marker was not unexpected. Despite the potential for deer upstream of station DSB3 (due to the presence of open land and small wetland area), the likelihood that a ruminant source is a primary contributor to fecal coliform pollution in this area is low. It is essential to consider that each source type was only detected by a MST marker at individual sites on a single occasion despite consistently elevated levels of indicator bacteria at some of those locations. This suggests that bacterial levels in this tributary generally reflect "background" levels contributed from wild animals and stormwater.

Investigations by the HCHD upstream of station SB2 revealed that the Stonebridge Mobile Home Park (Map 8) had several failing septic systems, possibly servicing up to 30 trailers, directly impacting the surface waters of Spartman Branch (personal communication, HCHD, March 26, 2008). According to the HCHD report, it is possible that these failures have been occurring since December 2007. If this was the case, these failures may account for the detection of human *Bacteroides* at this location during the December sampling event; however, elevated levels of bacteria were consistently observed throughout the sampling program. Surface water samples were collected near this location and analyzed by Dr. Harwood's laboratory on March 31, 2008. At this time, the actual site of leakage was inaccessible and samples were instead collected from immediately upstream of the contamination event where no pollution was expected, as well as approximately 1.5 miles downstream at station SB2, in order to determine if the contamination was transported downstream. Results indicate that fecal coliform levels were only slightly higher at the downstream site while enterococci concentrations were substantially elevated at station SB2 as compared to the upstream location. It should be noted that bacteria concentrations were within the range observed during the previous eight-month sampling period. No human fecal source markers were detected at either site, though tests for *esp* were inconclusive due to the potential for inhibition.

Multivariate analyses of bacterial community data did not detect a relationship between the MST results (presence or absence of MST markers) and per-sample bacterial communities for any marker within Spartman Branch. A chi-squared goodness of fit test,

used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for the individual MST markers); however, for each marker, greater than 20% of expected values were under 5 and were subsequently removed from the analysis. The scarcity of source-specific markers detected throughout the Spartman Branch basin in conjunction with the apparent lack of correlation between elevated bacterial levels and MST markers may be indicative of a chronic contaminant source such as stormwater and associated polluted sediments, or possibly contribution from wildlife that are not directly identified through the current MST tests. Other findings have been published (Whitlock et al. 2002, McQuaig et al. 2006b) which demonstrate that indicator bacteria levels are frequently not correlated with the finding of human contamination. This disconnect is no doubt due in part to the fact that elevated levels of indicator bacteria are frequently not directly related to a human source, but may be entering the water from stormwater or other sources, or the markers have a "patchy" distribution and are not always detected in the water column. Given the land use in this area, the potential for wildlife as a significant contributor is most likely around stations SB3, SB2, and DSB2.

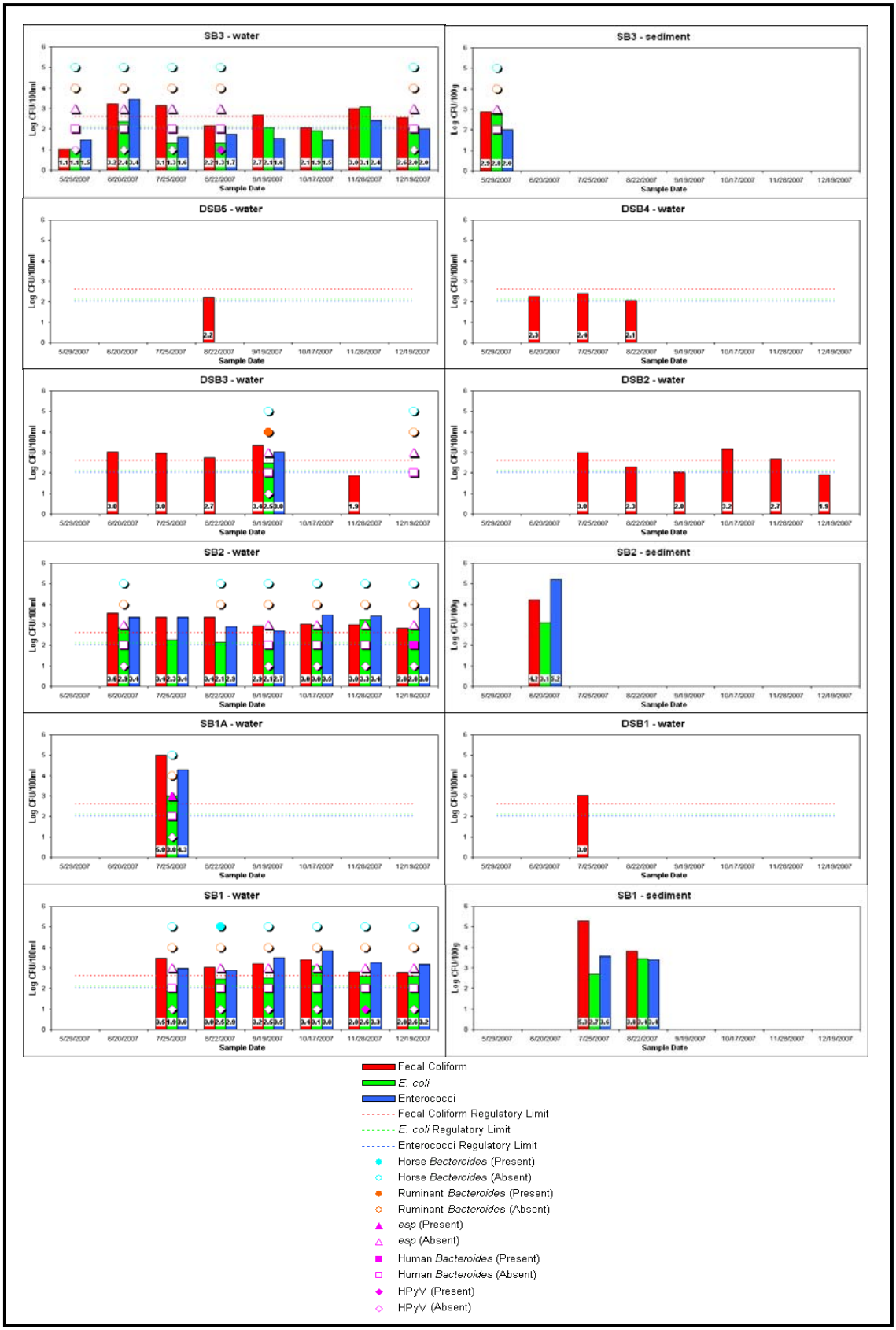


Figure 9. Indicator bacteria results at Spartman Branch from the May through December 2007 sampling events. Typically only water samples are collected and analyzed for fecal coliforms at the "flexible" stations, including DSB1, DSB2, DSB3, DSB4 and DSB5.

Table 21. Results of the source-specific microbial tracking tests utilized in this project as of December 2007. Results are reported as the number of positive results/total number of tests performed. For example, 0/1 = 0 positive tests out of 1 total test conducted. Unless otherwise indicated, analyses were conducted on water samples. Yellow shading indicates positive results.

Station	Human Bacteroides	*Enterococcus* (*esp*)	Human Polyomavirus	Ruminant Bacteroides	Horse Bacteroides
SB1	0/6	0/6	1/6	0/6	1/6
SB1A	0/1	1/1	0/1	0/1	0/1
SB2	1/5	0/5	0/5	0/6	0/6
DSB3	0/2	0/2	0/1	1/2	0/2
SB3	0/4	0/4	1/4	0/4	0/4
SB3 (sediment)	0/1	0/1	0/1	0/1	0/1

Section 7.5 Fecal Coliform Source Assessment Summary

Current data indicate that the most probable sources of fecal pollution vary throughout the Spartman Branch watershed and are, for the most part, spatially isolated. The most upstream segment, represented by station SB3, appears to reflect contributions from an episodic source of human-specific contamination or a more chronic source of pollution (possibly due to wildlife) that may be diluted by the occasional flow of surface waters from Walden Lake. Not surprisingly, it also appears that the pollutant concentrations at this site are higher under stagnant conditions.

In contrast to the most upstream portion of Spartman Branch, the mid- and downstream segments of the watershed demonstrated more consistently elevated IO concentrations. The detection of ruminant *Bacteroides* on one occasion, and the lack of detection of other makers (all markers tested only twice), together with the land use in this area, suggests that deer are a possible source of contamination at this site. Further downstream, at station SB2, only one source-specific marker (human *Bacteroides*) was detected after several tests despite the presence of chronically high IOs. This disconnect suggests that elevated levels of indicator bacteria in this location are entering the water from stormwater or other sources or that the markers have a "patchy" distribution and are not always detected in the water column. Given the land use in this area, the potential for wildlife as a significant contributor is possible. Still further downstream, chronically high IO concentrations appear to be due the presence of both human- and horse-related pollution. Illicit discharges, similar to that identified at station SB1 in July 2007, may be possible in this portion of the basin. Table 22 identifies the most likely sources of fecal contamination for each area of Spartman Branch.

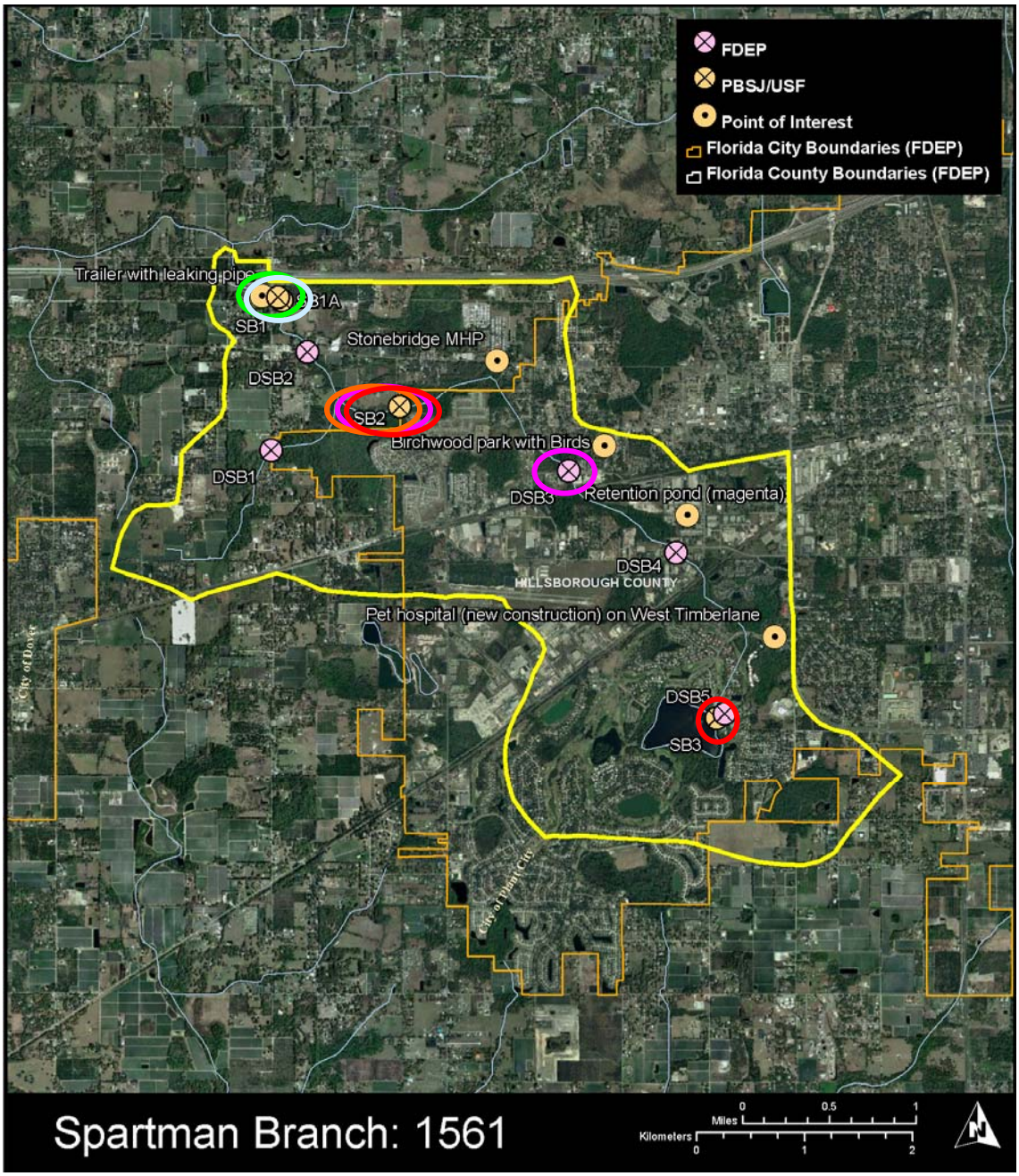
Suggested corrective actions, specifically to address potential OSTDS-related sources in the vicinity of station SB2, illicit discharges possibly in neighborhoods scattered throughout the basin (e.g., station SB1), and likely livestock- and wildlife-associated contributions near station SB1 and stations SB3, DSB3, SB2, and DSB2 respectively, are included in Sections 11.1 and 11.3.

Table 22. Summary of most probable sources of fecal contamination contributing to the Spartman Branch sampling locations (generally listed from upstream to downstream). N/A = Not Applicable.

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
SB3	Human Wildlife	Golfview Drive	N/A	Fixed	**Human** Human-specific MST **Wildlife** Only one MST marker detected and it did not coincide with IO exceedances Presence of large lake provides potential for wildlife in area	Headwater of Spartman Branch; account for any potential sources leaving Walden Lake Chronic source likely diluted by lake inputs (appears episodic) Bacteria concentrations in sediments only slightly elevated Detection of human-specific marker did not coincide with IO exceedances
DSB5	N/A	Golfview Drive Retention Pond)	N/A	Flexible	N/A	Limited data (n=1)
DSB4	N/A	Airport Road	N/A	Flexible	N/A	No observed exceedances Limited data (n=3)
DSB3	Wildlife	SR 574 (Dr. Martin Luther King Jr. Hwy)	N/A	Flexible	Ruminant-specific MST Detection of ruminant *Bacteroides* coincided with maximum IO levels at this site Potential for deer upstream	

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
SB2	Human Wildlife	Turkey Creek Road	STORET 28005778210285	Fixed	**Human** Human-specific MST Highest fecal coliform concentrations and only marker detected during non-flowing conditions, suggesting local or source transported via groundwater Consistently high IO exceedances **Wildlife** Only one MST marker detected Consistently high IO exceedances Surrounding land use indicates potential for wildlife	Extremely elevated bacterial levels in sediments may cause re-inoculation of surface waters Pattern of IO in sediments may suggest “aged” fecal pollution Episodic exceedances downstream at station DSB2 suggest limited, if any transport downstream. Failing OSTDS at Stonebridge Mobile Home Park observed by HCHD
DSB2	Human Livestock Wildlife	U.S. 92 (West Baker Street)	STORET 24030126	Flexible	Episodic exceedances Land use suggests multiple potential sources	Advanced MST analysis was not performed at this site Periodic transport from upstream is possible (both high and low IO concentrations coincide with flow at upstream station SB2)

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
SB1	Human Horse	Beauchamp Road	EPCHC 533	Fixed	Consistent IO exceedances **Human** Human-specific MST Detection of HPyV maker coincided with elevated levels of IOs Maximum fecal coliform concentrations coincided with discovery of leaking sewage from adjacent trailer (station SB1A) but remained high after leak was eliminated **Horse** Horse-specific MST Detection of horse *Bacteroides* coincided with elevated IOs Horses observed upstream	Episodic exceedances upstream at station DSB2 suggest presence of local pollution source Highly variable sediment loads over time may indicate recent source Extremely elevated bacterial levels in sediments may cause re-inoculation of surface waters
DSB1	N/A	Branch Forbes Road	N/A	Flexible	N/A	Only had sufficient water for sampling in July



Map 8. Map of Spartman Branch upon completion of Phase II, including identified sampling locations for Phase II, historical sampling locations, and general points of interest. Results of the May through December 2007 sampling events suggest the presence of chronic "hot spots" for fecal contamination at DSB3, SB2 and SB1 and episodic manifestations at SB3. The most likely sources in general areas are denoted by circles of different colors: red = sanitary sewer; orange = septic systems; green = illicit discharges; pink = wildlife; and grey = horses.

Section 8.0 Blackwater Creek – WBID 1482

Section 8.1 Background

The main channel of Blackwater Creek originates approximately 0.5 miles east of North Galloway Road, north of Knights Station Road in the City of Lakeland, Polk County. The headwater of a smaller branch is located roughly 1 mile north just east of North Galloway Road and flows west for approximately 3.5 miles before intersecting with the main channel (Map 9). Blackwater Creek continues west for about 2 miles before turning to the northwest for another 4 miles and again to the west for the last 8 miles before intersecting with the Hillsborough River. Several smaller tributaries join the main channel of Blackwater Creek, the most significant of which is the Itchepackesassa Creek which flows northward from the City of Plant City.

During field visits in January, February and May of 2007, low volumes of water and low flow were observed throughout the tributary; several upstream locations were dry during the May visit. Analysis of impervious surface (see Section 5.1) indicates that the entire Blackwater Creek tributary contains less than 10% impervious surface. A soils survey (USDA/NRCS, provided by SWFWMD 2000) illustrates that the majority of the WBID is comprised of soils with very slow infiltration rates. Small scattered areas throughout the tributary, primarily in the upstream portion where residences are using OSTDS, contain soils with high infiltration rates which may compromise the effectiveness of septic tank drainfields.

Section 8.2 Preliminary Assessment

Data received for WBID 1482 from EPCHC AWQM, STORET, Legacy STORET, and USGS NWIS Stations between 1990 and 2006 indicate typically moderate levels of fecal impairment, with evidence of episodic events with more heavily-elevated levels (geometric mean = 178 CFU/100mL; maximum = 8,600 CFU/100mL; n = 264).

Findings from the initial screening process reveal that Blackwater Creek can be divided into three main sections based on predominant land use type. The potential sources of fecal pollution differ among these regions. The most upstream portion of the tributary, essentially from the Polk County line east to the headwaters (a small maple swamp) is predominantly low- to medium-density residential communities (Appendix D, Photograph 24) mixed with pastureland and associated animals (e.g., horses, cows, goats). Deep swales, indicative of efforts to control localized flooding, are common along many of the residential streets. Homes with visible septic mounds tend to be closer to these stormwater conveyance structures than the main channel of Blackwater Creek. The most likely sources in this section include human impacts (septic and/or sewer) with the potential for stormwater and minor animal inputs. Field observations made on May 8, 2007, did not reveal any straight-pipe illicit discharges from OSTDS to Blackwater Creek. An active chicken house was identified on Rushing Road (Appendix D, Photograph 25).

The potential for human influence decreases and that for livestock impacts increase to the west in the midstream section of the tributary, from SR-39 east to the Polk County border. This area is essentially comprised of Cone Ranch, an active cattle ranch where cattle have direct access to the creek (Appendix D, Photograph 26), and a sod farm operation. A water sample taken from a site on Cone Ranch on May 9, 2007, and analyzed by the USF laboratory did not reveal elevated levels of fecal indicator organisms (e.g., fecal coliforms < 400 CFU/100mL), though a host-specific assay using ruminant *Bacteroides* was positive. Unlike the majority of this section of the tributary, the eastern-most portion near the Polk County line includes small communities of mobile homes and small "villas" with private wastewater treatment facilities (Appendix D, Photographs 27 and 28). A WWTF pond overflow was identified and sampled in this area during the May site visit. Results indicated elevated levels of indicator organisms (e.g., fecal coliforms >5,000 CFU/100mL); however, human-specific assays were negative. This may be a result of microbial die-off due to exposure to environmental stressors such as ultraviolet radiation. This midstream section of the WBID also contains several connecting tributaries (e.g., Itchepackesassa Creek, East Canal) from both the north and south, most of which have been channelized over the last 80 years, similar to Blackwater Creek. The East Canal historically drained discharge from a City of Plant City WWTF, though the volume has decreased since a portion was directed up to CF Industries (phosphate plant) where it is currently re-used for industrial processes. During dry periods, most of the flow up the East Canal is from the WWTF discharge (the point of discharge is at the junction of East Canal and Knights Griffin Road). CF Industries uses a water hyacinth system (giant ponds) to uptake nutrients. Chicken manure is also spread on fields as a fertilizer throughout the area around East Canal. Although it is generally understood that this practice does not occur adjacent to the water, it remains possible that heavy rainfalls in this area could transport these potential impacts to the tributary.

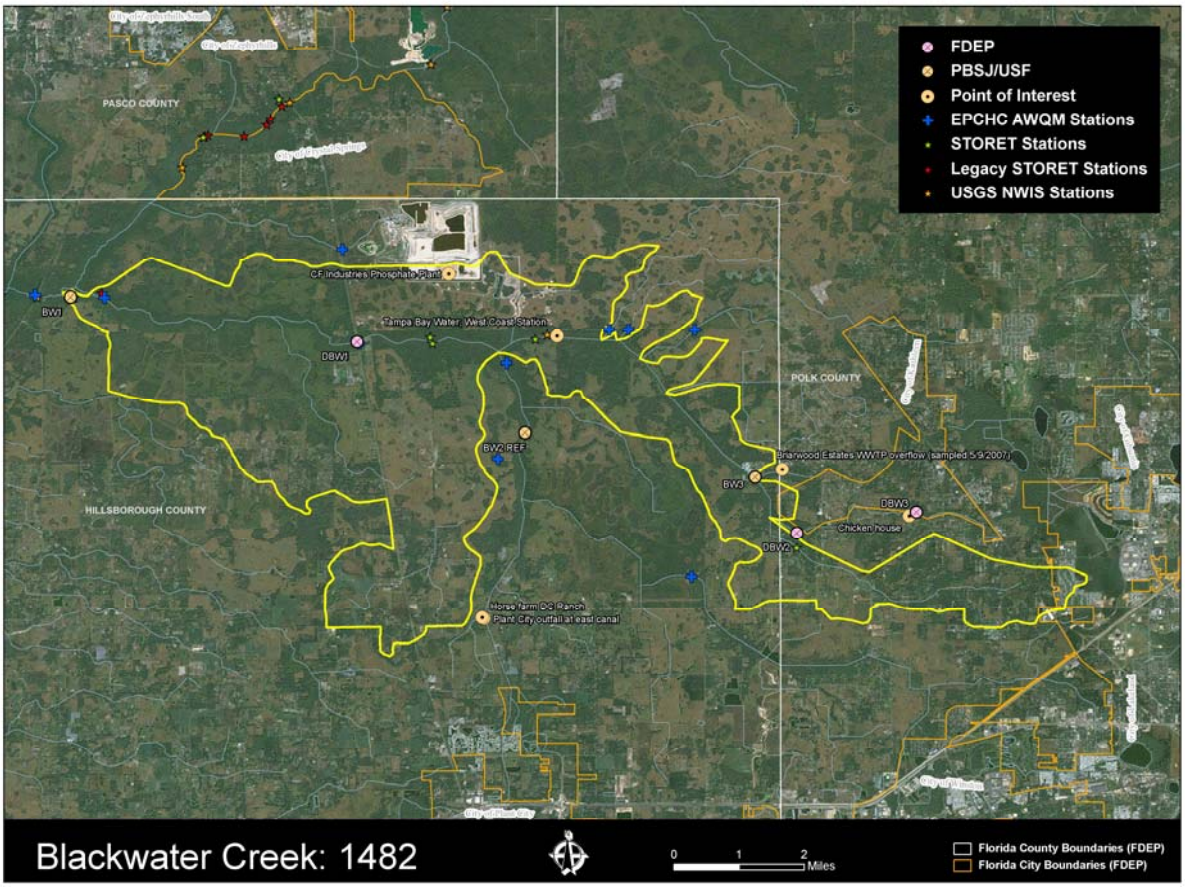
The most downstream area of the creek, upstream of the intersection with the Hillsborough River east to SR-39, is primarily floodplain forest, pasture, and native range (Appendix D, Photograph 29) controlled by Two Rivers Ranch and Hillsborough County Environmental Land Acquisition Protection Program (ELAPP). Four small tributary streams, with similar land use types, connect with the main channel of Blackwater Creek in this section. Very few rural homes and only a low density of cattle are present in this area, though they do have access to the creek on the ELAPP property. Wildlife such as deer, feral hogs, wild turkeys, foxes, and coyotes are present.

There are no septic system "hot spot" areas identified by the HCHD within the Blackwater Creek WBID boundaries or immediate contributing waters within Hillsborough County.

Section 8.3 Suggested Monitoring Stations

Sampling locations were determined using the same criteria as described in Section 4.0. The results of the initial screening process, as described in Section 8.2, demonstrated that the potential source-types of fecal contamination in Blackwater Creek are generally specific to certain portions of the tributary (Table 23). Upon completion of Phase I, it was recommended that six initial sampling stations (including three "fixed" stations and

three "flexible" stations) be utilized to better assess these sources (Map 9; Table 23). One of the stations (BW2 Ref), located in Cone Ranch, was designated not only to help identify potential impacts from livestock but also to provide a control for testing the MST techniques for human impacts (there are no suspected human sources present). These station locations were investigated and confirmed during the "Walk the WBIDs" portion of Phase II.



Map 9. Map of Blackwater Creek upon completion of Phase I, including identified sampling locations for Phase II, historical sampling locations, and general points of interest.

Table 23. A summary of confirmed sampling locations within the Blackwater Creek WBID for Phase II, Level I analysis. Stations BW1, BW2 and BW3 were identified for sampling by the USF and PBS&J staff and stations DBW1, DBW2 and DBW3 were identified for sampling by FDEP personnel. N/A = Not Applicable.

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Comments
DBW3	Human (OSTDS/sewer)	Lewellyn Road	N/A	Flexible	Stormwater and chicken house(s) may contribute to fecal pollution in this area
BW3	Human (OSTDS)	Deeson Road	N/A	Fixed	Stormwater may also contribute to fecal pollution in this area
DBW2	Human (OSTDS)	Shady Oak Drive	N/A	Flexible	Stormwater may also contribute to fecal pollution in this area
BW2 Ref	Livestock	Cone Ranch	N/A	Fixed	Reference station controlling for human impacts
DBW1	Livestock	Paul Buchman Highway (SR 39)	EPCHC 0	Flexible	Wildlife may contribute to fecal pollution in this area
BW1	Wildlife	Two Rivers Ranch	N/A	Fixed	Livestock may contribute to fecal pollution in this area

Section 8.4 Phase II, Level I Sampling Results

Level I sampling was greatly restricted at Blackwater Creek due to a lack of water and/or flow at all original stations, with the exception of BW2, at least once during the sampling period (Table 24). For example, only station BW2 had flowing water during the May sampling event. As of September 2007, station DBW2 was always observed to remain dry and was dropped for future analyses. Several stations were also added to assist in source identification. For instance, station BW4, located on Harrelson Road (Map 10; Table 24), was added to help detect the potential for human impacts from the upstream portion of Blackwater Creek when station BW3 was dry; flow was not observed at stations BW4. Although all stations with adequate water were sampled, the results should be read with the understanding that flow was not existent at times (see Section 5.4

for discussion). A summary of stations and their surface water status over the sampling period is provided in Table 24.

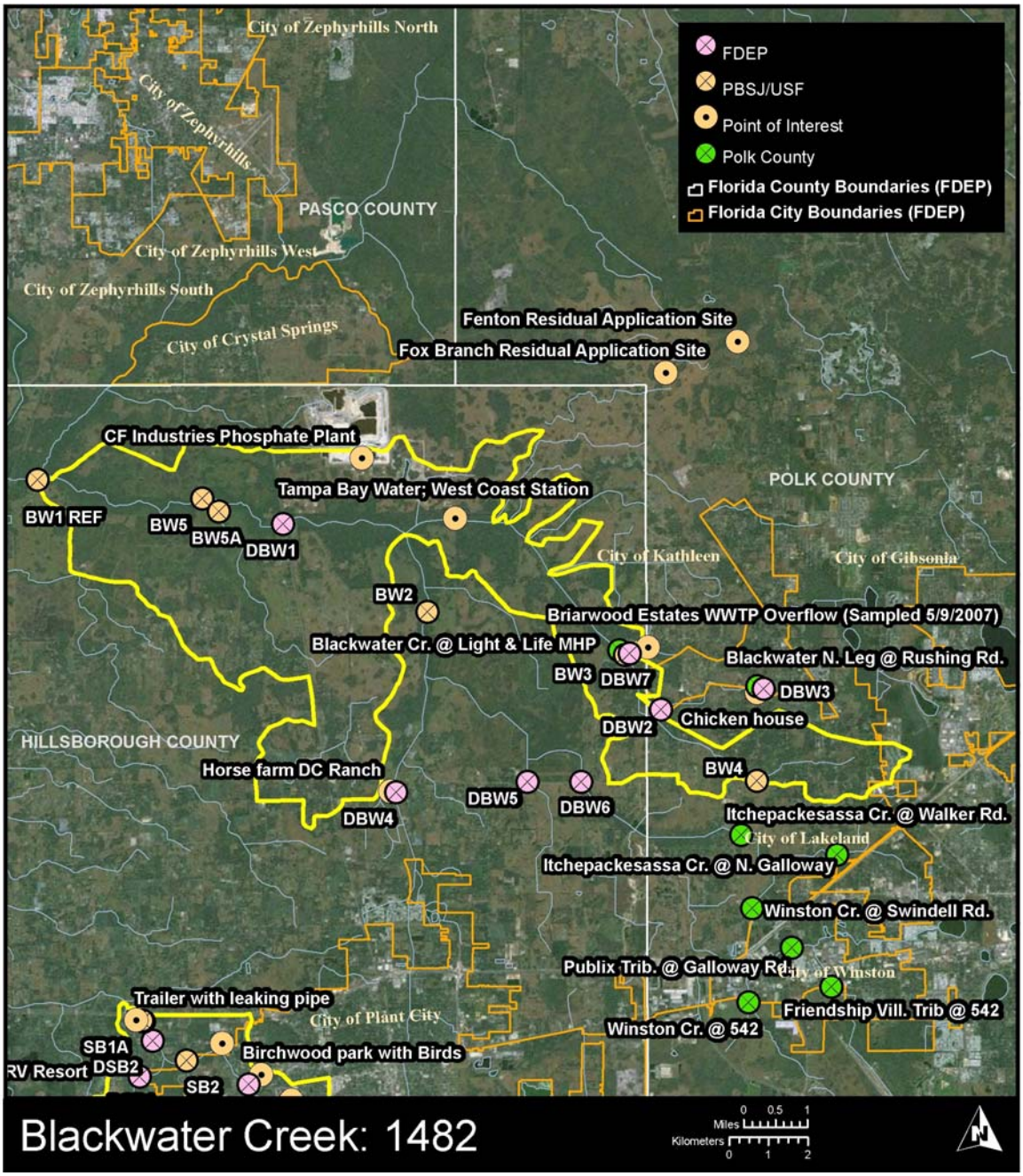
Table 24. Summary of water quality stations in Blackwater Creek and their surface water status over the sampling period (May – December). N/A = Not Applicable. All stations are identified on Map 10.

Station	Month Added	Months Sampled	Dry	No Flow
DBW3	May	July	May, June, August, October	July, September
BW4	June	June - July	N/A	June, July
DBW2	May	Never	Always	N/A
BW3	May	July - October	May, June, November, December	August
DBW7	September	September - October	November, December	N/A
DBW6	July	July - September	N/A	N/A
DBW5	July	August - September	July	N/A
DBW4	June	June - December	N/A	N/A
BW2	May	May - November	N/A	N/A
DBW1	May	June, July, September-December	May	N/A
BW5A	October	October-November	N/A	October
BW5	October	October-November	N/A	October
BW1 Ref	May	May - December	N/A	May, September, December

Results indicate that the surface waters of Blackwater Creek, consistently exceeded regulatory standards predominantly due to the water quality observed at stations BW2 and BW3 (Chapter 62-302 of the F.A.C.; Table 25; Figure 10). During flow, fecal coliform levels at all stations except BW2 and BW3, did not exceed 627 CFU/100mL, and often fell below 400 CFU/100mL. At those stations where *E. coli* and enterococci concentrations were also evaluated, similar patterns were observed such that these IOs did not exceed 225 CFU/100mL and 495 CFU/100mL, respectively. Stations BW2 and BW3, in contrast, commonly exceeded regulatory standards for all three indicator bacteria under both flowing and non-flowing conditions, thereby suggesting that a chronic source contributes to these areas. Fecal coliform levels, for example, detected at station BW2 ranged from 440-2,250 CFU/100mL (maximum value achieved on August 22, 2007) while those at BW3 were observed between 1,150-2,050 CFU/100mL, during flowing conditions (fecal coliform concentrations reached 5,200 CFU/100mL at BW3 in August when there was no water flow). *E. coli* concentrations at station BW2 also peaked during the August sampling events at 2,200 CFU/100mL while maximum enterococci levels reach 2,050 CFU/100mL in October. The highest levels of both *E. coli* and enterococci, under flowing conditions, were obtained at station BW3 in October (1,390 CFU/100mL and 10,400 CFU/100mL, respectively). Interestingly, this pollution does not appear to be transported to downstream stations, as evidenced by the lack of fecal coliform exceedances at station DBW1 for the duration of this project. The relationship among observed concentrations of the three IOs is further reflected in the

significant positive correlations evident in Blackwater Creek; fecal coliforms and *E. coli* ($r = 0.681$, $p<0.05$), fecal coliforms and enterococci ($r = 0,745$, $p<0.05$), and *E. coli* and enterococci ($r = 0.622$, $p<0.05$; Table 3). These results demonstrate that the IOs all exhibited similar behavior, in terms of potential persistence and/or growth, under the circumstances present throughout the basin over the duration of the project (see Section 4.0).

Evaluation of the potential impact of rainfall on bacterial concentrations could not be conducted for Blackwater Creek due to lack of proximity (>5 miles) to an actively-reported rain gauge (Map 2). Unlike the other basins, however, maximum concentrations of fecal coliforms in the surface waters of Blackwater Creek did not occur after the significant rainfall event recorded by USGS rainfall gauge 2303330 (Map 2) in July. Although this may indicate that rainfall plays a limited role in the bacterial loadings in this basin and that the primary sources of fecal contamination are not related to stormwater or overland flow, more recent sampling conducted by Polk County provides conflicting results. According to Polk County (personal communication, April 4, 2008), surface water samples were collected approximately 15 hours after a significant local rain event (3.5 inches). Results from sites in the immediate vicinity of stations BW3 and DBW3 (see Map 10) revealed extremely elevated levels of fecal coliforms (20,000 CFU/100mL and 9,500 CFU/100mL, respectively). Given the land use in this area, this pollution is likely of human decent. Additional samples, collected by Polk County from surface waters contributing to the Itchepackesassa Creek upstream of station DBW6 on the same day, resulted in fecal coliform concentrations ranging from 1,200-12,000 CFU/100mL; however, it is unknown how these results impact the waters of Blackwater Creek.



Map 10. Sites sampled by Polk County within the Blackwater Creek watershed in April 2008.

Seven sediment samples were collected from Blackwater Creek and analyzed for bacteria over the project duration, four of which, collected from stations DBW4, BW2, and BWRef, were obtained under flowing conditions. Regardless of the presence of flow, indicator organism concentrations in the sediments were always higher than those in the associated surface waters sampled on the same day. The most significant and chronic impairment of surface waters within Blackwater Creek appear to be specific to the areas around stations BW2 and BW3 where sediments also generally show the highest concentrations of indicator bacteria concentrations (fecal coliforms = 237,000 CFU/100g

and 19,000 CFU/100g, respectively; *E. coli* = 32,000 CFU/100g and 3,500 CFU/100g, respectively; and enterococci = 2,900 CFU/100g and 40,000 CFU/100g, respectively; Figure 10). Levels of indicator organisms in the sediments at DBW4, BW4 and BW1 Ref were also considerably elevated (fecal coliforms = 4,850 CFU/100g, 8,100 CFU/100mL, and 3,350 CFU/100g, respectively). These results suggest either recent and/or periodic inoculation or extended persistence of indicator bacteria in the sediments (Davies et al. 1995, Anderson et al. 2005).

Unlike the corresponding water samples, the differences in concentration among the indicator bacteria species in the sediments, specifically higher levels of both fecal coliforms and *E. coli* over enterococci at stations BW4, DBW4, BW2, BW5, and BW5A, may suggest the presence of a more recent source in these areas. In contrast, sediments at station BW3 exhibited considerably higher concentrations of enterococci than the other IOs and the sediments at station BW1Ref showed a similar, yet less extreme pattern (Figure 10). The results from these locations may reflect older sources of pollution and be attributed to the suggestion that enterococci appear to demonstrate greater persistence and growth in sediments under certain circumstances (personal communication, Dr. Valerie J. Harwood, October 3, 2007). Periodic re-inoculation of the water column from the sediment reservoir throughout the basin likely contributes to the elevated levels observed at the sampling locations.

Table 25. Geometric mean concentration of indicator organisms (IOs) in water and sediment samples collected in the Blackwater Creek basin. Calculations were performed on all available data collected under flowing conditions.

WBID	Fecal Coliforms				*E. coli*				Enterococci			
	GeoMean	N	SE	Max	GeoMean	N	SE	Max	GeoMean	N	SE	Max
Blackwater Creek (water)	2.49	37	0.09	3.36	2.23	16	0.18	3.34	2.57	16	0.18	4.02
Blackwater Creek (sediment)	3.90	5	0.39	5.37	3.57	5	0.30	4.51	2.63	5	0.29	3.46

IO Suite abundance values from three stations within Blackwater Creek (BW2, DBW1, and DBW4) were tested for correlations with available water quality parameters as described in Appendix C, Section 1.2. Similar to stations in Baker Creek and Flint Creek, a marginally significant negative correlation between pH and fecal coliform abundance was evident at station DBW4 ($r = -0.745$, $p = 0.054$; Appendix C, Table 2); however, the inconsistency in the direction of this correlation across WBIDs and individual stations suggests that the detection of significance may merely be the result of chance after numerous tests were performed or the presence of an interacting factor.

Human-specific markers were commonly detected throughout Blackwater Creek, providing strong evidence for a consistent human-related source (Figure 10; Table 26). As expected, all three of these markers were detected at station BW3, each on a different occasion and not in August, under no flow conditions. This indicates that the source of human-specific contamination is likely upstream of this sampling location. It should be noted, however, that these markers were also tested for at station BW4 in June; however, neither noticeable flow nor markers were evident at this time. Samples were collected

from station DBW7 during the September and October events to help discern a potential source of contamination entering Blackwater Creek from the east just downstream of station BW3, namely the Briarwood Estates Mobile Home Park located south of Deeson Road. A WWTF pond overflow was identified and sampled in this area during the May site visit (see Section 8.2). Since this time, a new, temporary treatment pond has been built to help prevent overflow events (though little freeboard remains) and the facility is currently undergoing re-permitting for a new five-year cycle (personal communication, EPCHC, February 26, 2008). Although station DBW7 was added to the sampling program in September, MST markers were not utilized. Given that elevated bacterial concentrations were evident at both station BW4 and DBW7, it is likely that at least two distinct sources of human-specific contamination were contributing to Blackwater Creek in this area.

Also, as predicted, was the consistent presence of ruminant *Bacteroides* at station BW2 (Figure 10, Table 26), indicating that animals such as cattle and deer (attributed, in this instance to cattle from upstream pastures) are responsible for the fecal contamination present at this location. Surprisingly, a human-specific marker was also identified at this site on two separate occasions, the May and July sampling events. In order to help determine the original source of the human-specific pollution detected at station BW2, three additional sites were added upstream on three different tributaries that flow into the main stem of Blackwater Creek (Map 11): 1) station DWB4, located just downstream of the City of Plant City WWTF outfall to East Canal, was added in June; 2) station DBW5, situated on Knights Griffin Road just east of Frazier Road, was added in August (dry in July); and 3) station DBW6 located on Knights Griffin Road at the crossing of the Itchepackesassa Creek, was added in July. A cost-effective strategy was employed in which MST markers were only used at DBW4 where a likely source was present and at DBW5 and DBW6 if elevated levels of fecal coliform pollution were detected. As a result, MST markers were only analyzed at DBW4 and only on one occasion. The *esp* marker was detected at this location in July, despite the relatively minor elevation of indicator bacteria levels (maximum fecal coliforms = 627 CFU/100mL). These data suggest that the source of the human-specific marker identified at station BW2 (also in July) originated near DBW4; however, it is likely that the human-related source of indicator bacteria is relatively minor compared to that contributed by the cattle on the local ranch. Nonetheless, the detection of human-specific markers at station BW2 early in the investigation prompted the designation of a different site, station BW1, to be used as the control for testing the MST techniques for human impacts.

Horse-specific *Bacteroides* was also identified at station DBW4 at this time. This is likely the result of horses present on DC Ranch adjacent to the sampling station, contributing to the total pollution observed at station DBW4. Lastly, as mentioned above, the July sampling event was the only one to have transpired after a rain event. Although this rainfall does not appear to have affected the levels of indicator species present throughout Blackwater Creek, it may contribute to transport of microbial pollutants between sites.

More recent investigations upstream of station BW2 took place on April 9, 2008 when the EPCHC conducted an inspection of the Country Village Flea Market, located upstream of the East Canal and station DBW4 and BW2, after receiving a complaint

(personal communication, EPCHC, April 30, 2008). As part of this inspection, the EPCHC collected samples from three points on and off the property. The sampling locations included a pond immediately adjacent to a mobile food vendor, a ditch on the west side of SR 39 approximately ten feet south of the highway culvert that carries discharge from the pond, and a ditch on the west side of SR 39 about 10 feet north of the highway culvert. Fecal coliform concentrations from these locations ranged from 4,400 CFU/100mL in the ditch to the south of the culvert to 16,000 CFU/100mL in the pond; enterococci levels varied from 3,400 CFU/100mL in the ditch to the north of the culvert to 78,000 CFU/100mL in the pond. According to a follow-up inspection by the EPCHC (personal communication, EPCHC, June 24, 2008), the location of all OSTDS on the property were identified and there were no observed discharges to the ditch on SR 39. EPCHC suggested that future sampling events transpire on the same day as a light/moderate rain event or the next day or within two days for a rain event of greater than one inch. It is suggested that downstream sampling locations such as station DBW4 be sampled concurrently so as to identify the potential for transport of pollution to Blackwater Creek.

The unpredicted presence of multiple human-specific markers and slightly elevated indicator bacteria levels at BW1Ref (Two Rivers Ranch, the negative control site for human sources), together with the relatively low fecal coliform concentrations detected at the closest upstream site (station DBW1), prompted the addition of two new sampling stations, BW5 and BW5A, in October (Map 11). In an effort to capture potential inputs from humans and cattle downstream of station DBW1, Valerie J. Harwood and Scott Emery conducted a reconnaissance trip on October 17, 2007. At this time, they identified a small tributary to Blackwater Creek that runs through ELAP property that is heavily grazed by cattle as well as an older residential area, presumably on OSTDS. During their visit, there was very little water in the tributary and virtually no flow; however, under rain events, this tributary is probably a significant contributor to Blackwater Creek. Immediately downstream of SR-39, Blackwater Creek runs through another run-down residential area that utilizes OSTDS. The water table in this area is clearly close to the surface. Station BW5A was added upstream of the confluence of the small tributary and Blackwater Creek to account for any impacts contributing to Blackwater Creek from the south. Station BW5 was located downstream of this confluence to discern any potential sources originating between station DBW1 and the southern branch represented by station BW5A. Although both stations were only flowing slightly, they both revealed relatively low levels of indicator bacteria and the human *Bacteroides* marker was identified at station BW5A, therefore explaining the presence of this indicator at BW1Ref, also identified at this site in October. Human contamination, likely the result of failing OSTDS, in the area of station BW5A also likely accounts for those markers identified at BW1Ref during the August sampling event. The lack of positive *esp* markers, together with the presence of both human *Bacteroides* and HPyV at station BW1Ref, corroborates the idea of an OSTDS source. Additional samples were collected from this area in November to help determine the spatial extent of the fecal coliform contamination contributing to Two Rivers Ranch. Similar bacteria concentrations were observed and no MST markers were detected (Figure 10). It should also be noted that a tributary draining the City of Crystal Springs joins the Hillsborough River before connecting to Blackwater Creek near station BW1Ref. This portion of the Hillsborough River also drains ~2,500 acres of residual application sites (Fox Branch and Fenton)

located to the east of the Blackwater Creek WBID (Map 11). It is possible that these areas are factors in the detection of human-specific pollution at station BW1Ref, though it is necessary to collect samples from this contributing waterbody to draw any specific conclusions.

Unlike in other watersheds, the detection of source-specific pollution always coincided with elevated levels of at least one IO. In addition, multivariate analyses of IO Suite concentration data detected a relationship between MST results (presence or absence of MST markers) and per-sample bacterial communities for ruminant *Bacteroides* in Blackwater Creek (Global R = 0.537, $p<0.05$; Appendix C, Table 5; Appendix C, Figures 3 and 4). Similar to the results in Baker Creek, SIMPER identified *E. coli* concentration as a major driver of 46.81% of group dissimilarity whereby higher bacteria abundance was correlated with the detection of the ruminant marker (see Section 4.0). The results of a chi-squared goodness of fit test, used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for the individual MST markers), also identified an association between the 400 CFU/100mL limit for fecal coliforms and the detection of human *Bacteroides* and *esp* in Blackwater Creek (X^2=1.33, df=1, P=0.249 and X^2=3.00, df=1, P=0.083, respectively); greater than 20% of expected values for the ruminant- and horse-specific markers were under 5 and were subsequently removed from the analysis.

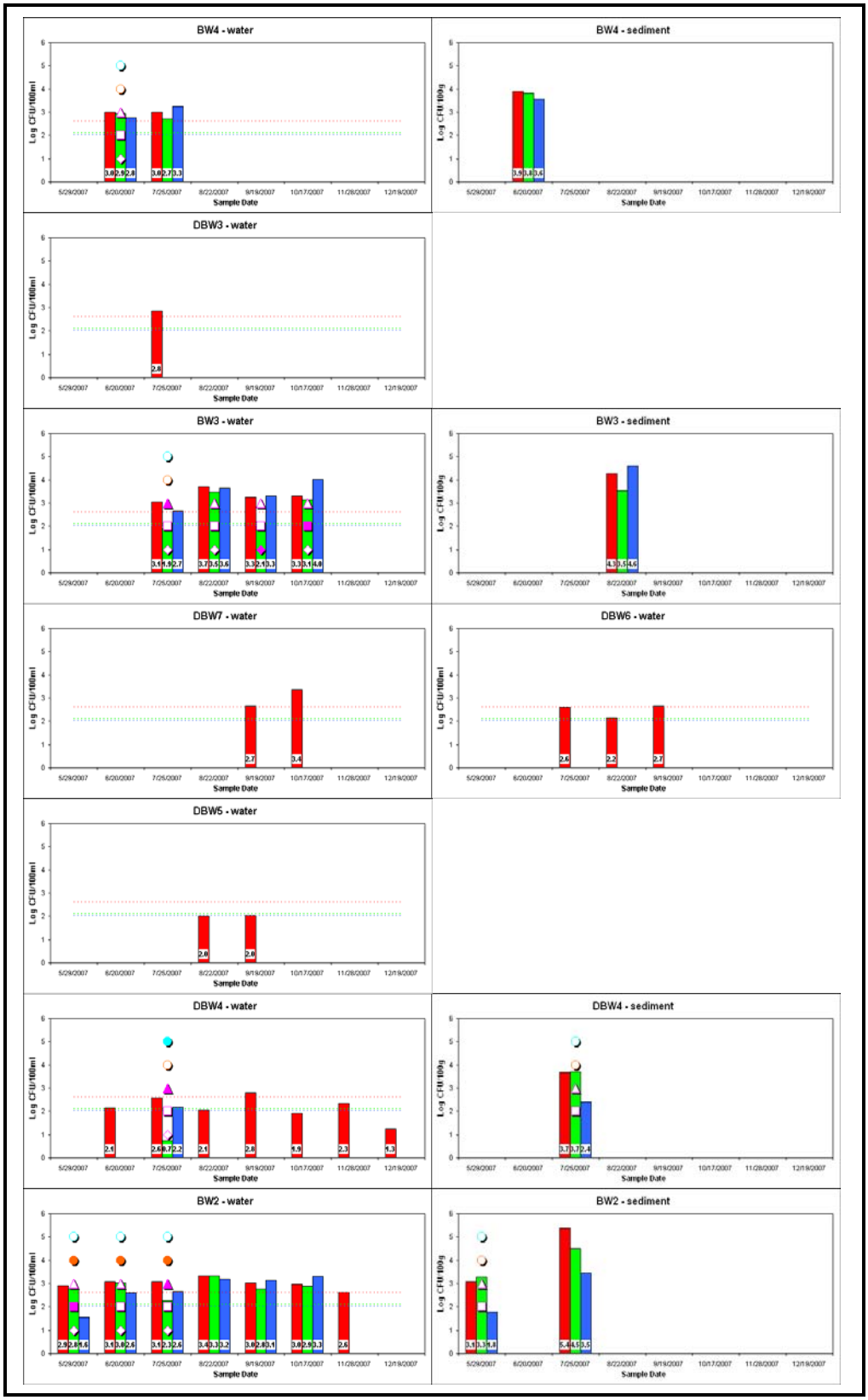


Figure 10. Indicator bacteria results at Blackwater Creek from the May through December 2007 sampling events. Typically only water samples are collected and analyzed for fecal coliforms at the "flexible" stations, including DBW1, DBW3, DBW4, DBW5, DBW6 and DBW7. This figure is continued below.

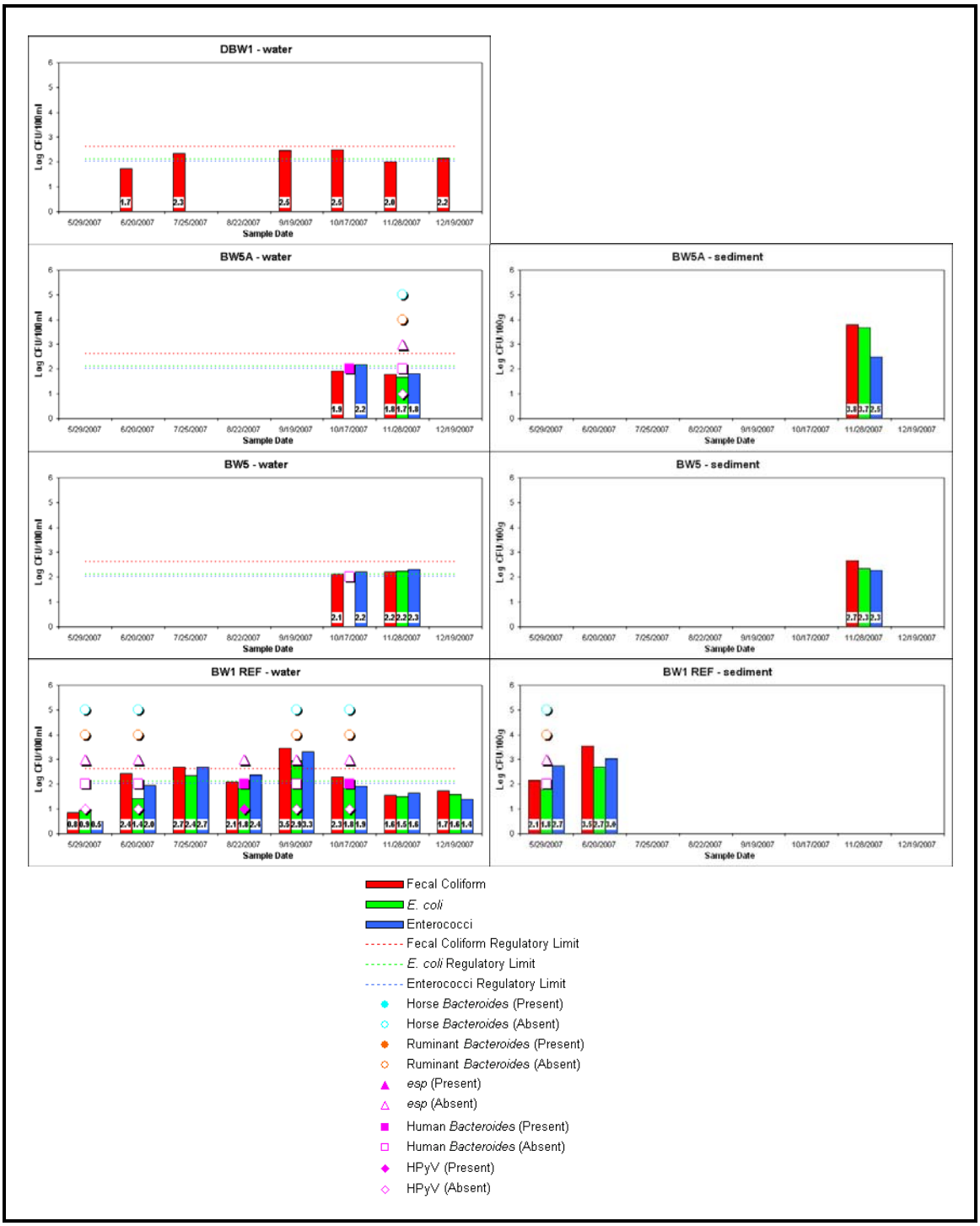


Figure 10 continued. Indicator bacteria results at Blackwater Creek from the May through December 2007 sampling events. Typically only water samples are collected and analyzed for fecal coliforms at the "flexible" stations, including DBW1, DBW3, DBW4, DBW5, DBW6 and DBW7.

Table 26. Results of the source-specific microbial tracking tests utilized in this project as of December 2007. Results are reported as the number of positive results/total number of tests performed. For example, 0/1 = 0 positive tests out of 1 total test conducted. Unless otherwise indicated, analyses were conducted on water samples. Yellow shading indicates positive results.

Station	Human Bacteroides	*Enterococcus* (*esp*)	Human Polyomavirus	Ruminant Bacteroides	Horse Bacteroides
BW1	2/5	0/5	1/5	0/4	0/4
BW1 (sediment)	0/1	0/1	0/0	0/1	0/1
BW5A	1/2	0/1	0/1	0/1	0/1
BW5	0/1	0/0	0/0	0/0	0/0
BW2	1/3	1/3	0/3	3/3	0/3
BW2 (sediment)	0/1	0/1	0/0	0/1	0/1
DBW4	0/1	1/1	0/1	0/1	1/1
BW3	1/4	1/4	1/4	0/1	0/1
BW4	0/1	0/1	0/1	0/1	0/1

Section 8.5 Fecal Coliform Source Assessment Summary

This assessment suggests that the most probable sources of fecal contamination vary throughout the Blackwater Creek watershed. The upstream portion of the basin appears to be affected by multiple human-related sources, evident along the main stem of Blackwater Creek at stations BW3 and BW4 and from a contributing tributary from the east, represented by station DBW7. Briarwood Estates Mobile Home Park located south of Deeson Road, was identified as one of the contributing sources and as a result, is currently undergoing modifications to their WWTF system.

As expected, pollution in the middle portion of the Blackwater Creek watershed, represented by station BW2, is primarily attributed to cattle. Although human-related contamination, originating near station DBW4, has been identified to impact station BW2, low levels of IOs at station DBW4 suggest that it is unlikely that this contribution is significant compared to that from cattle on the local ranch. Similar results were found for horses in this area, though horse *Bacteroides* was not detected at station BW2. Despite consistently elevated levels of IOs at station BW2, it is important to note that pollution levels decline considerably downstream at station DBW1.

Bacterial concentrations, together with the detection of multiple human-specific markers, suggest the presence of relatively low levels of human-specific pollution contributed by OSTDS in the downstream segment of Blackwater Creek. It is possible that the source of this contamination originates near station BW5A. Additional inputs may also be contributed by the Hillsborough River which drains both the City of Crystal Springs to the north and several residual application sites to the east. Table 27 identifies the most likely sources of fecal contamination for each area of Blackwater Creek.

Suggested corrective actions, specifically to address likely OSTDS-related sources in the vicinity of stations BW3, BW4, DBW7, and BW5A and livestock-associated contributions around station BW2, are included in Sections 11.1 and 11.3.

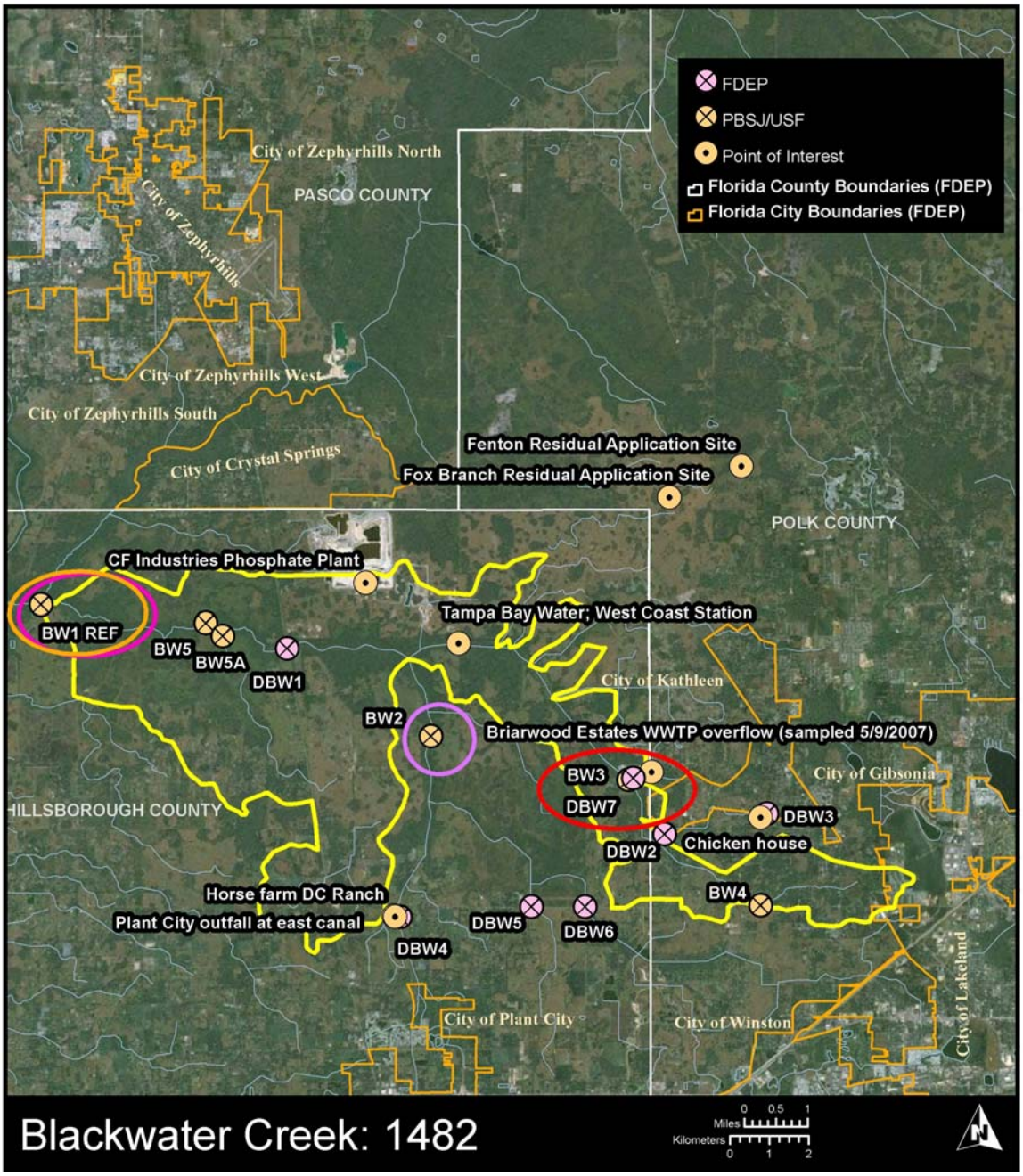
Table 27. Summary of most probable sources of fecal contamination contributing to the Blackwater Creek sampling locations (generally listed from upstream to downstream). N/A = Not Applicable.

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
DBW3	N/A	Lewellyn Road	N/A	Flexible	N/A	Often dry-limited data (n=1) Extremely elevated levels of fecal coliforms were detected by Polk County after April 2008 rain event
BW4	Human	Harrelson Road	N/A	Fixed	Elevated fecal coliform concentrations Land use suggests potential for human source	Limited data (n=2) Elevated bacterial levels in sediments may cause re-inoculation of surface waters Pattern of IO in sediments may suggest recent fecal pollution
BW3	Human	Bethel Drive, just above confluence with eastern branch and station DBW7	N/A	Fixed	Human-specific MST Detection of human-specific markers and consistently elevated IOs under both flowing and non-flowing conditions suggests transport of source from upstream (possibly public or private sanitary sewer source) Relationship identified between higher fecal coliform concentrations and the detection of human *Bacteroides* and *esp*	Extremely elevated bacterial levels in sediments may cause re-inoculation of surface waters Pattern of IO in sediments may suggest "aged" fecal pollution Stormwater may also contribute to fecal pollution in this area Extremely elevated levels of fecal coliforms were detected by Polk County after April 2008 rain event

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
DBW7	Human	Bethel Drive northeast of station BW3, just before confluence with Blackwater Creek	N/A	Flexible	Elevated fecal coliform concentrations WWTF pond overflow identified in May 2007	Limited data (n=2) MST markers not utilized
DBW2	N/A	Shady Oak Drive	N/A	Flexible	N/A	Always dry
DBW6	N/A	Intersection of Knights Griffin Road and Charlie Taylor Road	N/A	Flexible	N/A	Limited data (n=3) One minor fecal coliform exceedance observed
DBW5	N/A	Knights Griffin Road	N/A	Flexible	N/A	Limited data (n=2) No observed exceedances
DBW4	Human (Sewer) Horse	Knights Griffin Road at the East Canal	N/A	Flexible	**Human** Human-specific MST Located just downstream of City of Plant City WWTF outfall to East Canal **Horse** Horse-specific MST Observed horses on nearby ranch	Only one observed, relatively minor, exceedance Elevated bacterial levels in sediments may cause re-inoculation of surface waters Pattern of IO in sediments may suggest recent fecal pollution

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
BW2	Cattle	Cone Ranch	N/A	Fixed	Ruminant-specific MST Consistently detected ruminant *Bacteroides* marker and elevated IOs Relationship identified between higher *E. coli* concentrations and the detection of ruminant *Bacteroides* Located on cattle ranch Cow feces observed in and around surface waters	Extremely elevated bacterial levels in sediments may cause re-inoculation of surface waters Pattern of IO in sediments may suggest recent fecal pollution Human and horse contamination may also contribute to this site (markers identified upstream at station DHR7 but did not coincide with elevated bacteria concentrations) Relationship identified between higher fecal coliform concentrations and the detection of human *Bacteroides* and *esp*
DBW1	N/A	Paul Buchman Highway (SR 39)	EPCHC 0	Flexible	N/A	No observed exceedances Demonstrates lack of extended transport of pollution from upstream stations
BW5A	Human (OSTDS)	West of Tollar Road (southern branch)	N/A	Fixed	Human-specific MST Older residential area, presumably on OSTDS	Limited data (n=2) Detection of human-specific marker did not coincide with elevated bacteria levels Tributary is likely a significant contributor to Blackwater Creek during rain events Cattle may also contribute fecal coliform pollution at this station

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
BW5	N/A	West of Tollar Road (main stem of Blackwater Creek)	N/A	Fixed	N/A	Limited data (n=2) No observed exceedances
BW1Ref	Human (OSTDS) Wildlife	Two Rivers Ranch	N/A	Fixed	**Human** Human-specific MST, except *esp* marker Multiple human-specific markers observed under both flowing and non-flowing conditions Co-occurrence of human markers did not coincide with elevated levels of fecal coliforms Human impacts likely originate upstream **Wildlife** Detection of human-specific markers did not coincide with elevated bacteria concentrations Land use suggests potential for wildlife source	Reference station controlling for human impacts, although human impacts likely originate upstream (OSTDS from east and possibly the City of Crystal Springs to north) Elevated bacterial levels in sediments may cause re-inoculation of surface waters Pattern of IO in sediments may suggest "aged" fecal pollution Highest bacteria concentrations observed under non-flowing conditions Livestock may contribute to fecal pollution in this area



Map 11. Map of Blackwater Creek upon completion of Phase II, including identified sampling locations for Phase II, historical sampling locations, and general points of interest. Results of the May through December 2007 sampling events suggest the presence of "hot spots" for fecal contamination in the vicinity of stations BW3 and DBW7, BW2 and, to a lesser extent, station BW1Ref. The most likely sources in general areas are denoted by circles of different colors: red = sanitary sewer and mobile home park wastewater facilities; orange = septic systems; purple = cattle; and pink = wildlife.

Section 9.0 Lower Hillsborough River – WBID 1443E

Section 9.1 Background

The most upstream portion of what is termed the Lower Hillsborough River is located between I-275 and Nebraska Avenue, just south of the Tampa Greyhound Track and downstream of Rowlett Park and the associated dam structure (Appendix D, Photograph 30). Thick algal mats are common in this area (Appendix D, Photograph 31). The portion of the Hillsborough River included in this WBID is tidally influenced throughout and flows west to the confluence with Kirby Creek before turning to the south where it flows in one sinuous channel for approximately 6-7 miles and then empties into Hillsborough Bay. There are several minor tributaries, small spring discharges, and significant stormwater conveyance systems (Appendix D, Photograph 32) that join the main channel leading to a rapid discharge of stormwater as a result of rainfall events in this area. This release of urban stormwater is likely the cause of the high turbidity levels recorded at Platt Street.

During field visits in January, February and May of 2007, high volumes of water and flow were observed throughout the tributary. Analysis of impervious surface (see Section 5.1) indicates that the entire Lower Hillsborough River tributary contains 10-25% impervious surface. In addition, the majority of the river's shoreline throughout this WBID is comprised of "soft" edges that are somewhat buffered from urbanization. A soils survey (USDA/NRCS, provided by SWFWMD 2000) shows that the portion of the tributary west of the river is mainly comprised of soils with very slow infiltration rates, with scattered areas with high infiltration rates (especially between Columbus Drive and Cass Street), while the eastern part of the WBID primarily includes soils with high infiltration rates. The entire area located within the WBID boundary is serviced by the City of Tampa central sewer system; however, it is possible that individual residences still utilize OSTDS.

Section 9.2 Preliminary Assessment

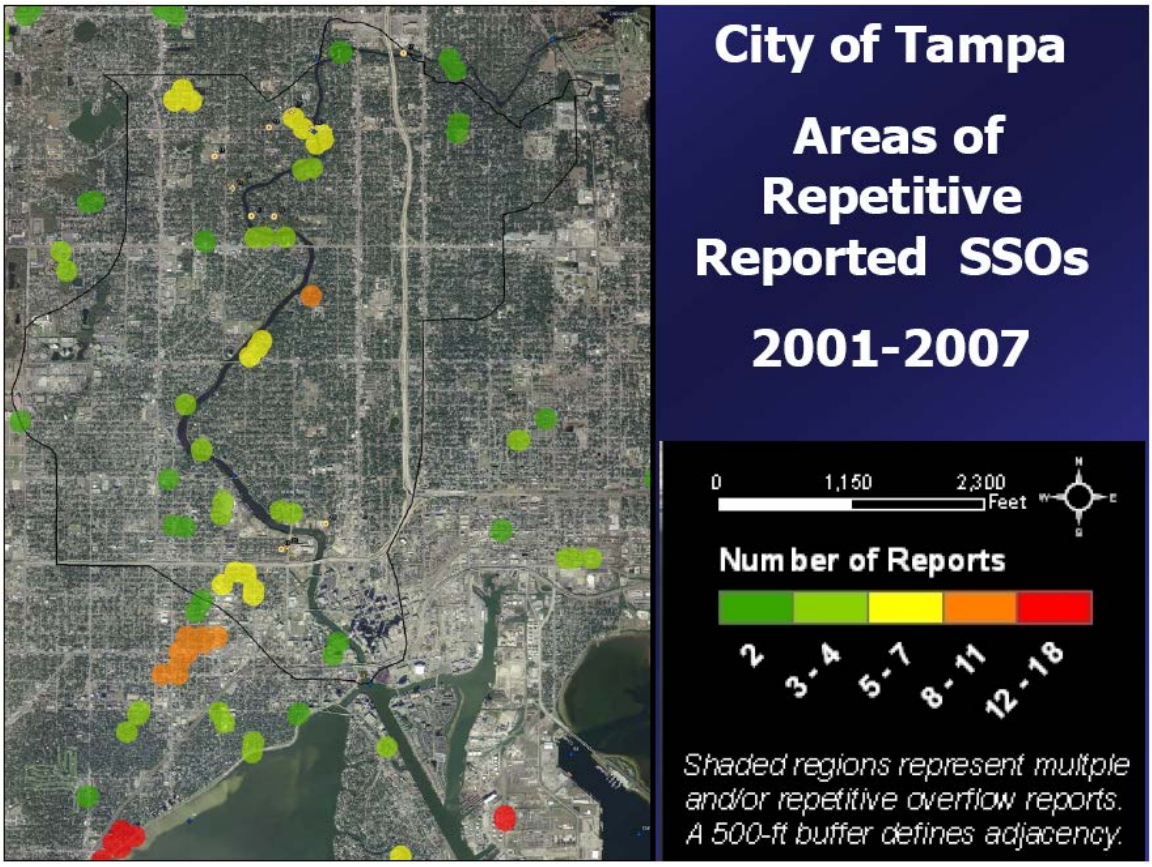
Data received for WBID 1443E from EPCHC AWQM, STORET, Legacy STORET, and USGS NWIS Stations between 1990 and 2006 indicate typically moderate levels of fecal impairment with evidence of episodic events with highly elevated levels (geometric mean = 123 CFU/100mL; maximum = 16,700 CFU/100mL; n = 424).

The initial screening assessment results reveal that the predominant land uses within the Lower Hillsborough River are high-density residential communities and commercial areas. The most significant potential source of fecal contamination, as a result, is primarily human-derived and essentially includes impacts from the sewer system and associated large-scale and repetitive SSOs. The reported contamination events range in volume but are dispersed throughout the entire area of the Lower Hillsborough River WBID (Map 12). Additional potential contributors of human-derived fecal pollution along this section of the river may include impacts from septic systems, homeless populations and live-aboard vessels docked at marinas along the river (Appendix D,

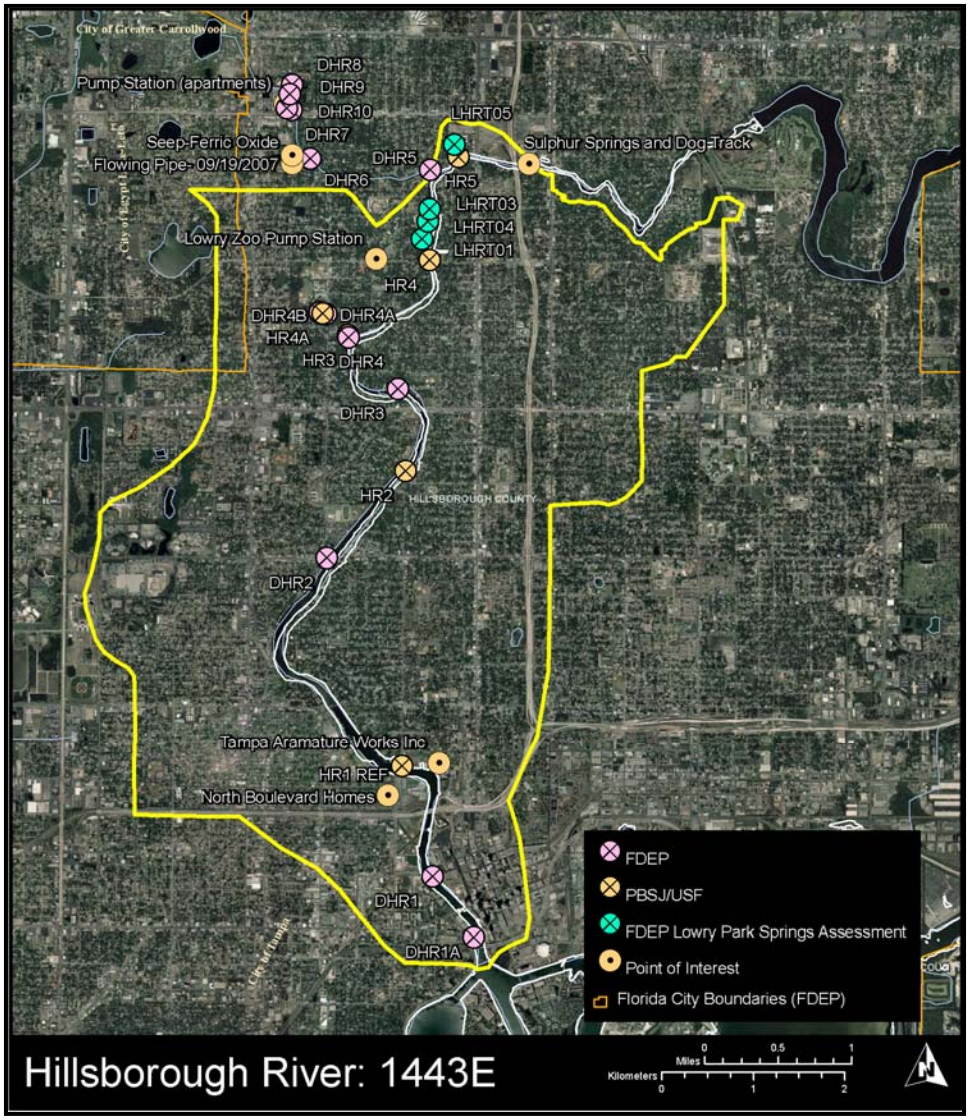
Photographs 33 and 34, respectively). It should be noted that these additional sources of fecal contamination are likely to be minor relative to the potential influence from SSOs. Similarly, the potential significance for non-human related impacts is expected to be comparatively low and may originate from stormwater, bird populations and other wildlife, dogs, and animals housed at the Lowry Park Zoo.

Although groundwater is usually not a major source of fecal contamination, the FDEP recently completed an investigation of three short spring runs and two tributaries of the Lower Hillsborough River for which there were limited or no data (personal communication, Kevin Petrus, April 16, 2008). This investigation took place between January and December 2007. Three of these spring runs and one tributary were identified as being located in the vicinity of the Lowry Park Zoo (Map 13). Fecal coliform concentrations reported from station LHRT05 (Spring Run #3), located just north of station HR5, were low (ranged from 2 to 706 CFU/100mL) and only exceeded 400 CFU/100mL on one occasion over eight sampling events from February-December 2007. In contrast, the two spring runs sampled further downstream between stations DHR5 and HR4, demonstrated highly variable levels of fecal coliforms ranging from 58 to 7,700 CFU/100mL while the unnamed stream (station LHRT01), just north of station HR4, had fecal coliform concentrations between 440 and 7,800 CFU/100mL. When sampling results from the two studies are compared over the same time periods (May – December 2007), a similar trend is apparent at stations HR4, HR5, LHRT01, LHRT02, and LHRT03; each site demonstrated maximum fecal coliform concentrations in July 2007. Results of this investigation indicate that the springs and unnamed tributary sampled by the FDEP may be contributing bacterial contamination to the Lower Hillsborough River. Additional data are necessary to further assess possible sources of pollution from this area.

There are no septic system “hot spot” areas identified by the HCHD within the Lower Hillsborough River WBID boundaries or immediate contributing waters within Hillsborough County.



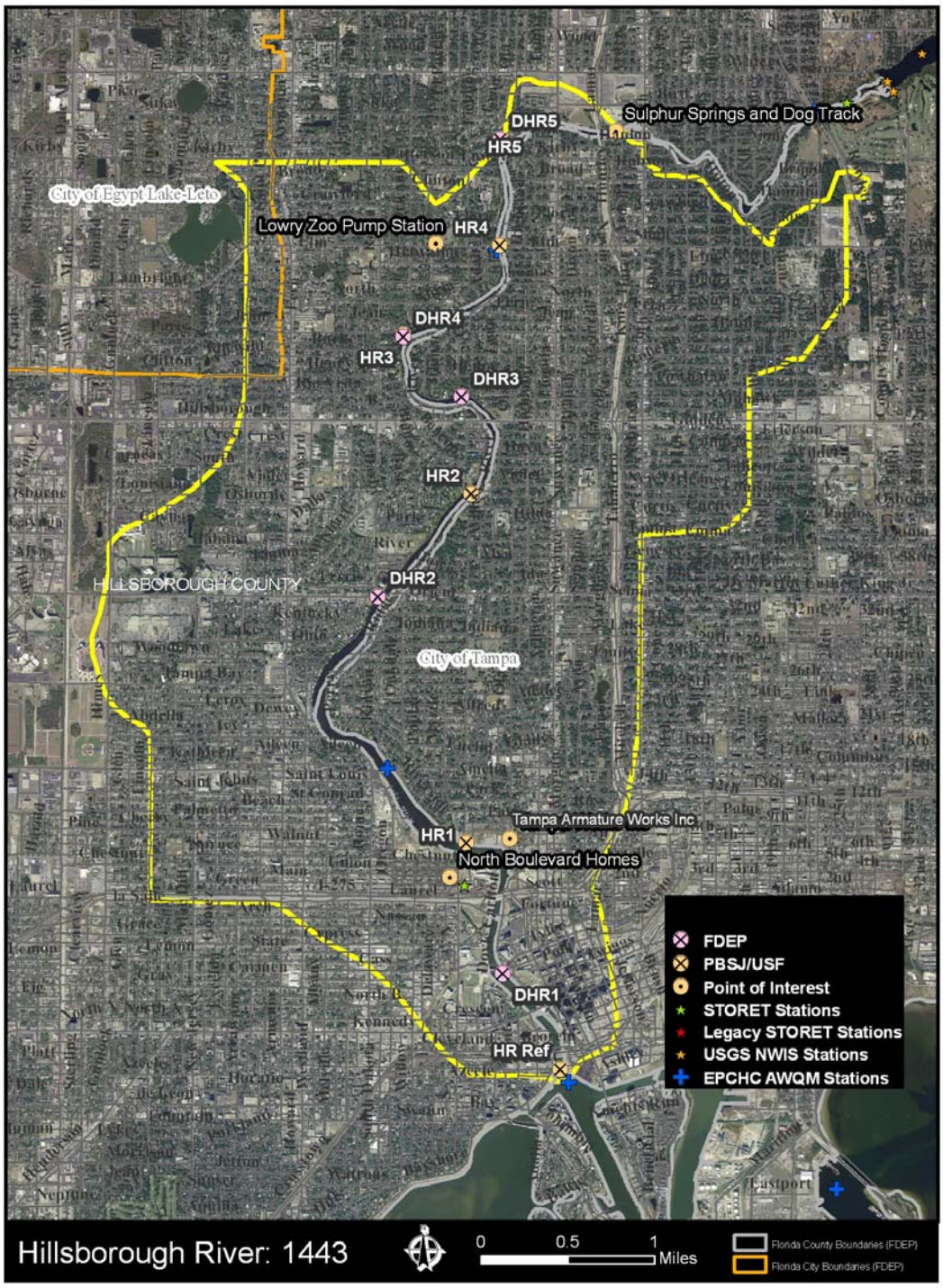
Map 12. Areas of repetitive sanitary sewer overflows along the Lower Hillsborough River as reported by the City of Tampa from 2001-2007.



Map 13. Sites sampled by the FDEP within the Lower Hillsborough River watershed between January and December 2007.

Section 9.3 Suggested Monitoring Stations

Sampling locations were determined using the same criteria as described in Section 2.4. The results of the initial screening process, as described in Section 9.2, demonstrated that due to the large size and numerous potential locations of primarily human sources, ten initial sampling stations (including five "fixed" stations and five "flexible" stations) would be required to better assess sources (Map 14; Table 28). One of the stations (HRRef), located at the southern WBID boundary (Platt Street), was designated not only to help identify potential impacts from humans but also to provide a control for testing the MST techniques for non-human impacts (e.g., there are no suspected significant animal sources, especially livestock, in this area). These station locations were investigated and confirmed during the "Walk the WBIDs" portion of Phase II.



Map 14. Map of the Lower Hillsborough River upon completion of Phase I, including identified sampling locations for Phase II, historical sampling locations, and general points of interest.

Table 28. A summary of suggested sampling locations within the Lower Hillsborough River WBID for Phase II, Level I analysis. Stations HR1, HR2, HR3, HR4 and HR5 were identified for sampling by the USF and PBS&J staff and stations DHR1, DHR2, DHR3, DHR4 and DHR5 were identified for sampling by FDEP personnel. N/A = Not Applicable.

Station	Potential Primary Source	Station Location	Corresponding Historical Station	Fixed or Flexible	Comments
HR5	Human (sewer)	North Highland Avenue	N/A	Fixed	SSOs at lift station on North Highland Avenue have been reported in the past
DHR5	Human (sewer)	West Whatley Place and River Shore Drive	EPCHC 514	Flexible	Account for input from Kirby Creek
HR4	Human (sewer)	Sligh Avenue	EPCHC 152	Fixed	Stormwater may also contribute to fecal pollution in this area
HR3	Human (OSTDS/sewer)	Small tributary off West Jean Street	N/A	Fixed	Account for input from west and frequent upstream SSOs
DHR4	Human (OSTDS/sewer)	Just downstream of HR3	N/A	Flexible	Account for potential impact of stormwater from adjacent outfall and frequent upstream SSOs
DHR3	Human (sewer)	Henry Street	N/A	Flexible	Account for input from east
HR2	Human (sewer)	River Boulevard (Hixon Park)	N/A	Fixed	Area of frequent SSOs and large stormwater outfall
DHR2	Human (sewer)	Dr. Martin Luther King Boulevard	N/A	Flexible	Area of frequent SSOs and large stormwater outfall
HR1	Human (sewer)	North Boulevard	N/A	Fixed	Across from Blake High School
DHR1	Human (sewer)	Cass Street	N/A	Flexible	Local homeless may also contribute to fecal pollution in this area
HR Ref	Human (sewer)	Platt Street	EPCHC 2	Reference	Reference station controlling for non-human impacts

Section 9.4 Phase II, Level I Sampling Results

Prior to the May sampling event, it was determined that it would be unnecessary to use the Platt Street location as a reference site since the expected lack of a ruminant- or horse-specific source is equally likely at HR1. Subsequently, HR1 at North Boulevard was defined as the reference location (HR1Ref). This replacement allows for a more efficient and effective use of available funds for sample analysis.

Unlike the other project WBIDs, sampling of the Lower Hillsborough River was not restricted by the absence of water or flow, with the exception of stations DHR6 and DHR4A which were not flowing during the October and December sampling events, respectively. In addition, station DHR4B was dry in both November and December. It is also important to note that water was flowing over the dam upstream of the WBID boundary during the August, September, and October sampling events. This may influence sampling results by either having a dilution effect at locations downstream or by contributing polluted waters to the Lower Hillsborough River.

Results indicate that the waters of the Lower Hillsborough River often exceeded state standards for IOs (Chapter 62-302 of the F.A.C; Table 29; Figure 11). In addition, the waters of the main stem of the Lower Hillsborough River vary in their level of impairment over both space and time (Figure 11; Map 18). For example, fecal coliform levels in surface waters of the main stem never exceeded 4,400 CFU/100mL (observed at station HR5 on July 25, 2007). Enterococci and *E. coli* concentrations peaked at 1,350 CFU/100mL and 2,450 CFU/100mL, respectively at station HR2 in July as well. In contrast, more chronic and considerably higher levels of indicator bacteria were observed in the smaller tributaries that flow into the main stem of the Lower Hillsborough River. For instance, fecal coliform concentrations at station DHR4A ranged from 17,850 to 99,667 CFU/100mL and were likely transported downstream to station HR3 which demonstrated fecal coliform levels from 550 to 7,500 CFU/100mL and exceeded 1,550 CFU/100mL in seven of eight sampling events. Similarly, fecal coliform concentrations at station DHR5 ranged from 2,600 to 5,900 CFU/100mL while those stations located further upstream along Kirby Creek (DHR6-DHR9) also oftentimes exceeded 2,000 CFU/100mL. These results suggest that contributing sources are located in both the upstream and downstream segments of Kirby Creek. In addition, enterococci and *E. coli* levels at station DHR4A peaked at 299,667 CFU/100mL and 97,333 CFU/100mL, respectively. Enterococci concentrations at station HR3 were also elevated to a maximum of 11,900 CFU/100mL in July 2007 while *E. coli* levels reached 5,000 CFU/100mL at this location in October. There were no obvious patterns in the concentrations of the indicator bacteria species to suggest the presence of an old or more recent source.

Statistical comparisons of IO concentrations within the Lower Hillsborough River did, however, demonstrate a significant positive correlation between all three IOs; fecal coliforms and *E. coli* ($r = 0.903$, $p<0.05$), fecal coliforms and enterococci ($r = 0.807$, $p<0.05$), and *E. coli* and enterococci ($r = 0.853$, $p<0.05$; Table 3). These results suggest that the IOs all exhibited similar behavior in terms of potential persistence and/or growth,

under the circumstances present throughout the Lower Hillsborough River for the duration of the project (see Section 4.0).

The difference in pollutant levels detected in the main stem surface waters compared to those in the tributaries may be the result of a dilution effect (decreased concentration of bacteria due to relatively greater volumes of water) present in the main stem portion of the river. It must be noted that in several instances, in both the main stem and tributaries of the river, the highest levels of contamination were identified during the July 2007 sampling event. According to USGS rain gauge 02304500 and COT Sulphur Springs/13th Street and Dazzo rain gauges (Map 2), this was the only sampling date that directly followed a significant rain event (the rain lasted six days and totaled 3.89, 0.91, and 3.53 inches, respectively). Multiple statistical correlations between IO concentration and rainfall corroborate this relationship. For example, a positive correlation was identified between seven-day cumulative rainfall and fecal coliform concentration at stations HR2, HR4, and DHR3 (Appendix C, Table 14). Similar relationships were also identified for enterococci levels at stations HR1Ref (despite non-normal rainfall data), HR2, and HR4. In addition, a positive correlation was also detected between 14-day cumulative rainfall and all three IOs at station HR2 and with fecal coliforms at station DHR3. These results suggest that stormwater, sanitary sewer failures associated with the inflow of stormwater or infiltration of groundwater into the lines, or possibly malfunctioning equipment and pumps due to power failures, are contributing sources of pollution throughout the Lower Hillsborough River basin. The lack of an identified relationship between rainfall and the abundance of *E. coli* despite the detection of correlations with the other IOs may be suggestive of older sources in these areas.

Thirteen sediment samples were obtained from stations throughout the basin, in both the main stem and tributaries of the river, and analyzed for IOs over the duration of this study. Although bacteria concentrations in the sediments were typically higher than those in the associated water column sampled on the same day, there were some exceptions (Figure 11). For example, at stations HR3 and DHR5, fecal coliform levels were higher in the surface waters (1,550 CFU/100mL and 5,900 CFU/100mL, respectively) than in the sediments (740 CFU/100g and 2,400 CFU/100g, respectively) during the June sampling event. Enterococci concentrations at station DHR6 showed a similar pattern in August with higher abundances in the surface waters (2,850 CFU/100mL) than the sediments (820 CFU/100g). Lastly, in both August and October, station DHR7 also exhibited higher enterococci levels in the water column (9,000 CFU/100mL on both dates) than the associated sediments (6,600 CFU/100g and 4,400 CFU/100g, respectively) on the same day. These results may suggest that there are recent sources of contamination impacting stations HR3, DHR5, DHR6 and DHR7. Furthermore, the observed differences among IOs in the sediments at stations DHR6 and DHR7, specifically the higher concentrations of fecal coliform and *E. coli* over enterococci, corroborate the potential for contributions by a relatively recent source of pollution in these areas. This interpretation, however, must be viewed with a great deal of caution as few sediment samples have been analyzed among the Florida sites.

It should also be noted that at least one IO reached levels over 50,000 CFU/g in the sediments at stations HR 2, HR4, and HR5, and over 100,000 CFU/g in the sediments at stations DHR4A, DHR7. Those stations with the highest levels of fecal coliforms in the

sediments (all exceeding corresponding levels of enterococci) included HR5, (fecal coliforms = 24,000 CFU/100mL), DHR7 (fecal coliforms = 104,500 CFU/100mL) and DHR4A (fecal coliforms = 176,000 CFU/100mL). These results suggest either recent and/or periodic inoculation or extended persistence of indicator bacteria in the sediments (Davies et al. 1995, Anderson et al. 2005). Each of these stations is suspected of being subject to recent wastewater leaks and is currently being more closely investigated with the help of COT and EPCHC representatives. In contrast, those stations with the highest levels of enterococci (all exceeding corresponding levels of fecal coliforms and *E. coli*) in the sediments included HR2 and HR4. Both of these stations have a history of localized and repetitive SSOs (over 5 incidents reported each between 2001 and 2007; Maps 12 and 14). In either case, it is apparent that there are significant reservoirs of bacteria in the sediments throughout the Lower Hillsborough River that have the potential to re-inoculate the water column and contribute to chronic and elevated levels of surface water contamination. Three exceptions (where no single IO concentration reached over 2,500 CFU/100g) were stations HR1Ref, HR3, and DHR5. Maximum indicator bacteria levels only reached 1,320 CFU/100g (enterococci), 2,120 CFU/100g (enterococci), and 2,400 CFU/100g (fecal coliforms), respectively at these locations; enterococci and *E. coli* were not analyzed at station DHR5.

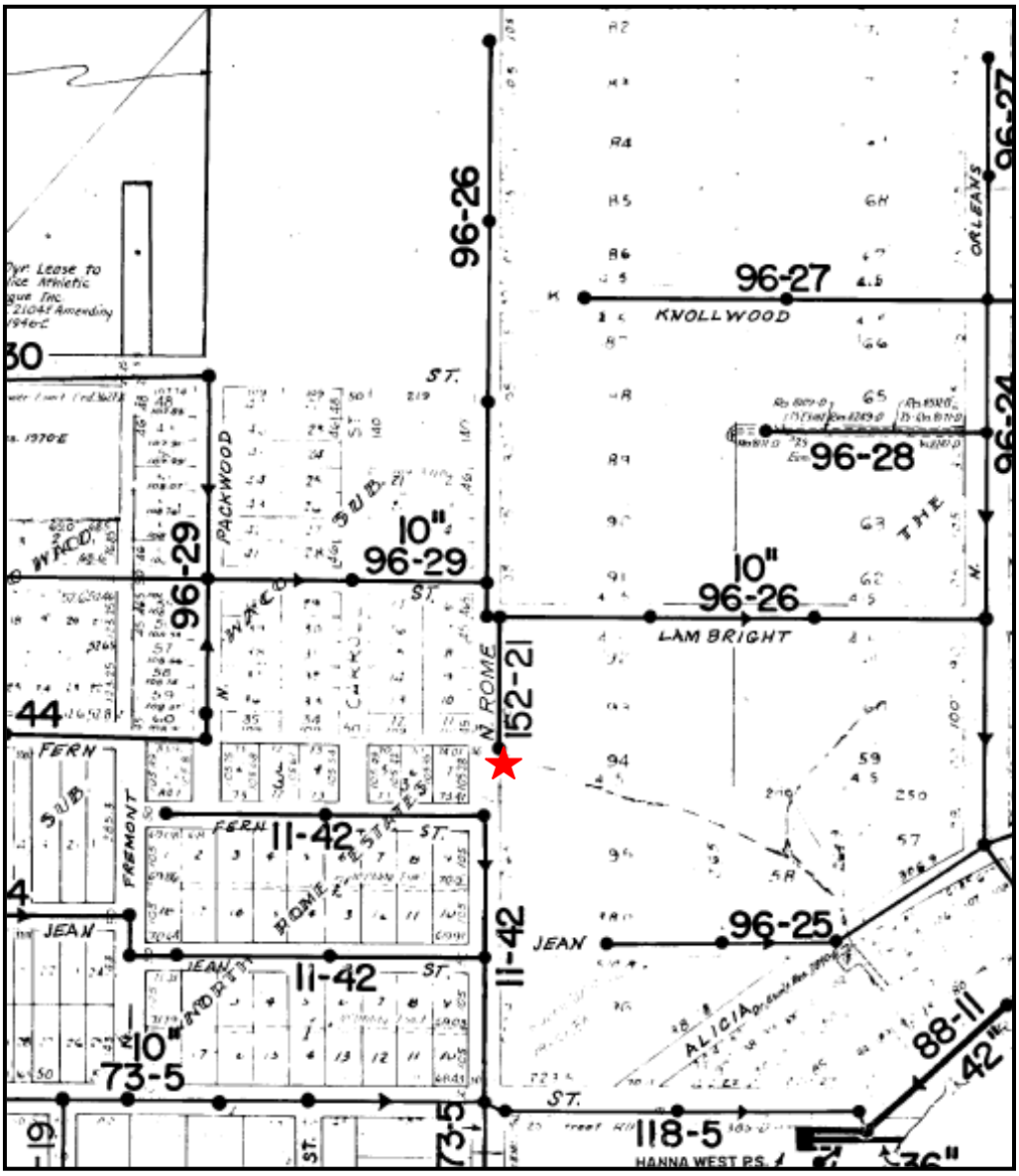
Table 29. Geometric mean concentration of indicator organisms (IOs) in water and sediment samples collected in the Lower Hillsborough basin. Calculations were performed on all available data collected under flowing conditions.

WBID	Fecal Coliforms				*E. coli*				Enterococci			
	GeoMean	N	SE	Max	GeoMean	N	SE	Max	GeoMean	N	SE	Max
Lower Hillsborough River (water)	2.76	100	0.09	5.00	2.52	53	0.12	4.99	2.70	53	0.14	5.48
Lower Hillsborough River (sediment)	3.96	13	0.29	5.25	3.70	12	0.26	5.20	4.04	12	0.23	5.06

IO Suite abundance values from nine stations within the Lower Hillsborough River watershed (HR1Ref, HR2, HR3, HR4, HR5, DHR2, DHR3, DHR4, and DHR5) were tested for correlations with available water quality parameters as described in Appendix C, Section 1.2. Unlike the other project basins, but as expected for this tidally-influenced system, significant relationships were identified for salinity. A significant negative relationship was identified between salinity and fecal coliform concentration at stations HR3 ($r = -0.852$, $p<0.05$), DHR3 ($r = -0.742$, $p<0.05$), and DHR4 ($r = -0.908$, $p<0.05$) while *E. coli* and salinity were also negatively correlated at station HR3 ($r = -0.889$, $p<0.05$; Appendix C, Table 2). This is likely due to the high inactivation ("die-off") rate of both fecal coliforms and *E. coli* in saline waters (Anderson et al. 1979, Solic & Krstulovic 1992, Bordalo et al. 2002, Anderson et al. 2005). In contrast, a significant positive relationship was detected between fecal coliforms and salinity at station DHR5 ($r = 0.853$, $p<0.05$; Appendix C, Table 2). Interestingly, the negative relationships were observed only at stations along the midstream portion of the basin (Map 18), perhaps where fluctuations of salinity were greatest and most influential.

As predicted, human-specific MST markers were detected in the surface waters throughout the Lower Hillsborough River basin (Figure 11; Table 30). More specifically, human *Bacteroides*, *esp*, and HPyV marker were detected in 39%, 24%, and 7%, of the samples analyzed for these markers, respectively. The failure to regularly detect HPyV is not surprising since this organism is present in more dilute concentrations in sewage than the other human markers. Despite the relatively analogous frequency of identification of the human *Bacteroides* and *esp* markers, it is interesting to note that the *esp* marker was noticeably absent from station DHR4A (added in August 2007 to help determine the source of pollution identified at station DHR4) despite the regularity of human *Bacteroides* detection and extremely elevated levels of contamination at this location. Similar patterns were observed at stations DHR7 and DHR8, though to a lesser degree. It should be noted that although *esp* is commonly found in human sewage resulting from sanitary sewer systems, it rarely survives through the storage tanks and associated drainfields of septic systems [personal communication, Dr Valerie J. Harwood, September 24, 2007; (Whitman et al. 2007)]. This disparity has not been observed for human *Bacteroides*. Therefore, these results suggest that OSTDS may be contributing to the fecal coliform contamination at station DHR4A.

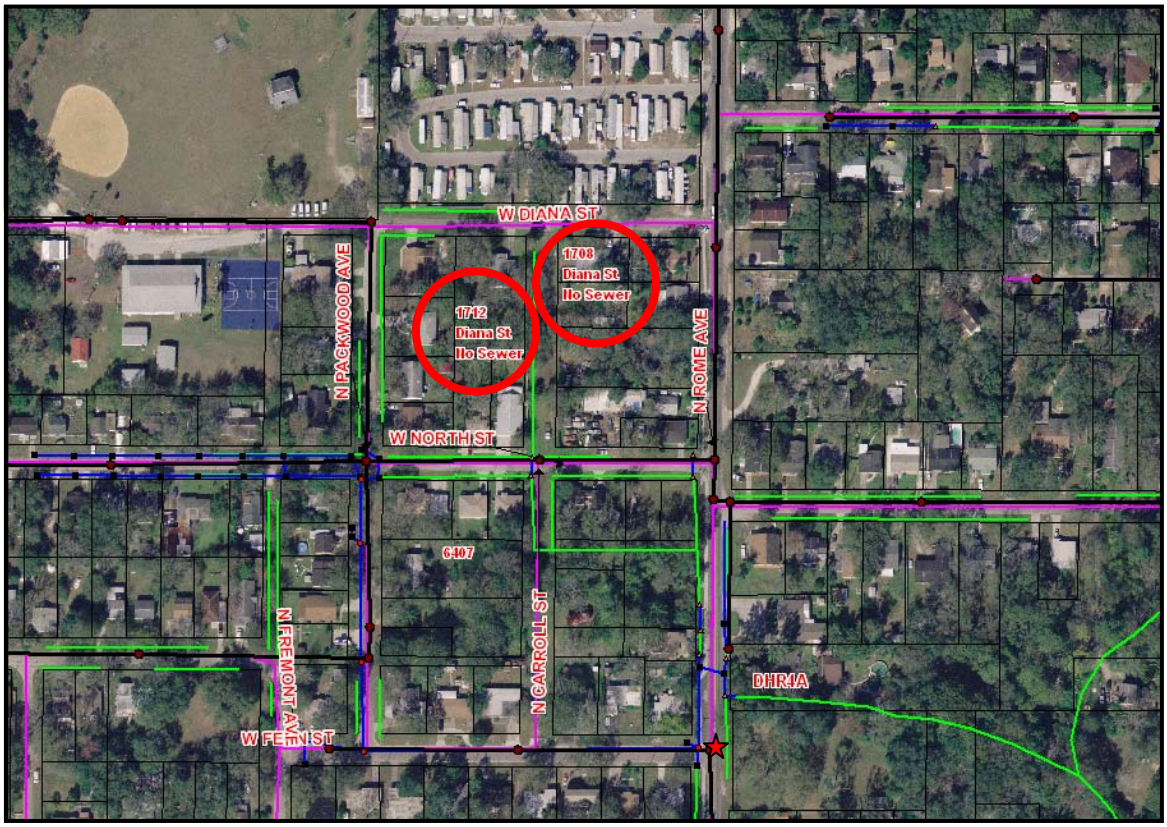
Given the abundance of sanitary sewer infrastructure and stormwater conveyance systems in the area surrounding station DHR4A, additional investigations of these structures were still necessary to better determine the specific source of pollution in this area. According to Michael Burwell, (COT, personal communication, October 5 and 8, 2007), representatives from the COT conducted a remote visual camera inspection of sewer lines in the area following the September 2007 sampling event. This procedure allowed the viewer to look for broken pipes, slipped joints, utility conflicts (e.g., holes in the gravity lines from boring machines associated with cable or water re-use lines), and leaking joints. A counter informs the operator of the position in the line so that if a problem is found a crew can be dispatched to the exact location to make the repair. Results of this investigation indicate that the majority of the system was in sufficient condition; however, a slipped joint near section 11-42 (approximately 225 feet west of North Rome Avenue on West Fern Street), was identified (Map 15). Since the sewer lines are deeper underground than the bottoms of the ditches in the area, it is more likely that this line would let in water rather than discharge any. Despite this possibility, necessary repairs to the lines were completed by the COT on January 24, 2008 (personal communication, Michael Burwell, February 27, 2008). In addition, supplemental sampling stations DHR4B and HR4A were added to help determine the source and spatial extent of pollution detected at station DHR4A. Elevated bacteria levels were identified at station DHR4B (fecal coliforms = 5,900 CFU/100mL) in September; however, concentrations were considerably lower than those observed downstream at station DHR4A (37,000 CFU/100mL) on the same day. Furthermore, DHR4B was dry in both November and December, at which time, extremely elevated levels of bacteria were still observed at station DHR4A. Similarly, station HR4A (located directly across the street from station DHR4A) demonstrated extremely low fecal coliform levels in October (2.5 CFU/100mL) when all corresponding IO levels at station DHR4A exceeded 28,000 CFU/100mL. The close proximity of these two stations together with the severity of the exceedances indicate that the primary source impacting station DHR4A is not being transported via the tributary itself but is likely being carried a relatively short distance through groundwater and/or wastewater or stormwater infrastructure.



Map 15. Sanitary sewer lines in the area around station DHR4A (denoted by the red star) that were investigated using remote camera equipment following the September 2007 sampling event. Segments 152-21, 11-42 and 96-26 were examined.

The severity of the bacteria concentrations and the consistent detection of human-specific markers at station DHR4A prompted additional investigations by local stakeholders. On February 13, 2008, the COT identified two parcels within City limits in the area upstream of station DHR4A that do not have an existing sanitary sewer account with the City (Map 16). The presumption is then that they are utilizing some type of onsite system. These parcels abut the ditch leading to North Street and eventually south towards station DHR4A. The potential for these systems to be failing and the associated impacts to the surface waters at station DHR4A remains to be determined. In addition, during a site visit by the COT on February 11, 2008, a slight sewage odor was detected at station DHR4A and although there was no flow observed in the drainage system upstream of the site, there was standing water at the station DHR4A. City personnel also located a kennel

with 4-5 dogs on a parcel along North Packwood Avenue which abuts the ditch system contributing to station DHR4A. Although there were also two tents in the rear of the parcel, according to neighbors, these belonged to youth who live at the house. It should be noted that a riparian ditch system drains into the tributary between stations DHR4A and HR3. Although additional water volume entering the tributary from this ditch may potentially dilute any signal that is transported downstream from station DHR4A, the detection of the *esp* marker at station HR3 (and not at upstream station DHR4A) suggests the presence of an additional source between stations. However, the *esp* marker was not tested for at station DHR4A at the time of detection at station HR3.



Map 16. Two parcels located upstream of station DHR4A, identified by the COT as not having an existing sanitary sewer account. The parcels are denoted by red circles. This map was provided by the COT (February 13, 2008). A red star indicates the approximate location of the sanitary sewer overflow reported by the COT on June 10, 2008.

On June 9, 2008, approximately six months after the sampling program was completed, a raw sewage discharge was identified by the EPCHC in the vicinity of station DHR4A. Water samples collected that day from the east side of Rome Avenue, just north of West Fern Street, showed extremely elevated fecal coliform and enterococci concentrations (1,430,000 CFU/100mL and 220,000 CFU/100mL, respectively). Upon receipt of the laboratory results, the EPCHC notified the COT on June 10, 2008. The COT officially reported the SSO to be associated with a grease blockage at a manhole at 6401 North Rome Avenue, located just south of station DHR4A. An indeterminate volume of raw sewage was discharged to the adjacent stormwater ditch for approximately one hour and twenty minutes before a vacuum was used to unblock the main line stoppage and clean up the overflow and the area was disinfected. As a result, as of June 10, 2008 this section of the sewer system has been scheduled to be cleaned and televised so as to eliminate or

reduce future grease-related overflows. Samples collected from station DHR4A and analyzed by Dr. Harwood's laboratory on June 17, 2008 demonstrated that elevated levels of fecal coliforms, *E. coli,* and enterococci (78,000 CFU/100mL, 30,000 CFU/100mL, and 35,000 CFU/100mL, respectively) remained in the surface waters after one week at this location. Furthermore, the human-specific markers *Bacteroides,* HPyV, and *M. smithii,* were all detected at this time. Interestingly, *esp* was not identified, suggesting the possibility of a septic system source. Additional samples collected in this area by the EPCHC on June 19, 2008 resulted in fecal coliform and enterococci concentrations of 60,000 CFU/100mL and 69,000 CFU/100mL, respectively. These results suggest that there is still a contributing source affecting this location.

Additional investigations were conducted by the EPCHC and HCHD concerning a potential sanitary nuisance in the vicinity of station HR3. According to EPCHC (personal communication, March 31, 2008), a black corrugated pipe (drainfield type) was observed in the ditch upstream of station HR3 on March 25, 2008. A lush, dark green line of grass "pointing" west suggested that nutrients were more readily available in the vicinity of the pipe than elsewhere. This line of grass eventually led towards a residence and an area of the yard was observed to be "soft and spongy". Samples were collected approximately five feet downstream of the corrugated drain pipe and indicated elevated levels of fecal coliform and enterococci (1,800 CFU/100mL and 5,300 CFU/100mL respectively). The HCHD visited the area on April 2 and 4, 2008 and did not find any evidence of a sanitary nuisance involving the property (personal communication, HCHD, April 4, 2008). Although a spongy area was observed, there was no sewage or effluent on the surface and no odor detected (except that from the adjacent COT lift station). It could not be determined if the pipe continued all the way to the property. The HCHD suggested that there may be a COT Utility right-of-way in that location and possibly a gravity sewer line that the black pipe crosses very close to the river. HCHD will attempt to contact the owner of the residence and notify them that, if currently utilizing OSTDS, the Florida statute would require mandatory connection to the sewer system due to proximity to the available infrastructure.

A follow-up investigation of the area near station HR3 was performed by the EPCHC on June 9, 2008. At this time, the OSTDS serving the residence on Alicia Avenue still produced a clearly defined growth of lush, dark green grass in two areas, suggesting the potential for two drainfields. One sample was collected from approximately 20 feet upstream and another from roughly 15 feet downstream of the black corrugated drain pipe upstream of station HR3. Fecal coliform and enterococci levels at both locations were similar (fecal coliforms = 1,800 and 1,500 CFU/100mL and enterococci = 7,500 and 6,100 CFU/100mL, respectively), suggesting that the pipe is not the primary contributor to the local bacteria levels in this area.

Despite the preponderance of human-specific MST markers throughout the Lower Hillsborough River basin, sites HR5, DHR5 and DHR6 only identified these markers 11-20% of the times they were analyzed (Table 30). In each of these cases, with the exception of station HR5 in November, the markers were detected simultaneously with elevated levels of indicator bacteria; the markers were identified at station DHR6 only during maximum fecal coliform concentrations. This may suggest a recent and episodic source of contamination at these sites. Supplemental stations, DHR6, DHR7, DHR8

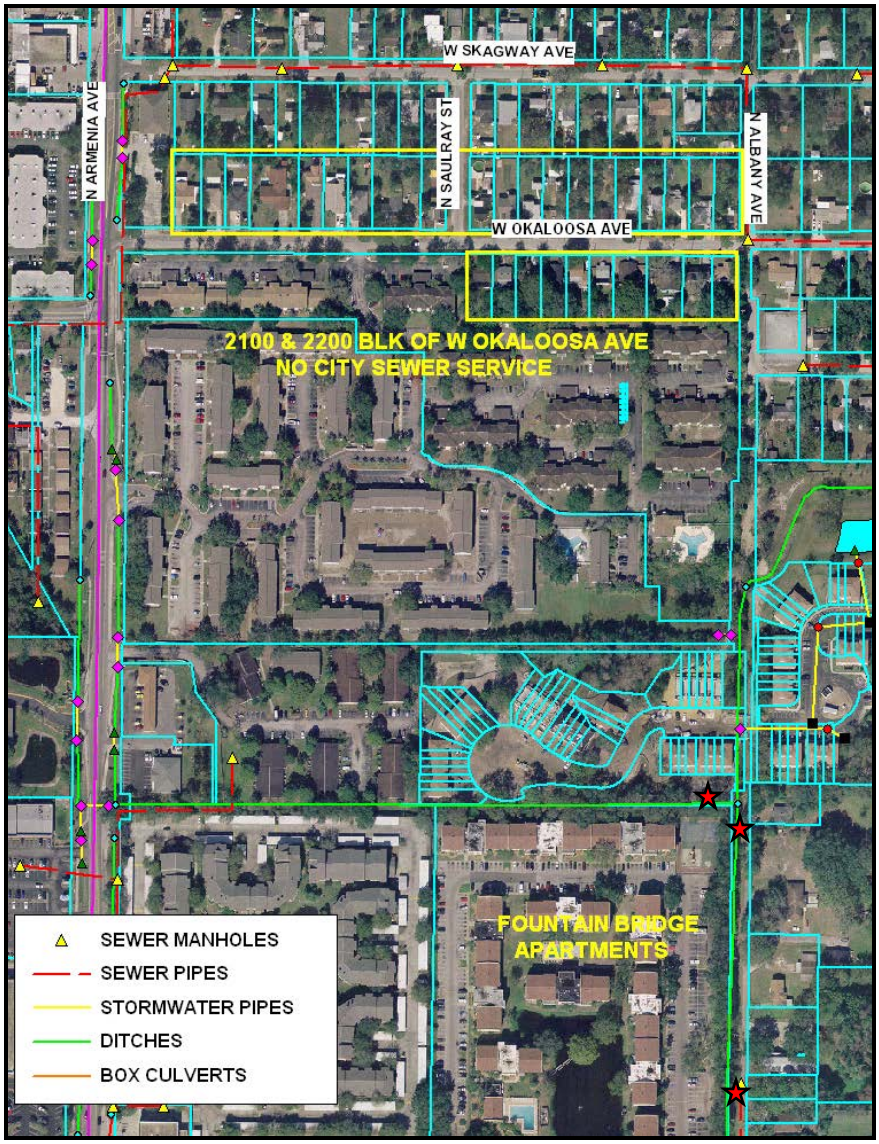
DHR9, and DHR10 (a stormwater grate in the Fountain Bridge Apartment complex parking lot consistently found with high water; Appendix D, Photograph 34) were all added along the Kirby Creek tributary between July and August 2007 to help identify the source of pollution detected at station DHR5 (Figure 11; Map 17).

In addition to the supplemental sampling sites, field reconnaissance efforts and associated investigations were conducted in conjunction with COT and EPCHC representatives to assist in the process of identifying sources contributing to the pollution in Kirby Creek, prior to entering the Lower Hillsborough River. For example, the COT shared knowledge of several redline properties between stations DHR6 and DHR5 that would be prone to flooding if developed in the usual way. In addition, in November 2007, the COT also identified parcels within City limits in the area upstream of the Fountain Bridge Apartment Complex (adjacent to stations DHR7-10) that do not have existing sanitary sewer accounts with the City, and are presumably using OSTDS (Map 16). These parcels are located in a residential area presumably built in the 1950s and therefore do not likely adhere to recent codes for such systems. A field investigation of the drainage system performed by COT personnel on February 11, 2008, revealed flow in the ditch system paralleling North Albany Avenue (between stations DHR7 and DHR8) after approximately two weeks without rainfall. In addition, the stormwater drain (station DHR10) was surcharged though there was no evidence of sewage or odor; the onsite retention pond appeared normal. Although not relevant to fecal coliform contamination, the COT responded to additional inquiries associated with sampling and field reconnaissance events. One of these incidents resulted in a letter being sent to a resident to disconnect a pipe running from a water softener to the tributary near station DHR6.

The EPCHC also visited the Fountain Bridge Apartment complex on March 25, 2008 at which time a water sample was collected in close proximity to station DHR9. The following information was received by EPCHC on March 31, 2008. Sample results demonstrated slightly elevated concentrations of fecal coliforms (700 CFU/100mL) and enterococci (1,500 CFU/100mL). During their field visit, EPCHC staff observed that at least two storm drains in the complex were surcharged and that the stormwater pond serving the complex has a pumped discharge. EPCHC reported that if the pump (there is apparently only one) fails or the floats are not set properly, the groundwater table could be impacted. In addition, a collapse in the pavement in front of building 18 was noticed that may impact the gravity sewer line. If there is a partial collapse in the collection system, or if the lines get plugged with grease, they can become surcharged and exfiltration of sewage into the ground water table can occur. This could appear in the retention pond in the middle of the complex and could account for the detection of the human *Bacteroides* marker in the overflowing waters of the stormdrain (station DHR10). EPCHC performed a follow-up investigation on June 9, 2008. At this time, the pump station associated with the stormwater pond was observed as not being maintained so as to function as intended. For example, storm drains in the parking lot, especially on the south end of the property (station DHR10) were surcharged. A sample collected from the stormwater pond demonstrated fecal coliform and enterococci concentrations of 400 CFU/100mL.

Further investigations by the EPCHC in this area resulted in the identification of a discharge from Tampa Tent and Rental Company, Inc. Samples collected near station

DHR7 demonstrated fecal coliform and enterococci levels of 9,200 CFU/100mL and 300 CFU/100mL, respectively (personal communication, EPCHC, March 31, 2008). The source of the bacteria remains unknown. According to the EPCHC, as of June 9, 2008, the discharge at this site continued. A consulting engineer for Tampa Tent indicated that they plan to connect to the City's sewage collection system.



Map 17. Parcels, outlined in yellow, located upstream of stations DHR7, DHR8, DHR9, and DHR10, identified by the COT as not having an existing sanitary sewer account. This map was provided by the COT (November 28, 2007). The red stars indicate the approximate locations of stations DHR7, DHR8, and DHR9. Blue lines denote parcel boundaries.

Ruminant- and horse-specific *Bacteroides* markers were unexpectedly detected in upstream portions of the Lower Hillsborough River basin (Figure 11; Table 30). Although a ruminant marker was identified on one occasion each at stations HR3 and DHR4A, in both cases, human-specific markers were also detected during the same sampling event as well as on at least two different occasions. These results, together with information pertaining to land use in these areas, suggest that the ruminant-specific markers resulted from small, transient deer populations, or possibly goats owned by local

residents, that contribute relatively small amounts of fecal coliform contamination. In contrast, the ruminant *Bacteroides* marker was regularly identified at station DHR7 and on two occasions, the human and ruminant *Bacteroides* markers co-occurred. Given that this station is located adjacent to a relatively large wooded area, the potential for a larger deer population is apparent; however, it must be noted that human-specific markers have also been detected in this area, as well as upstream, indicating the probability of a larger human-related source.

The horse-specific *Bacteroides* marker was also identified on one occasion each at stations DHR4A and DHR6 (Table 30); however, the presence of this marker at these stations is difficult to explain due to the lack of supporting land use information. The specificity of this marker was further tested by Dr. Valerie J. Harwood's laboratory as it is a relatively new MST tool for the lab. The assay was optimized for horse over the other non-target groups (e.g., dog, bird) in order to validate the test's sensitivity and accuracy in October 2007. Since this revision, the horse marker was no longer identified at these locations.

As mentioned above, the detection of human-related pollution oftentimes coincided with elevated levels of indicator species (e.g., stations DHR5 and DHR6). Multivariate analyses of bacterial community data showed a significant effect of MST results (presence of absence of MST markers) on per-sample bacterial communities for human *Bacteroides* in the Lower Hillsborough River (Global R = 03115, $p<0.05$), though this relationship was quite weak. Despite this relationship, SIMPER was unable to identify a major driver for group dissimilarity. A chi-squared goodness of fit test was used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for the individual MST markers); however, no significant relationships were identified.

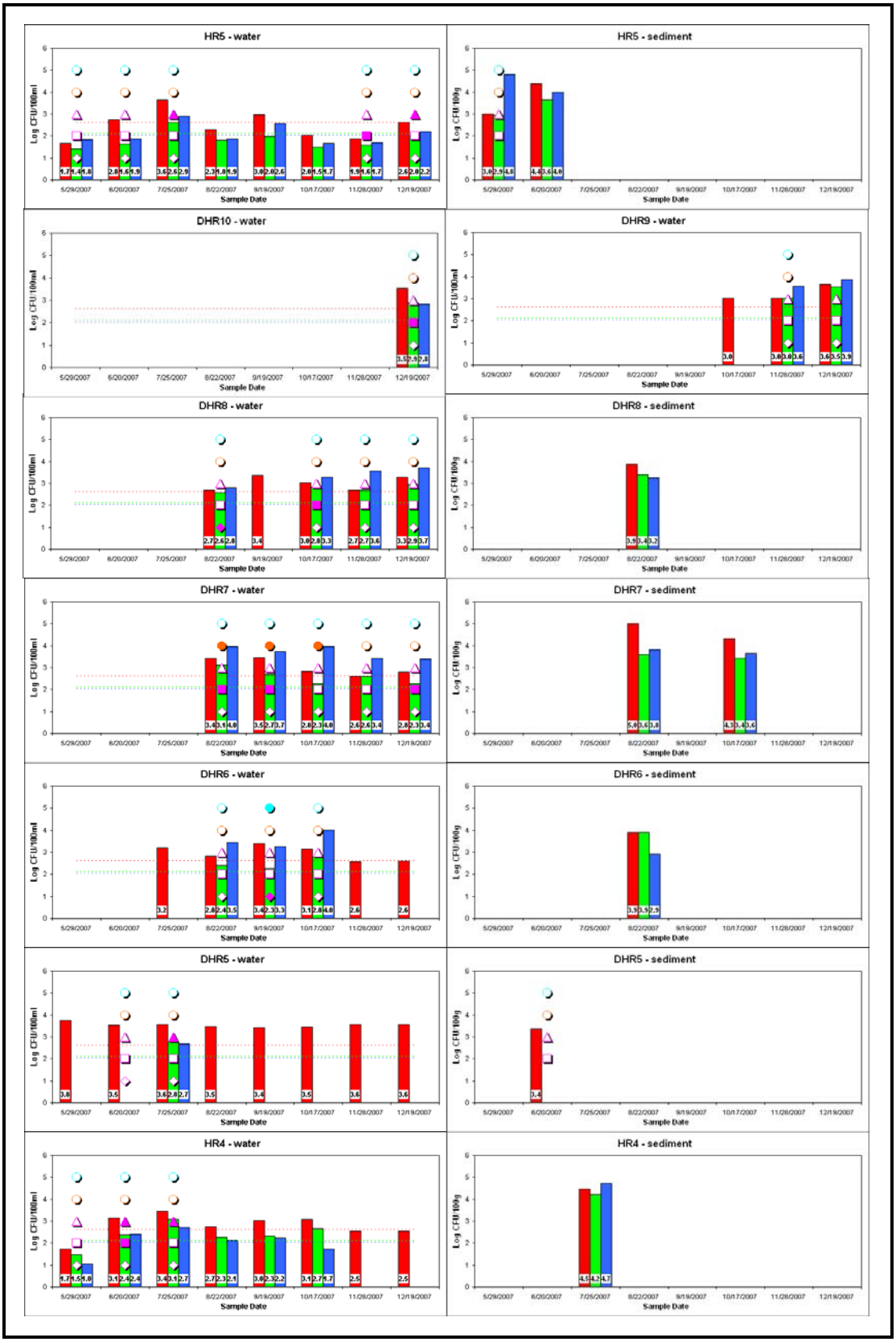


Figure 11. Indicator bacteria results at the Lower Hillsborough River from the May through December 2007 sampling events. Typically only water samples were collected and analyzed for fecal coliforms at the "flexible" stations, including DHR1, DHR2, DHR3, DHR4, and DHR5. This figure is continued below.

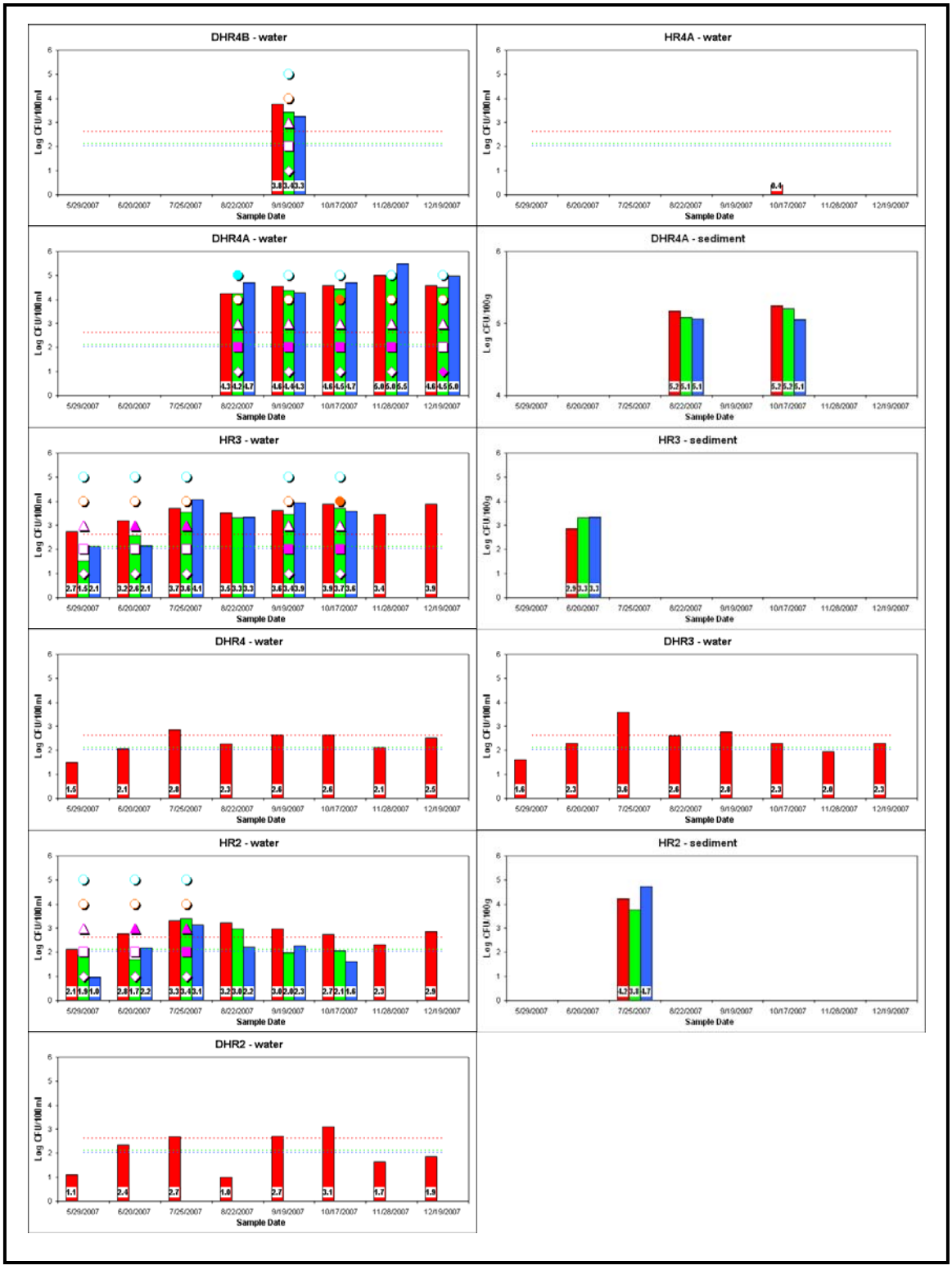


Figure 11 continued. Indicator bacteria results at the Lower Hillsborough River from the May through December 2007 sampling events. Typically only water samples were collected and analyzed for fecal coliforms at the "flexible" stations, including DHR1, DHR2, DHR3, DHR4, and DHR5. This figure is continued below.

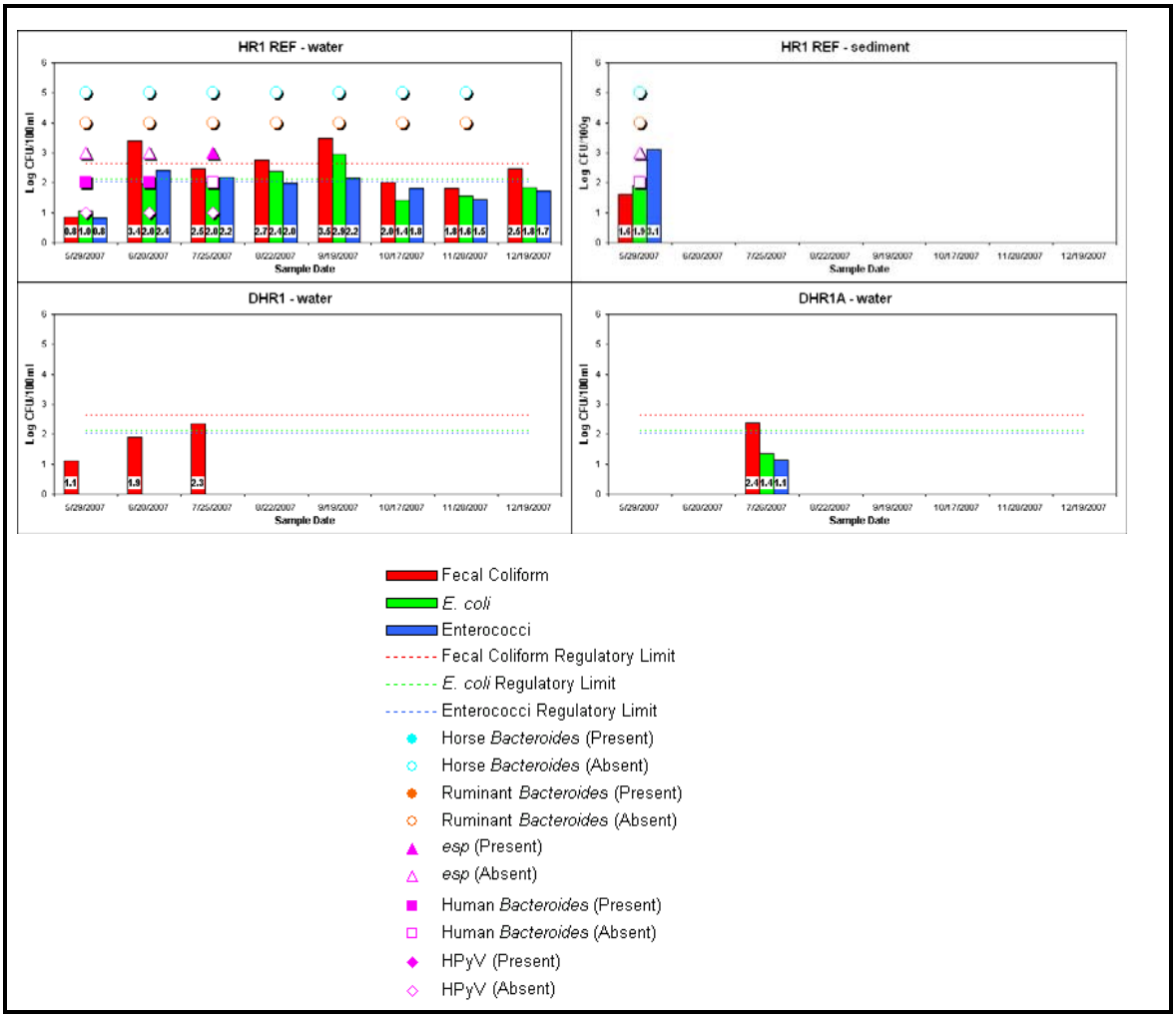


Figure 11 continued. Indicator bacteria results at the Lower Hillsborough River from the May through December 2007 sampling events. Typically only water samples were collected and analyzed for fecal coliforms at the "flexible" stations, including DHR1, DHR2, DHR3, DHR4, and DHR5.

Table 30. Results of the source-specific microbial tracking tests utilized in this project as of December 2007. Results are reported as the number of positive results/total number of tests performed. For example, 0/1 = 0 positive tests out of 1 total test conducted. Unless otherwise indicated, analyses were conducted on water samples. Yellow shading indicates positive results.

Station	Human Bacteroides	*Enterococcus* (*esp*)	Human Polyomavirus	Ruminant Bacteroides	Horse Bacteroides
HR1	2/3	1/3	0/3	0/8	0/8
HR1 (sediment)	0/1	0/1	0/0	0/1	0/1
HR2	1/3	2/3	0/3	0/3	0/3
HR3	2/5	2/5	0/5	1/5	0/5
DHR4A	4/5	0/5	1/5	1/5	1/5
HR4	1/3	2/3	0/3	0/3	0/3
HR5	1/5	2/5	0/5	0/5	0/5
HR5 (sediment)	0/1	0/1	0/0	0/1	0/1
DHR5	0/2	1/2	0/2	0/2	0/2
DHR5 (sediment)	0/1	0/1	0/0	0/1	0/1
DHR6	0/3	0/3	1/3	0/3	1/3
DHR7	3/5	0/5	0/5	3/5	0/5
DHR8	1/4	0/4	1/4	0/4	0/4
DHR9	0/2	0/2	0/2	0/1	0/1
DHR10	1/1	0/1	0/1	0/1	0/1

Section 9.5 Fecal Coliform Source Assessment Summary

This assessment indicates that human-related contamination is the leading cause of fecal pollution identified within the Lower Hillsborough River. Despite a difference in pollutant levels detected in the main stem surface waters compared to those in the tributaries, likely due to a dilution effect, human-specific markers were detected at every site tested, with the exception of station DHR9. In addition, the identification of these markers oftentimes coincided with elevated levels of indicator species and a significant relationship was observed between bacterial communities and the detection of the human *Bacteroides* marker. Furthermore, the sediments at several stations demonstrated extremely elevated IO concentrations suggestive of either recent and/or periodic inoculation or extended persistence of indicator bacteria. Each of these stations is suspected of being subject to recent or past wastewater leaks. In either case, it is apparent that there are significant reservoirs of bacteria in the sediments throughout the Lower Hillsborough River that have the potential to re-inoculate the water column and contribute to chronic and elevated levels of surface water contamination.

Although both ruminant- and horse-specific markers were identified on occasion, existing land use data, as well as the simultaneous detection of human-specific markers, indicate that a human source is both more likely and more significant.

The current data indicate the presence of the most significant "hot spots" for fecal coliform pollution in the vicinity of stations DHR4A as well as along Kirby Creek near stations DHR5 and DHR6-10. The most likely source at station DHR4A appears highly localized and, as indicated by the pattern of MST marker detection, is likely OSTDS-related, though this remains to be confirmed. The data at stations DHR5-10 indicates that there may be multiple sources contributing to the surface waters in these locations. It is probable that pollution originating in these areas is responsible for the elevated levels of indicator bacteria detected downstream. Lastly, human-specific sources, such as SSOs, likely play a more episodic role in the contamination of the main stem sites, especially around stations HR2, HR4, and HR5. Table 31 identifies the most likely sources of fecal contamination for each area of the Lower Hillsborough River.

Suggested corrective actions, specifically to address: 1) potential OSTDS-related sources in the vicinity of stations DHR7, DHR8, DHR9, DHR4A, and HR3; 2) likely sanitary sewer-impacted areas (including those effected by SSOs) near stations DHR5, DHR7, DHR8, DHR9, HR5, HR4, DHR4A, HR3, DHR3, HR2, DHR2, and HR1Ref; 3) neighborhoods with possible illicit discharges that may be present upstream of station DHR6; and 4) probable impacts associated with stormwater contributions at stations HR4, DHR3, HR2, and HR1Ref are included in Section 11.0.

Table 31. Summary of the most probable sources of fecal contamination contributing to the Lower Hillsborough River sampling locations (generally listed from upstream to downstream). N/A = Not Applicable.

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
HR5	Human (sewer)	North Highland Avenue	N/A	Fixed	Human-specific MST Episodic bacteria exceedances No known OSTDS in area	SSOs at lift station on North Highland Avenue have been reported in the past Abundant sewer infrastructure throughout area Extremely elevated bacteria levels in sediments may cause re-inoculation of surface waters Exceedances with and without flow over dam
DHR10	Human	Fountain Bridge Apartment Complex (stormwater grate)	N/A	Flexible	Human-specific MST Elevated levels of IOs	Limited data (n=1) Collapse in parking lot pavement (observed by EPCHC) may impact gravity sewer lines and account for human-specific marker detected in storm drain
DHR9	Human	North Albany Avenue (north of Fountain Bridge Apartment Complex)	N/A	Flexible	Consistently elevated levels of IOs Land use suggests human source	Account for input from west Limited data (n=3) Parcels likely using OSTDS, in older residential area, identified by COT upstream of site Abundant sewer infrastructure throughout area

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
DHR8	Human	North Albany Avenue (east of Fountain Bridge Apartment Complex)	N/A	Flexible	Human-specific MST, except *esp* marker may suggest OSTDS source Consistently elevated levels of IOs Detection of human-specific markers coincided with elevated bacteria levels Parcels likely using OSTDS, in older residential area, identified by COT upstream of site	Account for input from north Abundant sewer infrastructure throughout area
DHR7	Human	North Albany Avenue and West Waters Avenue	N/A	Flexible	**Human** Human-specific MST, except *esp* marker may suggest OSTDS source Consistently elevated levels of IOs Detection of human-specific markers coincided with elevated bacteria levels Parcels likely using OSTDS, in older residential area, identified by COT upstream of site	Higher enterococci concentrations in surface waters than associated sediments and pattern of IO concentrations in sediments may suggest recent source Extremely elevated bacteria levels in sediments may cause re-inoculation of surface waters Connection to Fountain Bridge Apartment Complex retention pond Collapse in parking lot pavement (observed by EPCHC) may impact gravity sewer lines and account for human-specific marker detected in storm drain Flowing water typically observed in tributary after long periods without rainfall Abundant sewer infrastructure throughout area Deer or goats may contribute fecal coliform pollution to this site Family of ducks regularly observed upstream

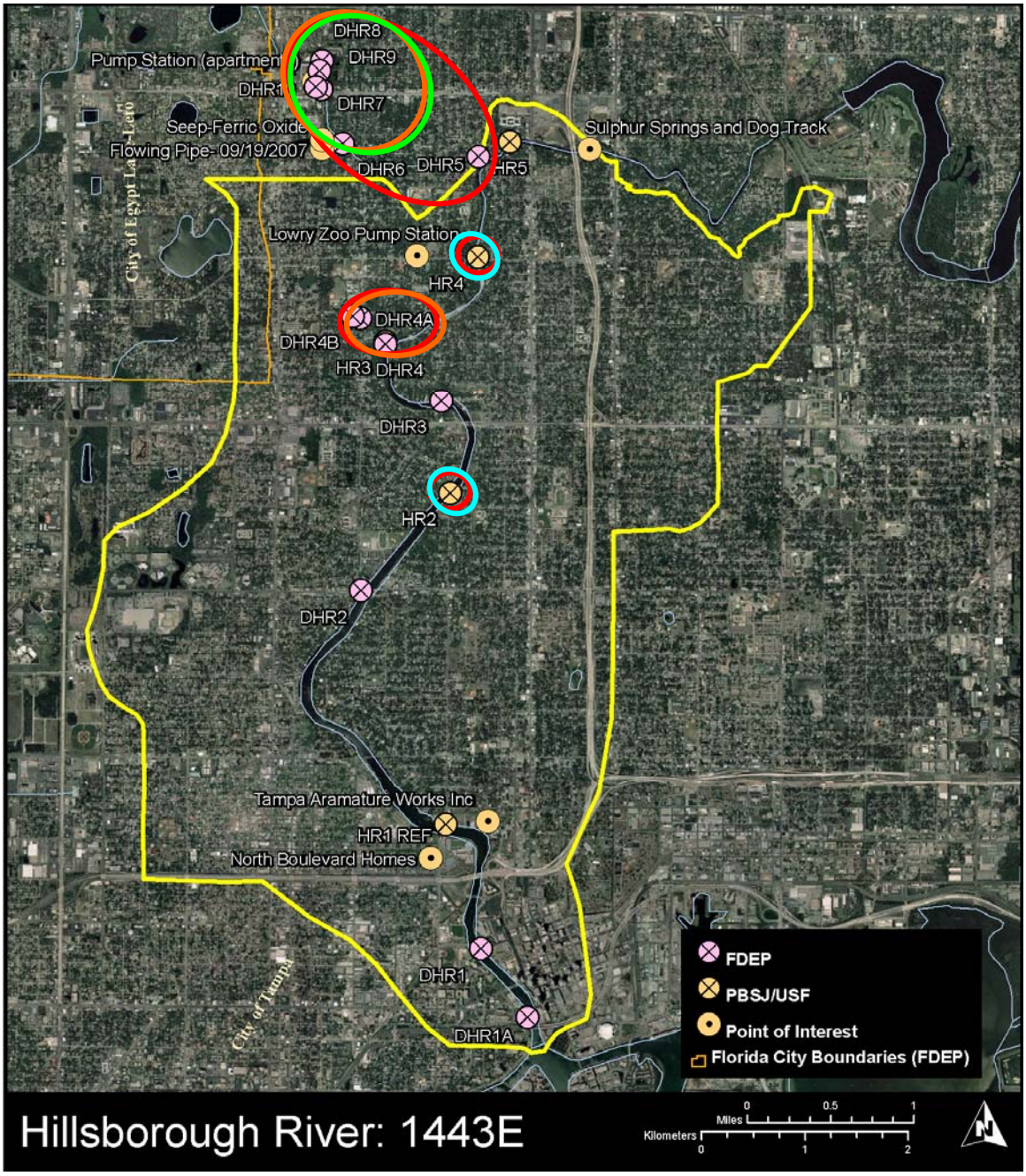
Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
DHR6	Human	West Sitka Street	N/A	Flexible	Human-specific MST Bacteria typically exceeded standards Only human –specific marker detected coincided with maximum fecal coliform concentration, may suggest recent source	Higher enterococci concentrations in surface waters than associated sediments and pattern of IO concentrations in sediments may suggest recent source Abundant sewer infrastructure throughout area
DHR5	Human	West Whatley Place and River Shore Drive	EPCHC 514	Flexible	Human-specific MST Consistently elevated levels of IOs Land use suggests human source	Account for input from Kirby Creek Higher fecal coliform concentrations in surface waters than associated sediments may suggest recent source Relatively low bacteria concentrations in sediments suggest that they do not likely cause re-inoculation of surface waters Abundant sewer infrastructure throughout area
HR4	Human Stormwater	Sligh Avenue	EPCHC 152	Fixed	**Human** Human-specific MST Co-occurrence of human markers is strong evidence of human contamination Detection of human-specific markers coincided with elevated bacteria levels **Stormwater** Positive correlation between 7-day cumulative rainfall and fecal coliform and enterococci concentrations may suggest "aged" fecal pollution Large stormwater outfall at site	History of localized and repetitive SSOs Abundant sewer infrastructure throughout area Extremely elevated bacteria levels in sediments may cause re-inoculation of surface waters

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
DHR4B	Human	Northwest of North Rome Avenue and West Fern Street	N/A	Flexible	Extremely elevated IO concentrations Land use suggests human source	Limited data (n=1)
DHR4A	Human (OSTDS/sewer)	North Rome Avenue	N/A	Flexible	Human-specific MST, except *esp* marker, may suggest OSTDS source Detection of human-specific markers always coincided with extremely high and consistent levels of IOs Pattern of IO concentrations in surface waters suggests recent source Source independent of flow in tributary from across Rome Avenue (station DHR4B) Slipped joint identified in sewer lines west of site (not likely to have discharged water) Two parcels likely using OSTDS identified by COT in vicinity of site	High bacteria levels in sediments may cause re-inoculation of surface waters Slipped joint identified in sewer lines west of site (not likely to have discharged water) Two parcels likely using OSTDS identified by COT in vicinity of site Standing water typically observed in stormwater ditch and at station DHR4A when tributary upstream of DHR4A is dry Deer or goats may contribute fecal coliform pollution to this site

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
HR3	Human (OSTDS/sewer)	Small tributary off West Jean Street	N/A	Fixed	Human-specific MST Detection of *esp* at this site and not at upstream station DHR4A may suggest an additional contributing source (possibly transported by riparian ditch)	Account for input from west Higher fecal coliform concentrations in surface waters than associated sediments may suggest recent source Relatively low bacteria concentrations in sediments suggest that they do not likely cause re-inoculation of surface waters Negative correlation between salinity and fecal coliform and *E. coli* concentrations suggests "die-off" of bacteria Deer or goats may contribute fecal coliform pollution to this site Possible sanitary nuisance observed upstream by EPCHC and HCHD
DHR4	N/A	Just downstream of HR3	N/A	Flexible	N/A	Only two very minor exceedances Account for potential impact of stormwater from adjacent outfall and frequent upstream SSOs Negative correlation between salinity and fecal coliform concentration suggests "die-off" of bacteria
DHR3	Stormwater	Henry Street	N/A	Flexible	**Stormwater** Positive correlation between 7-day and 14-day cumulative rainfall and fecal coliform concentrations Tributary draining urban landscape enters river from east at this site	MST markers not utilized Negative correlation between salinity and fecal coliform concentration suggests "die-off" of bacteria

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
HR2	Human (sewer) Stormwater	River Boulevard (Hixon Park)	N/A	Fixed	**Human** Human-specific MST Co-occurrence of human markers is strong evidence of human contamination Detection of human-specific markers coincided with elevated bacteria levels **Stormwater** Positive correlation between 7-day cumulative rainfall and fecal coliform and enterococci concentrations may suggest "aged" fecal pollution Positive correlation between 14-day cumulative rainfall and all three IOs may suggest more recent pollution Large stormwater outfall at site	History of localized and repetitive SSOs Abundant sewer infrastructure throughout area No known OSTDS in area Extremely elevated bacteria levels in sediments may cause re-inoculation of surface waters
DHR2	Human (sewer)	Dr. Martin Luther King Boulevard	N/A	Flexible	Episodic exceedances of IOs Land use suggests human source	MST markers not utilized Area of frequent SSOs and large stormwater outfall Abundant sewer infrastructure throughout area No known OSTDS in area No observed correlations with rainfall

Station	Most Probable Source	Sampling Location	Corresponding Historical Station	Fixed or Flexible	Weight-of-Evidence for Source Identification	Comments
HR1Ref	Human (sewer) Stormwater	North Boulevard	N/A	Fixed	**Human** Human-specific MST **Stormwater** Positive correlation between 7-day cumulative rainfall and enterococci concentrations, may suggest "aged" fecal pollution	Detection of human-specific markers did not always coincide with elevated bacteria levels Relatively low bacteria concentrations in sediments suggest that they do not likely cause re-inoculation of surface waters Abundant sewer infrastructure throughout area No known OSTDS in area
DHR1	N/A	Cass Street	N/A	Flexible	N/A	No observed exceedances of IOs



Map 18. Map of the Lower Hillsborough River upon completion of Phase II, including identified sampling locations for Phase II, historical sampling locations, and general points of interest. Results of the May through December 2007 sampling events suggest the presence of "hot spots" for fecal contamination in the vicinity of stations DHR6-DHR10, DHR5, DHR4A, and to a lesser extent, possibly resulting from dilution effects, stations HR4 and HR2. The most likely sources in general areas are denoted by circles of different colors: red = sanitary sewer and associated overflows; orange = septic systems; green = illicit discharges; and blue = stormwater.

Section 10.0 Application of Decision-Support Tool

Section 10.1 Background

Through an existing Department-funded project, PBS&J and its sub-contractors (Terra Ceia Consulting LLC and the University of South Florida) have developed a decision-support tool to help guide BMAP implementation in a group of Class III WBIDs in the Hillsborough River watershed that are impaired due to elevated fecal coliform concentrations. As explained in a companion report (PBS&J et al. 2008), the decision-support tool is based on the "Annapolis protocol" recommended by the (WHO 2003) and the "phased monitoring approach" recommended by the (NRC 2004) to address bacteriological impairments in recreational waters. The (WHO 2003) and (NRC 2004) methodologies acknowledge the limitations that affect the use of existing bacterial water quality indicators, such as fecal coliforms, *E. coli*, and enterococci, and use a weight-of-evidence approach to help compensate for those limitations. They use two independent categories of information — bacterial indicator data to identify locations with potential fecal contamination, combined with site-specific surveys to identify and classify indicator sources on the basis of their potential human health risks — to help prioritize and guide management actions to address bacteriological water quality impairments.

The fecal coliform BMAP decision-support tool (PBS&J et al. 2008) is applied by completing the following steps:

- First, microbial water quality conditions within each WBID are categorized based on fecal coliform concentrations observed in the available monitoring data. (A minimum of 30 samples per station, collected at a regular [e.g., monthly or bimonthly] frequency over a five-year period, is recommended for this purpose. If existing monitoring programs do not provide this amount of data, additional monitoring is recommended to ensure that adequate data will be available for future assessments);

- For characterization purposes, each monitoring station within a WBID is assigned to a microbial water quality assessment (MWQA) category. The MWQA categories are symbolized as letter grades (A through E) reflecting how frequently the State's fecal coliform criterion of 400 CFU/100 mL is exceeded at a given site. Because sites with higher frequencies of criterion exceedances also tend to exhibit higher overall concentrations of fecal coliforms and enterococci, MWQA categories A through E also represent progressively higher indicator organism concentrations and, potentially, an increasing likelihood of human health risk;

- Next, for sites at which more than 10% of the samples exceed the State's 400 CFU/100 mL criterion, contaminant source surveys (CSS) are carried out in order to identify the types of probable sources that could contribute to the elevated

- bacterial concentrations occurring at the site and to characterize their potential human health risks;

- Following the concepts outlined in the Annapolis protocol, each surveyed site is placed in a CSS category (ranging from "very low" to "very high" levels of potential risk) reflecting the types of probable bacterial sources found in the vicinity of the site and the estimated likelihood that those sources pose human health risks;

- Following the phased monitoring concept recommended by the (NRC 2004), the intensity of CSS investigation that a site receives is based on its MWQA classification. That is, sites that exhibit more frequent (and higher magnitude) exceedances of the State's 400 CFU/100 mL fecal coliform criterion (e.g., sites in MWQA categories C, D or E) are subject to more intensive CSS investigations than sites exhibiting less frequent (and lower magnitude) exceedances;
- Once the MWQA and CSS analyses are completed, each site receives a two-part classification, based on the MWQA and CSS categories into which it has been placed;

These classification outcomes can be summarized in a "classification matrix" format (PBS&J et al. 2008) , which can be included in annual progress reports to give policy-makers and the public a summary of the locations where management actions are deemed necessary to address the fecal contaminant sources identified in the impaired WBIDs, the management actions that have been or will be implemented in those locations, and the changes in water quality that occur in response to those actions.

In addition to developing the decision-support tool, the Department has also contracted with PBS&J and the University of South Florida to carry out detailed CSS and MST assessments in six impaired WBIDs in the Hillsborough River basin. MST identifies the presence of certain microorganisms (generally bacteria, although viruses are also used) that are restricted to the gastrointestinal tract of certain animals or humans. The detection of deoxyribonucleic acid (DNA) from these host-specific microorganisms identifies fecal contamination from particular sources. The specific genes DNA detected are frequently called "markers". The six WBIDs targeted for the Hillsborough River study MST were:

- Spartman Branch (WBID 1561);

- Baker Creek (WBID 1522C);

- Flint Creek (WBID 1522A);

- Blackwater Creek (WBID 1482);

- New River (WBID 1442); and

- Lower Hillsborough River (WBID 1443E).

The supplemental CSS and MST information was collected in these pilot WBIDs during 2007 and has been summarized by PBS&J.

The next step in this overall work effort is to integrate the results of the decision-support tool (PBS&J et al. 2008) and microbial source tracking projects. The present report initiates that process. Sections 1 through 3 describe ways in which the MWQA and CSS information generated by the two projects can be combined and evaluated. Section 4 uses the decision-support tool and the supplemental CSS and MST information to suggest a BMAP implementation strategy for the six WBIDs. It prioritizes the WBIDs, and the monitoring locations within them, for follow-up action based on the estimated likelihood of public health risk. It also recommends management actions that can be carried out at each location to address fecal coliform impairments.

The MST project has provided supplementary data on concentrations of indicator bacteria, more detailed CSS ratings, and new MST information on contamination sources that was not available when the decision-support tool was initially developed by (PBS&J et al. 2008). Appendix E of this report gives a brief summary of statistical relationships between these groups of variables in the MST data set, which may be helpful in the future.

Appendix E provides worked examples of the site classification methods used in the report, in a case-study format.

Section 10.2 Assigning Monitoring Stations to MWQA categories

Section 10.2.1 Using multi-year (2001 – 2007) EPCHC data

During the development of the decision-support tool, monitoring data provided by the EPCHC were used to assign MWQA categories to long-term monitoring stations based on the fecal coliform concentrations observed at each station during the years 2001 through 2007. The EPCHC monitoring data were collected on a monthly basis, providing relatively large sample sizes (82 to 84 samples per station) from the 2001-2007 period that were available for this purpose.

MWQA category assignments are based on the percentage of samples that exceed the State's 400 CFU/100 mL fecal coliform criterion, as summarized in Table 32. The MWQA categories range from A (≤ 10% of samples exceeding the standard) to E (>75% of samples exceeding the standard). MWQA categories assigned to the long-term EPCHC monitoring stations located in the pilot Hillsborough River WBIDs using this approach are shown in Table 32.

Two WBIDs (Spartman Branch and New River) contained no long-term EPCHC stations, and therefore received no MWQA designations (Table 33). In each of the remaining WBIDs, all stations were classified in MWQA categories A or B based on the 2001-2007 monitoring data (Table 33).

Table 32. Microbial water quality assessment (MWQA) categories, based on the percentage of samples exceeding the State's 400 CFU/100 mL fecal coliform criterion. "Break points" separating the MWQA categories are at exceedance frequencies of 10%, 30%, 50% and 75%. (Source: (PBS&J et al. 2008).

MWQA Category	Break Point (percentage of samples exceeding the 400 CFU/100 mL fecal coliform criterion)	Range of exceedance frequencies (percentage of samples exceeding the 400 CFU/100 mL fecal coliform criterion) included in category
A	≤ 10%	0% to 10%
B	> 10%	>10% to 30%
C	> 30%	>30% to 50%
D	> 50%	>50% to 75%
E	> 75%	>75% to 100%

Table 33. MWQA categories initially assigned to monitoring stations in the Hillsborough River WBIDs, based on monthly EPCHC monitoring data from the years 2001 through 2007.

Sub-basin	WBID No.	EPCHC Station Number	Sample Size (n)	MWQA Category Based on 2001 – 2007 EPCHC data
Spartman Branch	1561	--	--	--[1]
Baker Creek	1522C	107	84	B
Flint Creek	1522A	118	82	A
		135	82	A
		148	84	B
Blackwater Creek	1482	143	82	B
New River	1442	--	--	--[1]
Lower Hillsborough River	1443E	2	84	A
		152	84	B
		137	84	B

Note: 1. No long-term EPCHC monitoring stations present in WBID

Section 10.3 Using recent (2007) MST data

As noted earlier (Section 10.1), in cases where MWQA category assignments are calculated for use in the decision-support tool, it is recommended that they be based on a minimum of 30 fecal coliform measurements collected at regular (e.g., monthly or bi-monthly) intervals over a five-year period. This amount of data is needed to ensure that the MWQA characterization is based on a representative range of seasonal and multi-year variability that occurs at a site. The process of assigning MWQA categories to sites can also be helpful, however, for making shorter-term, between-site comparisons using smaller data sets. For that purpose, MWQA category assignments were calculated for a number of the stations that were monitored as part of the MST project during the months of May through December, 2007. Those results are shown in Table 34. Because of the much smaller sample sizes on which they are based, and their lack of long-term seasonal and year-to-year variability, the MWQA assignments shown in Table 34 are not expected to be comparable to those shown in Table 33. However, they are helpful for comparing

fecal coliform concentrations among the WBIDs and stations that were monitored during the eight-month sampling period addressed by the MST project. (When calculating these MWQA category assignments, stations that were sampled fewer than six times during the MST project were omitted from the analysis to avoid potential biases that could arise due to the smaller sample sizes.)

One point that is apparent from Table 34 is that fecal coliform concentrations showed substantial spatial variability within WBIDs during the MST sampling period. Within a single WBID there were often considerable between-station differences (e.g., differences of two or more "letter grades") in MWQA classifications (Table 34). These indicate large between-station differences in the frequency of exceedance of the State's 400 CFU/100 mL fecal coliform standard (see Table 32), and suggest that the fecal coliform sources present within several of the surveyed WBIDs had localized effects on concentrations of the indicator bacteria.

Another point that is apparent from Table 34 is that, for the individual monitoring stations for which both long-term EPCHC and short-term MST project data are available, the MWQA "letter grades" assigned using data from the MST monitoring period were often poorer (i.e., reflect a higher exceedance frequency of the 400 CFU/100 mL fecal coliform criterion) than the grades assigned using the EPCHC data. As noted earlier, given the differences that exist between the data sets on which they are based, it would not be appropriate to make detailed comparisons between the two sets of MWQA scores. The differences between the scores could potentially be influenced by a large number of factors, such as year-to-year fluctuations in fecal coliform concentrations that are reflected in one data set but not the other, variations in salinity (for stations in the Lower Hillsborough River; see Appendix E) and, for the 2007 data, chance effects due to the different dates on which samples were collected at the same stations by the long-term monitoring program and the MST project. One factor that does not appear to be involved is a long-term increasing trend in fecal coliform concentrations. Nonparametric trend tests (using Kendall's tau) indicate that annual geometric mean fecal coliform concentrations at the long-term EPCHC stations shown in Table 34 have exhibited either no trends or downward trends over various time periods during the past three decades (Table 35).

In addition to fecal coliforms, concentrations of two other bacterial indicators (*E. coli* and enterococci) were also measured as part of the MST project. Nonparametric correlation analyses (Kendall's tau) indicate that positive associations exist between the MWQA categories assigned to the MST monitoring sites (based on the frequency of exceedance of the State's 400 CFU/100 mL fecal coliform criterion) and the geometric means of the three bacterial indicators monitored there. Tau values and their significance levels for these associations were:

- MWQA category and geometric mean fecal coliform concentration, tau = 0.80, $p<0.0001$ (n=27 stations);

- MWQA category and geometric mean *E. coli* concentration, tau = 0.83, p<0.0001 (n=21 stations); and

- MWQA category and geometric mean enterococci concentration, tau = 0.59, p<0.001 (n=21 stations).

Similar associations between MWQA category assignments and geometric mean fecal coliform and enterococci concentrations were found in the long-term EPCHC data (PBS&J et al. 2008) . The results of the MST study support those earlier findings, and suggest that MWQA scores can also be used to predict geometric mean *E. coli* concentrations at monitoring stations within the Hillsborough River watershed.

Table 34. MWQA categories assigned to monitoring stations in the six Hillsborough River WBIDs, based on fecal coliform data collected as part of the MST project during the months of May through December, 2007. Long-term EPCHC monitoring stations located at the same sites as MST stations are indicated in parentheses. (Sources: EPCHC, (PBS&J et al. 2008))

Sub-Basin	WBID No.	MST Project (and EPCHC) Station Numbers	MST Project Sample Size (n)	MWQA Category based on MST Project Data	EPCHC Sample Size (n)	MWQA Category based on EPCHC Data
Baker Creek	1522C	BK1 (=EPCHC 107)	8	C	84	B
		BK2	8	D		
		BK3	6	C		
		DBK1	8	B		
New River	1442	NR1	6	C		
		NR2	7	C		
Spartman Branch	1561	DSB2	6	B		
		SB1	6	D		
		SB2	7	D		
		SB3	8	B		
Lower Hillsborough River	1443E	DHR2	8	B		
		DHR3	8	A		
		DHR4	8	B		
		DHR5	8	D		
		DHR6	6	C		
		HR1REF (=EPCHC 2)	8	B	84	A
		HR2	8	C		
		HR3	8	D		
		HR4 (=EPCHC 152)	8	C	84	B
		HR5	8	B		
Flint Creek	1522A	FL1 (=EPCHC 148)	8	C	84	B
		FL2	8	C		
		FL3 (=EPCHC 118)	8	A	82	A
Blackwater Creek	1482	BW1REF	8	A		
		BW2	8	D		
		DBW1 (=EPCHC 143)	6	A	82	B
		DBW4	7	A		

Table 35. Long-term trends (Kendall's tau and associated *p*-value) in annual geometric mean fecal coliform concentrations at selected EPCHC stations over various time periods in the previous three decades. Significant ($p<0.05$) trends shown in bold font. No significant increasing trends in fecal coliform concentrations are apparent at this group of monitoring stations over the time periods evaluated.

EPCHC Station No.	Time Period			
	2001 – 2007	1998 - 2007	1988 - 2007	1978 - 2007
2	- 0.05 (0.88)	0.20 (0.42)	- 0.12 (0.48)	**- 0.46 (<0.01)**
107	0.33 (0.29)	0.33 (0.17)	- 0.26 (0.10)	**- 0.40 (<0.01)**
118	0.14 (0.65)	- 0.20 (0.42)	**- 0.55 (<0.01)**	**- 0.56 (<0.01)**
143	- 0.33 (0.29)	- 0.11 (0.65)	**- 0.48 (<0.01)**	N/A (n=20 years)
148	0.05 (0.88)	0.11 (0.65)	**- 0.42 (0.01)**	N/A (n=20 years)
152	0.24 (0.45)	0.00 (1.0)	N/A (n=9 years)	N/A (n=9 years)

Section 10.4 Assigning CSS categories

Following the (WHO 2003) "Annapolis protocol" approach, information from contaminant source surveys can also be used to categorize microbial water quality conditions in the immediate vicinity of individual sampling locations. The (PBS&J et al. 2008) report recommended the use of the following CSS categories in the Hillsborough River watershed, based on the estimated likelihood that fecal contamination from the described sources would pose human health risks:

1. **Very Low**: No visual evidence of potential sources of human pathogens; natural environment; no or minimal anthropogenic land uses; wildlife present (any density)

2. **Low:** Low density agricultural and residential sources, including pets, livestock (without direct access to surface waters), or poultry operations; residences on septic systems

3. **Moderate**: Urban stormwater sources (including pet waste) present; well-functioning wastewater infrastructure (both sewer and septic); episodic/low volume sanitary sewer overflows (SSOs) reaching surface waters; moderate-density livestock with little direct access to surface waters; Class A residual and/or septage spreading areas may be present

4. **High**: Major stormwater outfalls present; history of failing wastewater infrastructure (central sewer or onsite systems); episodic or chronic/high volume SSOs reaching surface waters; concentrated livestock without direct access to surface waters; residual/septage spreading (Class B)

5. **Very High**: Current failing wastewater infrastructure; chronic/high volume SSOs reaching surface waters; concentrated livestock with direct access to surface waters; evidence of direct sewage inputs (e.g., confirmed illicit discharges)

During the development of the decision-support tool initial CSS categories were assigned to the EPCHC monitoring locations, and generically to the WBIDs that lacked long-term monitoring stations, based on a Phase 2 CSS investigation (PBS&J et al. 2008). This investigation included the evaluation of long-term data sets, GIS information, and observations from site visits made by the members of the project team and local partners during a "walk the WBID" exercise that was conducted during May 7-10, 2007. These initial ratings are shown in Table 36. The CSS category assignments were generally "low" for the more rural WBIDS, and "moderate" to "high" for the highly-urbanized Lower Hillsborough River WBID (Table 36), reflecting the land use characteristics and the types and densities of potential fecal contamination sources observed in the different areas during the site surveys.

Table 36. Initial (Phase 2) CSS categories assigned to monitoring stations in the six Hillsborough River WBIDs, based on site evaluations made during the "Walk the WBID" exercise in May 2007. (Source: (PBS&J et al. 2008)

Sub-basin	WBID No.	Location (EPCHC Station Number)	Initial (Phase 2) CSS Category (Estimated Likelihood of Fecal-Related Human Health Risk)
Lower Hillsborough River	1443E	152 137	4 (High) 3 (Moderate)
Blackwater Creek	1482	143	3 (Moderate)
New River	1442	--	3 (Moderate)
Spartman Branch	1561	--	3 (Moderate)
Baker Creek	1522C	107	2 (Low)
Flint Creek	1522A	135 118	2 (Low) 2 (Low)

More recently, as part of the MST project, a Phase 3 CSS investigation (PBS&J et al. 2008), including the use of appropriate MST tools, as necessary, to identify and characterize sources was conducted. Resulting CSS categories were assigned to each monitoring location used in that project. These CSS ratings, which are based on more detailed site assessments, are shown in Table 37. The ratings were updated to reflect information gained through MST analyses and field observations made during sampling events and site visits performed in collaboration with local stakeholders to better determine specific sources of contamination. Information and reports of specific suspicious activities that may impact bacterial levels in the six Hillsborough WBIDs, provided by local stakeholders throughout the project duration, were also used to determine appropriate CSS ratings.

A combination of fecal coliform measurements (updated MWQA categories; Section 10.2) and CSS information (based on detailed site assessments conducted as part of the MST project) was used to assign each monitoring location used in the MST project to a classification matrix outcome (PBS&J et al. 2008) that is designed to rank sites based on the apparent likelihood of human health risk. It should be noted that the MWQA scores for stations with MST data were calculated using limited sample sizes, and are not expected to be comparable to those shown in Table 33. Small sample sizes are anticipated in these cases, due to the exploratory nature of the stations that are used to identify sources of bacteria as well as the cost of performing MST analysis. The data are helpful, however, for comparing fecal coliform concentrations among the WBIDs and stations that were monitored during the eight-month sampling period addressed by the MST project.

Table 37. Updated (Phase 3) CSS categories assigned to monitoring stations in the six Hillsborough River WBIDs, based on more intensive contaminant source surveys conducted as part of the MST project. Within the table, WBIDs are arranged in order of decreasing mean CSS score. (Data source: PBS&J).

Sub-Basin	WBID No.	Location (MST Project Station Number)	Updated (Phase 3) CSS Category (Estimated Likelihood of Human Health Risk)
Lower Hillsborough River	1443E	HR1REF	3
		HR2	4
		HR3	4
		HR4	4
		HR5	3
		DHR4	3
		DHR4A	5
		DHR4B	4
		DHR5	4
		DHR6	3
		DHR7	3
		DHR8	3
		DHR9	3
		DHR10	4
			(Mean = 3.57)
Blackwater Creek	1482	BW1REF	2
		BW2	4
		BW3	4
		BW4	3
		BW5	3
		DBW4	3
		BW5A	3
			(Mean = 3.14)
Baker Creek	1522C	BK1	3
		BK2	3
		BK3	2
		BK4	4
		BK5	3
			(Mean = 3.00)
New River	1442	NR1	3
		NR2	3
		NR3	3
			(Mean = 3.00)
Flint Creek	1522A	FL1	2
		FL2	3
		FL3	2
			(Mean = 2.33)
Spartman Branch	1561	SB1	3
		SB2	2
		SB3	2
		DSB3	2
			(Mean = 2.25)

Section 10.5 Moving to BMAP Implementation: Identifying Priority Sites, Probable Sources, and Recommended Management Actions

Based on the detailed CSS and MST information provided by the MST project, and the prioritization strategies described in the decision-support tool (PBS&J et al. 2008), WBIDs and the individual monitoring locations within them can be prioritized for follow-up management actions to address fecal coliform impairments.

Epidemiological evidence indicates that, as a general rule, water contaminated by human fecal material poses a substantially higher risk to public health than water contaminated by fecal material from non-human organisms (WHO 2003, NRC 2004). Among non-human sources, livestock and household pets are thought to pose the next highest levels of risk. This is due to their periodic excretion of potential human pathogens (e.g., *Giardia*, *Cryptosporidium*, and several pathogenic bacteria), and the fact that bacterial pathogens excreted by these animals can exhibit resistance to antibiotics that are commonly used in human and veterinary medicine. Wildlife species can also act as sources of *Giardia*, *Cryptosporidium* and other human pathogens. However, wildlife populations are present at lower densities than livestock or pets in many agricultural, suburban and urban areas, and are not treated with the antibiotics that can encourage the development of antibiotic-resistant strains. Under most circumstances the presence of fecal material from wildlife sources in recreational waters is therefore thought to represent a lower risk to human health than the other source categories (WHO 2003, NRC 2004).

Several biological markers were used in the MST project to identify sources of fecal contamination, including:

- Human polyomavirus (HPyV), a nonpathogenic virus with a high carrier rate among humans;
- A genetic marker for a bacterial virulence factor (an enterococcal surface protein [*esp*] found in the bacterium *Enterococcus faecium*) which is specific to human sources; and
- Genetic markers for human-specific, ruminant-specific and horse-specific forms of *Bacteroidetes*, a family of anaerobic bacteria found in human and animal intestines (and which includes the well-known genus *Bacteroides*);

The presence of one or more of these markers in surface water samples provides evidence of contamination by human, horse, or ruminant (e.g., cattle, deer) fecal sources. Markers for additional livestock-related sources, such as hogs and poultry, were not included in the MST study. It appears that neither of these species is currently raised on a commercial scale in the Hillsborough River watershed. Chicken manure is applied to pasturelands as a fertilizer source and soil amendment in portions of the Blackwater

Creek WBID, however, and inclusion of a marker capable of identifying this source (if available) would be appropriate for future MST projects.

The percentage of sampling dates on which the human, horse, and ruminant-specific markers were detected can be used to estimate the relative frequency of fecal contamination from these sources at a given site. Because of the generally higher public health risk posed by human fecal material, monitoring locations exhibiting high frequencies of one or more of the human markers (HPyV, *esp*, or human-specific *Bacteroides*) should be given the highest priority for follow-up management actions to identify and address specific contaminant sources. Locations showing elevated frequencies of the ruminant-specific or horse-specific *Bacteroides* markers should be given the second highest priority. Because the ruminant-specific *Bacteroides* marker may be an indicator of a wildlife-based (e.g., deer) source, additional information will be needed to confirm the types of sources present at locations showing high frequencies of this marker. At locations where the CSS provides no evidence of human or livestock-based sources of fecal contamination, wildlife or other environmental factors are assumed to be the primary sources of any indicator bacteria or ruminant-specific MST markers observed in monitoring data. These locations can be given the lowest priority for follow-up management action, and it is assumed that under most circumstances no actions will be taken to address wildlife sources affecting Class III waters.

A prioritized list of WBIDs, and of MST monitoring locations within WBIDs, which was produced using this approach is shown in Table 38. Within a WBID, individual monitoring sites were ranked from higher to lower priority based on classification matrix outcomes (PBS&J et al. 2008) (determined using updated, Phase 3, CSS category assignments and MWQA scores) and the percentage of samples showing evidence of the presence of the human-specific or ruminant-specific markers. (Confirmed detections of the horse-specific *Bacteroides* marker were uncommon in this study, occurring only once each in samples from the Spartman Branch and Blackwater Creek WBIDs, and were therefore not used in the prioritization process.) Summaries of the most probable sources of fecal contamination and recommended management actions for each site are provided in Table 39.

At the WBID scale, the Lower Hillsborough River (WBID 1443E) appears to be the highest priority for follow-up management action. This section of the river receives a great deal of recreational use, had a large number of sites falling in CSS category 4 and generated the largest mean CSS rating in the updated surveys (Table 37), exhibited high frequencies of human MST markers at several monitoring sites (Table 38), and appears to receive inputs from a number of sources of human fecal contamination (Table 39a). Sanitary sewer infrastructure, malfunctioning septic systems, homeless residents and stormwater sources appear to be the highest priorities for management action in this WBID (Table 39a).

The Blackwater Creek (WBID 1482) and Baker Creek (WBID 1522C) WBIDs form a second priority group, with a preponderance of MST monitoring sites falling in CSS categories 3 and 4 (Table 37) and high frequencies of human and ruminant fecal markers

detected at several locations (Table 38). The Hillsborough River Reservoir, a Class I waterbody from which public supply withdrawals are made by both the City of Tampa and Tampa Bay Water, is located downstream of both WBIDs. In addition, Baker Creek also discharges to Lake Thonotosassa, a highly-eutrophic Class III waterbody that is on the State's SWIM priority list due to poor water quality (FDEP 2005). Primary sources of MST markers in the Blackwater Creek WBID appear to be septic systems, livestock, and discharges of treated effluent from either publicly or privately owned wastewater treatment plants, while malfunctioning septic systems and livestock appear to be the primary sources of markers detected at sites in the Baker Creek WBID (Table 39b).

The Flint Creek (WBID 1522A), Spartman Branch (WBID 1561) and New River (WBID 1442) WBIDs form a lower priority group, with most MST monitoring sites classified in CSS group 2 (Table 37) but with elevated frequencies of human and ruminant fecal markers sporadically detected at some locations (Table 38). All three discharge to the Class I Hillsborough River Reservoir. Sources in the Flint Creek WBID have not yet been clearly defined but appear to include a mixture of human, agricultural, and wildlife contributions (Table 39c). Primary sources in the New River WBID appear to be septic systems, livestock and wildlife. A mixture of human and wildlife sources are suspected in the Spartman Branch WBID.

Regardless of the priority group in which a WBID falls, it is recommended that significant human and livestock fecal sources identified at any of the MST monitoring locations should be addressed as a means of reducing pollutant loads to downstream receiving waters such as the Hillsborough River Reservoir.

Table 38. Proposed priority rankings (from higher to lower priority) of WBIDs and MST sampling locations for further management action, based on updated classification matrix outcomes (using updated CSS and MWQA categories), and frequencies of MST marker detection. Outcomes can be color-coded, ranging from green (least potential for human health risk) to purple (greatest potential for human health risk); stations in grey represent those areas where the CSS and MWQA scores provide different assessments and require further evaluation (PBS&J et al. 2008). Only stations with MST data are included. (Data source: PBS&J.)

					Human Fecal Markers		Ruminant Fecal Marker	
Sub-Basin	WBID	Monitoring Location	Updated Classi-fication Matrix Outcome	Sample Size (n) for Updated MWQA Score	No. Sampling Dates	% Dates Marker(s) Detected	No. Sampling Dates	% Dates Marker Detected
Lower Hillsborough River	1443E	**DHR4A**	D5	5	5	100 %	5	20 %
		HR3	D4	8	5	80 %	5	20 %
		DHR5	D4	8	2	50 %	2	0 %
		DHR8	D3	5	4	50 %	4	0 %
		HR2	C4	8	3	67 %	3	0 %
		HR4	C4	8	3	67 %	3	0 %
		DHR7	C3	5	5	60 %	5	60 %
		DHR6	C3	6	3	33 %	3	0 %
		DHR9	C3	3	2	0 %	1	0 %
		HR1 REF	B3	8	3	100 %	8	0 %
		HR5	B3	8	5	60 %	5	0 %
		DHR10	A4	2	1	100 %	1	0 %
		DHR4B	A4	1	1	0 %	1	0 %
Blackwater Creek	1482	**BW3**	D4	4	4	75 %	1	0 %
		BW2	D4	8	3	67 %	3	100 %
		BW4	C3	2	1	0 %	1	0 %
		DBW4	A3	7	1	100 %	1	0 %
		BW5A	A3	2	2	50 %	2	0 %
		BW5	A3	2	1	0 %	1	0 %
		BW1 REF	A2	8	7	43 %	6	17 %
Baker Creek	1522C	**BK2**	D3	8	6	67 %	6	67 %
		BK1	C3	8	7	57 %	7	29 %
		BK3	C2	6	2	50 %	2	0 %
		BK4	A4	1	1	100 %	1	0 %
		BK5	A3	2	2	100 %	2	0 %
Flint Creek	1522A	**FL2**	C2	8	6	50 %	6	0 %
		FL1	C2	8	5	20 %	5	0 %
		FL3	A3	8	6	33 %	6	0 %
Spartman Branch	1561	**SB1**	D3	6	6	17 %	6	0 %
		SB2	D2	7	5	20 %	6	0 %
		DSB3	C2	5	2	0 %	2	50 %
		SB3	B2	8	4	25 %	4	0 %
New River	1442	**NR2**	C3	7	7	29 %	7	29 %
		NR1	C3	6	2	0 %	2	50 %
		NR3	C3	4	2	0 %	2	50 %

Table 39a. Priority ranking, most probable source categories, and recommended management actions for Lower Hillsborough River (WBID 1443E) MST sampling locations. (Data source: PBS&J)

Sub-Basin	WBID	Monitoring Location	Classification Matrix Outcome	Fecal Source(s) of Concern	% of Sampling Dates Markers Detected	Most Probable Source Categories and Recommended Management Actions
Lower Hillsborough River	1443E	DHR4A	D5	Human	100 %	Septic and possibly sanitary sewer; confirm and address specific source locations and provide public outreach explaining potential presence of health risk at site
		HR3	D4	Human	80 %	Septic and sanitary sewer; confirm and address specific source locations and provide public outreach explaining potential presence of health risk at site
		DHR8	D4	Human	50 %	Multiple sources suspected; identify and address specific source locations; provide public outreach explaining potential presence of health risk at site
		DHR5	D3	Human	50 %	Multiple sources suspected; identify and address specific source locations; provide public outreach explaining potential presence of health risk at site
		HR2	C4	Human	67 %	Sanitary sewer and stormwater; identify and address specific source location(s)
		HR4	C4	Human	67 %	Sanitary sewer and stormwater; identify and address specific source location(s)
		DHR7	C3	Human	60 %	Multiple sources suspected; identify and address specific source locations
		DHR6	C3	Human	33 %	Unknown; identify and address specific source location(s)
		DHR 9	C3	Human	0 %	Unknown; identify specific source location(s)
		HR1 REF	B3	Human	100 %	Homeless camp, sanitary sewer and possibly stormwater; confirm and address specific source locations
		HR5	B3	Human	60 %	Sanitary sewer; confirm and address specific source locations
		DHR10	A4	Human	100 %	Multiple sources suspected; identify and address specific source locations
		DHR4B	A4	Human	0 %	Unknown; identify specific source location(s)

Table 39b. Priority ranking, most probable source categories, and recommended management actions for Blackwater Creek (WBID 1482) and Baker Creek (WBID 1522C) MST sampling locations. (Data source: PBS&J)

Sub-Basin	WBID	Monitoring Location	Classification Matrix Outcome	Fecal Source(s) of Concern	% of Sampling Dates Markers Detected	Most Probable Source Categories and Recommended Management Actions
Blackwater Creek	1482	BW3	D4	Human	75 %	Public or privately owned wastewater facilities; confirm and address specific source locations; provide public outreach explaining potential presence of health risk at site
		BW2	D4	Human Ruminant	67 % 100%	Unknown human source(s); confirm and address specific source location(s); provide public outreach explaining potential presence of health risk at site Cattle; install fencing around watercourse
		BW4	C3	Human	0 %	Unknown; identify specific source location(s)
		DBW4	A3	Human	100 %	Sanitary sewer; ensure that upstream WWTP (City of Plant City) maintains adequate disinfection of treated effluent
		BW5A	A3	Human	50 %	Septic; confirm and address specific source locations
		BW5	A3	Unknown	0 %	Unknown; identify specific source location(s)
		BW1 REF	A2	Human	43 %	Septic; confirm and address specific source locations
Baker Creek	1522C	BK2	D3	Human Ruminant	67 % 67 %	Septic; confirm and address specific source locations; provide public outreach explaining potential presence of health risk at site Cattle and/or deer; install fencing around watercourse if cattle are confirmed
		BK1	C3	Human Ruminant	57 % 29 %	Septic; confirm and address specific source locations Cattle; install fencing around watercourse if confirmed
		BK3	C2	Human	50 %	Septic; confirm and address specific source locations
		BK4	A4	Human	100 %	Septic; confirm and address specific source locations
		BK5	A3	Human	100 %	Septic; confirm and address specific source locations

Table 39c. Priority ranking, most probable source categories, and recommended management actions for Flint Creek (WBID 1522A), Spartman Branch (WBID 1561) and New River (WBID 1442) MST sampling locations. (Data source: PBS&J)

Sub-Basin	WBID	Monitoring Location	Classification Matrix Outcome	Fecal Source(s) of Concern	% of Sampling Dates Markers Detected	Most Probable Source Categories and Recommended Management Actions
Flint Creek	1522A	FL2	C2	Human	50 %	Likely septic; identify and address specific source location(s)
		FL1	C2	Human	20 %	Possibly upstream septic and livestock sources; identify and address specific source location(s)
		FL3	A3	Human	33 %	Not clearly defined; identify and address specific source location(s)
		SB1	D3	Human	17 %	Unknown; identify and address specific source location(s); provide public outreach explaining potential presence of health risk at site
Spartman Branch	1561	SB2	D2	Human	20 %	Septic; confirm and address specific source locations; provide public outreach explaining potential presence of health risk at site
		DSB3	C2	Ruminant	50 %	Wildlife sources suspected; no management actions suggested
		SB3	B2	Human	25 %	Unknown; identify and address specific source location(s)
		NR2	C3	Human Ruminant	29% 29%	Septic; confirm and address specific source locations Cattle; install fencing around watercourse upstream if confirmed
New River	1442	NR1	C3	Ruminant	50%	Cattle and/or wildlife; install fencing around watercourse where cattle present
		NR3	C3	Ruminant	50%	Cattle; install fencing around watercourse upstream if confirmed

Section 11.0 Suggested Corrective Actions

The evaluation of available data discussed above indicates that the dominant sources of fecal coliform contamination in the Hillsborough River tributaries are basin dependent and include contributions from: 1) humans (e.g., OSTDS, existing utility infrastructure, and stormwater as a conveyance system for non-point sources); 2) livestock; and 3) wildlife.

Section 11.1 Remediation of Onsite Treatment and Disposal Systems

Properly designed, functioning, and maintained septic systems can effectively remove nearly all suspended solids, bio-degradable organic compounds, and fecal coliforms (EPA 2002); however, old and poorly maintained septic systems are a major source of fecal coliform contamination. Few programs exist to address onsite system operation and maintenance which result in failures leading to otherwise preventable costs and risks to public health and water resources (EPA 2002).

In some situations, OSTDS provide a better health protection alternative than the public sewer system as, by their nature, they include the provision for passive wastewater treatment and are relatively cost-effective. However, since even one failing OSTDS may be a large and chronic source of bacteria contamination, the situation conveys a need to have consistent city-wide measures to educate property owners to properly maintain their systems, to repair failing systems in a timely manner and/or connect to sanitary sewer for proper treatment, to educate professionals in the business of installing and repairing OSTDS, and to carefully monitor and inspect properties when there are complaints.

If not already being done, it may be beneficial to implement the following suggestions in those areas most likely impacted by fecal contamination originating from OSTDS. These areas primarily include the older residential neighborhoods and mobile home parks near: 1) station NR2 in the New River; 2) stations BK2, BK3, BK4, and BK5 in Baker Creek; 3) station FL2 in Flint Creek; 4) stations BW3, BW4, DBW7, and BW5A in Blackwater Creek; 5) station SB2 in Spartman Branch; and possibly 6) stations DHR7, DHR8, DHR9, DHR4A, and HR3 in the Lower Hillsborough River.

As these systems are privately owned, one of the most important aspects to increasing their effectiveness is public education and outreach. It is suggested that the HCHD and other local groups [(e.g., the Florida Onsite Wastewater Association (FOWA)] continue to focus a great deal of effort towards educating the public on various levels ranging from the legislators who vote on State and City statutes to local plumbers and environmental health professionals. One suggestion is to increase the direction of these outreach efforts to homeowners in communities with a high incidence or likelihood of OSTDS failures (e.g., homeowners in close proximity to surface waters) and educate the owners on proper preventative maintenance practices.

In areas where traditional OSTDS systems are not a viable option due to environmental conditions, new performance-based systems may provide a practical and cost-effective alternative. According to Scott Turner, Duval County Health Department (personal communication, March 5, 2007), newer performance-based systems are now available to meet advanced secondary treatment levels. These systems are permitted in areas with decreased setbacks and smaller drainfields. As a result, failure of these systems can be more problematic and are therefore subject to annual inspections. It is suggested that owners of these systems be subject to a mandatory educational course instructing them in the proper maintenance of their systems.

In areas where isolated and problematic septic systems are contaminating the neighboring surface waters, the following alternative programs may be beneficial:

- a Department of Health-sponsored certification program for haulers responsible for the proper pumping, transportation, disposal and documentation of OSTDS maintenance procedures;
- a program in which septic tanks must be inspected when home ownership changes or in regular time intervals, such as five years;
- a community-based onsite management system program in which clusters of conventional septic tanks are drained into centralized advanced wastewater treatment areas and are managed by a designated responsible party (e.g., local utility or health department) (NESC 2006); and
- a subsidized septic tank-pumping program that could be used in conjunction with septic-tank phase out programs (transition to centralized sewer) to help immediately alleviate impacts during the transition period.

Section 11.2 Rehabilitation of Existing Utility Infrastructure

Severe utility infrastructure collapses are rare; however, as the infrastructure ages, failures become more likely. Continued inspection and rehabilitation of existing utility infrastructure, including proper preventative and predictive maintenance and the identification of problem areas, is important in order to help reduce or eliminate wastewater conveyance system leaks and SSOs. These measures should be implemented by all area utility providers. Oftentimes, as is the case in Hillsborough County and the City of Tampa, a single utility is not responsible for all sewer infrastructure throughout the area. Private sewer infrastructure (e.g., lift stations) for instance, may be a potential source of fecal coliform contamination to area tributaries; however, currently there is no comprehensive geographic information available to identify these private systems. In addition, service laterals that are part of every centralized sewer connection (e.g., for residences, hospitals, institutions) have a private component. It is suggested that in order to increase the accountability of these facilities, explicit testing and maintenance requirements be employed and a program be implemented to arrange for inspections and repairs to be made by the same entity, with all reports sent to the City.

The majority of households within the Lower Hillsborough River use centralized sewer and the abundance of associated infrastructure (e.g., lift stations, manholes, gravity mains, force mains) may fail on occasion resulting in varying levels of environmental

impacts. Those areas that would most likely benefit from the following suggestions, if not already being implemented, are contained within this basin. The specific stations include: DHR5, DHR7, DHR8, and DHR9. Those corrective actions pertaining to the prevention of SSOs are recommended for the infrastructure impacting stations HR5, HR4, DHR3, HR2, DHR2, and HR1Ref.

Predictive and preventative maintenance programs are crucial to the reduction of environmental impacts associated with leaking sewer infrastructure or more severe SSOs. The identification of particular areas that have been determined to be problematic and/or have a known root cause could therefore be instrumental in revealing the primary sources of contamination in the Lower Hillsborough River watershed. For example, it is recommended that the COT conduct regular inspections of sanitary lift stations for which it issues construction permits. Additionally, it should be mandatory that sanitary lift stations have certified operator attendance at least once a month and that all lift stations located in close proximity to surface waters have back-up generators to prevent spills during power failures.

Since large-scale replacements of existing systems have significant capital costs, it is necessary to routinely conduct investigations including but not limited to infiltration and inflow studies to evaluate the integrity of the infrastructure and use this type of assessment to locate severe problem areas with a high probability of impacts related to the utility. Examples of investigations and maintenance procedures include regularly using remote camera equipment to inspect the lines as well as the cleaning of lines to ensure proper function. Local utilities should use "mean time to failure" estimates to replace pumps at lift stations prior to failing as well as warning systems for failure of utilities in order to help minimize impacts when failures occur. Additional preventative programs may include a Fats, Oils, and Grease (FOG) Reduction Program, a SSO Root Cause Program, and a Pop-Top Program whereby all manholes are inspected.

Section 11.3 Stormwater Treatment and Non-point Sources

"Non-point sources" are used to describe intermittent, rainfall driven, diffuse sources of pollution (e.g., stormwater runoff) associated with everyday human activities, including runoff from urban land uses, agriculture, and silviculture; discharges from failing septic systems; and atmospheric deposition. Additional non-point sources may include areas with concentrated wildlife (e.g., bird rookeries) or domestic animals (e.g., dog parks).

Typical fecal coliform bacteria concentrations for untreated stormwater runoff are within a range of 10,000 to 100,000 MPN/100mL (Metcalf & Eddy 2003). As a result, it is important to consider stormwater as a potential and highly probable conveyance system for a variety of more specific sources of bacterial contamination. Although the reduction and elimination of bacteria sources entering the stormwater system is critical, stormwater must also be considered as a source unto itself in order to manage it more effectively.

Management actions to provide additional stormwater treatment will benefit a tributary by reducing the sediment, oil and grease, and nutrient and bacteria loadings discharged

into the affected waterbody. When focusing efforts on stormwater treatment, it is important to realize that the reduction of sediment loads to a tributary is critical because such particles frequently adsorb bacterial colonies thereby facilitating their survival, growth, and transportation from one area to another (Gerba & McLeod 1976, LaLiberte & Grimes 1982, Marino & Gannon 1991, Davies et al. 1995).

The addition of programs to address stormwater treatment, if not already being implemented, are most likely to benefit the following project areas: 1) highly urbanized landscapes such as those in the Lower Hillsborough River (e.g., stations HR2 and HR4); 2) neighborhoods with a high propensity for illicit discharges or where illicit discharges have been identified such as within Spartman Branch (e.g., station SB1) and the Lower Hillsborough River (e.g., upstream of station DHR6); 3) rural areas associated with livestock and agricultural practices in the New River (e.g., station NR1), Baker Creek (e.g., downstream of station DBK1 and between stations DBK2 and BK2), Spartman Branch (e.g., station SB1), and Blackwater Creek (e.g., station BW2) basins; and 4) more natural settings supportive of wildlife populations in the New River (e.g., station NR1), Flint Creek (e.g., stations FL1 and FL3), and Spartman Branch (e.g., stations SB3, DSB3, SB2, and DSB2) watersheds.

Section 11.3.1 Stormwater BMPs for Highly Urbanized Landscapes

Four BMPs have been identified as possible stormwater treatment and/or retrofit alternatives for the urbanized area within the Lower Hillsborough River basin: 1) filtration/dry detention with filtration; 2) infiltration/dry retention; 3) bioretention; and 4) baffle boxes. The basic structures, mechanisms, and treatment efficiencies of these four BMPs are provided below.

Filtration/Dry Detention with Filtration

Stormwater filtration BMPs (also known as dry detention with filtration) capture stormwater runoff, temporarily store the water in a small settling basin, and then allow the water to filter through an engineered media, most commonly a natural sand filter. The filtered water is then collected in an underdrain and transported back to the storm drain system. Because of the short detention times in this type of structure, biological processes such as nutrient uptake do not play a role in pollutant removal. Alternatively, physical processes such as sedimentation, and chemical processes, such as precipitation and absorption, are the major mechanisms of pollutant removal.

Filtration systems are useful BMPs in urban retrofit projects because they do not require large areas and can be easily constructed into road right-of-ways and medians, parking lot perimeters and medians, within recreational facilities, as part of commercial landscaping, and within existing shallow swales. In addition, these methods are practical in areas where open space is not available because the filter system can be located underground.

Filtration systems are not designed to serve as flood storage. As a result, treatment processes requiring longer exposure times, such as biological uptake necessary to remove

dissolved constituents, are extremely limited in this type of BMP. Furthermore, although this BMP can be constructed in areas with high water tables, the removal efficiencies will be reduced. This is because the primary mechanisms of pollutant removal used with this method are physical and chemical processes resulting from the infiltration of the water through the permeable soil layer before entering the drain system below. As a result, a deeper pipe system will generate greater pollutant removal efficiencies from the stormwater before it enters the drain.

Generally, filtration provides good removal of most pollutants with the exception of soluble nutrients and they are best suited to remove total suspended solids and oil and grease from stormwater flows (EPA 2007c). Ranges of average pollutant removal efficiencies for filtration/dry detention with filtration BMPs are given in Table 40. It should also be noted that this system should not be utilized where large amounts of trash or debris can cause the system to become clogged.

Table 40. Ranges of Pollutant Removal Rates for Filtration/Dry Detention with Filtration BMPs (EPA 2007c).

Pollutant	Pollutant Removal Efficiency (25th Percentile - 75th Percentile) (%)
Total Suspended Solids	80 - 90
Total Phosphorus	40 - 65
Soluble Phosphorus	(-10) - 65
Total Nitrogen	30 - 50
Organic Carbon	40 - 70
Total Zinc	70 - 90
Total Copper	35 - 70
Bacteria	25 - 70
Hydrocarbons	80 - 95
Chloride	0 - 0
Trash/Debris	85 - 95

Infiltration/Dry Retention

Infiltration BMPs (also known as dry retention) capture and store stormwater runoff in a dry basin before allowing the water to infiltrate into underlying soils and eventually return to the local aquifer. The stormwater runoff, along with the pollutants of concern, are then trapped in the dry retention area and underlying soils and not allowed to be transported into the storm drain system. In this type of system, biological, physical, and chemical processes all contribute to pollutant removal. Sedimentation and limited evaporation occur in the standing water immediately following a storm event. Sides and bottoms of all infiltration systems must be fully vegetated over sandy soils. The vegetation serves to stabilize the soils and maintain permeability in the underlying bed while also removing pollutants through nutrient uptake. The sandy bed then works to

remove pollutants through both physical and chemical processes (Harper & Baker 2007). The bottom of the infiltration basin can often be covered in loose stones to provide storage for the stormwater before it enters the soils. In this case, the stormwater runoff will first pass through some type of pretreatment, such as a swale or sediment basin, before passing into the infiltration chamber to prevent the loose stone layer from becoming clogged (EPA 2007c).

Infiltration systems are useful BMPs in urban retrofit projects because they do not require large areas and can be easily constructed into road right of ways and medians, parking lot perimeters and medians, within recreational facilities, as part of commercial or residential landscaping, and within existing shallow swales. Because all flow is retained within the infiltration basin, pollutants and nutrients in the stormwater flow are removed from the storm drain system while also reducing runoff volumes. Another advantage to this type of BMP is groundwater recharge. One disadvantage to this method is that infiltration systems can only be constructed in areas with low groundwater tables and permeable soils. In addition, because all stormwater is retained in the infiltration basin, the runoff volumes treated are limited by the available storage in the treatment area (EPA 2007c).

In theory, because all water is returned to the native soils and groundwater, all pollutant loads should be removed from the storm drain system as a result of infiltration. Infiltration systems are best suited to remove total suspended solids, metals, possibly bacteria, organic carbon, and oil and grease from stormwater flows. Pollutant removal data for infiltration systems are limited, so the ranges of average pollutant removal efficiencies for bacteria, hydrocarbons, chloride and trash/debris in Table 41 are largely based on the assumption that pollutant removal efficiencies will be similar to those in filtration/dry detention with filtration studies (EPA 2007c). Also, similar to the filtration/dry retention with filtration BMP, infiltration/dry retention is not suitable where there are large amounts of trash and debris as they can cause the system to become clogged.

Table 41. Ranges of Pollutant Removal Rates for Infiltration/Dry Retention BMPs (EPA 2007c).

Pollutant	Pollutant Removal Efficiency (25th Percentile - 75th Percentile) (%)
Total Suspended Solids	60 - 95
Total Phosphorus	50 - 95
Soluble Phosphorus	55 - 95
Total Nitrogen	0 - 65
Organic Carbon	80 - 95
Total Zinc	65 - 85
Total Copper	60 - 90
Bacteria	25 - 70
Hydrocarbons	60 - 95
Chloride	0 - 0
Trash/Debris	85 - 95

Bioretention

Bioretention is a landscape feature that serves as a stormwater BMP and typically treats watersheds less than 1 acre in area. The bioretention cell is a shallow landscaped depression that treats stormwater in much the same way as a forested area. The cell contains trees and shrubs planted in a mulch layer above an 18- to 48-inch deep sand/soil bed. Stormwater is routed to the bioretention cell where it ponds temporarily and then filters through the mulch layer and into the sand/soil bed below. The bioretention cell generally stores approximately 6-9 inches of standing water during a storm event. The trees and vegetation in the landscape feature clean the standing water through biological uptake of nutrients by plant surfaces and roots. Sedimentation and limited evaporation occur in the standing water immediately following a storm event. Sides and bottoms of all infiltration systems must be fully vegetated over sandy soils. The sandy bed then works to remove pollutants through both physical and chemical processes. The water filters through this bed and into either a perforated drain to carry the stormwater downstream into the drainage system, or else the stormwater drains directly into the native soils and continues to infiltrate into the local aquifer (EPA 2007c). Bioretention is a landscaped and aesthetic use of infiltration and/or filtration stormwater BMPs.

Bioretention systems are useful BMPs in urban retrofit projects because they do not require large areas and can be easily constructed into road right-of-ways and medians, parking lot perimeters and medians, within recreational facilities, as part of commercial landscaping, and within existing shallow swales. They are aesthetic and are often easily blended into a residential or commercial area. When a bioretention cell is built to infiltrate all water into the native soils, it provides substantial water quality treatment. Because all flow is retained within the bioretention cell, all pollutants and nutrients in the stormwater flow are removed from the storm drain system while also reducing runoff volumes. In this case, another advantage of bioretention is groundwater recharge. When a

bioretention cell is built to filter the stormwater into the storm drain system below, care must be taken to ensure that the groundwater table is low and the soils are permeable to provide the maximum removal of pollutants from the stormwater before it enters the storm drain system. Similar to infiltration systems, one disadvantage is that because all stormwater is retained in the bioretention cell, the runoff volumes treated are limited by the available storage in the treatment area. However, this BMP has more storage and will provide more flood attenuation than a standard underground filtration system.

Bioretention systems are best suited to remove metals, bacteria, and oil and grease from stormwater flows. Bacteria removal data for bioretention systems are limited, so the ranges of average pollutant removal efficiencies in Table 42 are largely based on the assumption that they will be similar to those in filtration/dry detention with filtration studies (EPA 2007c).

Table 42. Ranges of Pollutant Removal Rates for Bioretention BMPs (EPA 2007c).

Pollutant	**Pollutant Removal Efficiency (25th Percentile - 75th Percentile) (%)**
Total Suspended Solids	15 - 75
Total Phosphorus	(-75) - 30
Soluble Phosphorus	(-10) - 50
Total Nitrogen	40 - 55
Organic Carbon	40 - 70
Total Zinc	40 - 95
Total Copper	40 - 95
Bacteria	25 - 70
Hydrocarbons	80 - 95
Chloride	0 - 0
Trash/Debris	80 - 95

Baffle Box

Baffle boxes use the physical process of settling to remove trash, sediment, suspended particles and associated pollutants from stormwater runoff. The BMP is comprised of ,a concrete or fiberglass structure that contains several settling basins separated by baffles. An initial trash screen can be installed to prevent large debris, floatables or trash from passing into the assembly with the inflow of stormwater. Once flow enters the box, sediment is removed from the runoff as the water enters each settling chamber and is impeded by the baffle walls. As the settling chamber begins to fill, flow velocity is slowed and allows the sediment to settle out of the water. This continues in series removing the majority of the sediment from the flow (EPA 2001).

Baffle boxes are generally placed in-line or at the outfall of a storm drain system. They are particularly well suited for urban retrofit projects because of the ease and low cost of

installing the boxes directly into existing storm drain systems. While installation cost can be very low if pre-cast boxes are used, maintenance costs can be rather large. The accumulated sediment must be removed from the baffle boxes on a regular basis to prevent the sediment basins from filling. If sediment is allowed to accumulate in the basins, re-suspension into the stormwater flow may occur during subsequent storms. The frequency of cleaning depends on the landuse, rainfall, and accumulation of sediment. Another disadvantage to baffle boxes is they are not designed for nutrient removal, so this would not be a suitable BMP if nutrients are the main concern in the area. However, nutrients, similar to bacteria, attached to suspended sediment will be removed from the flow as the sediment settles out (EPA 2001).

Baffle Boxes are best suited for the removal of sediments from stormwater. Average pollutant removal efficiencies for baffle box BMPs are given in Table 43; removal efficiencies for bacteria are unknown.

Table 43. Average Pollutant Removal Efficiencies for Baffle Box BMPs (ASCE 2001).

Pollutant	Pollutant Removal Efficiency (%)
Total Suspended Solids	80
Total Phosphorus	30
Total Nitrogen	0

Section 11.3.2 Illicit Discharges

Contamination of the stormwater drainage system and receiving waters by illegal and/or improper discharges occurs in a variety of ways. Such discharges may include, but are not limited to: sanitary sewer flow; industrial process water; chlorinated pool water; and laundry releases. Sanitary sewer flow, for example, may result from improper connections to sanitary sewage pipes or the direct connection of septic systems to stormwater conveyance networks, thus short-circuiting treatment provided by the drainfield.

It is suggested that programs dedicated to the mapping, detection and elimination of illicit discharges be recognized as mandatory components of stormwater management plans. In addition, educational outreach should be utilized to inform the public of the associated impacts on water quality. Outreach can be implemented through the distribution of educational pamphlets and informational door hangers and through a storm drain-stenciling program. Volunteers can also be trained to perform a variety of tasks to improve the environmental quality of their local watersheds. These tasks can include: water quality monitoring; surveying streams for pollution sources; and the distribution of public outreach materials.

Section 11.3.3 BMPs for Livestock and Agricultural Practices

The reduction or prevention of pollutant discharges associated with livestock and agricultural practices can include a combination of both structural and nonstructural practices. The following examples of BMPs are recommended by the Florida Department of Agricultural and Consumer Services (DACS) for beef cow/calf operations in Florida and are described in the *draft* manual for Water Quality Best Management Practices for Florida Cow/Calf Operations (DACS 2008).

- Maintenance of adequate vegetation cover;
- Develop alternative water sources to attract animals away from streams, drainage canals, and lakes as much as possible;
- When feasible, re-establish natural flow patterns, plug drainage canals and restore water through internal marshes, cypress ponds, or other natural wetlands that can assimilate nutrients and filter bacteria;
- Use practices such as grassed waterways, filter strips, diversions, sediment traps, swales, and retention and detention ponds;
- When cleaning ditches, use turbidity screens in the water at discharge points so turbid water does not leave your property; and
- Properly train and re-train employees annually and when changes are made.

Section 11.3.4. Wildlife and Natural Sources

Lastly, since natural (i.e., wildlife-related) impairments are considered relatively low risk to human health and may be difficult or unreasonable to restore, it is suggested that signs alerting the public to the potential for health risk be posted at the necessary locations.

All of the above, alone or in combination, can be effective in reducing coliform loadings to surface waters; however, each must be evaluated based on the area in question, the cost required, and the level of public acceptance. It is recommended that analysis be conducted on a life-cycle cost basis and factors such as capital costs, operation and maintenance costs and regulatory issues be considered in order to determine the most feasible approach.

Section 12.0 Lessons Learned

Several important lessons were learned during this project regarding both the practical application and interpretation of the developed methodology, as well as the logistical requirements of a successful study. There are four main categories in which these lessons could be placed: 1) collaboration with local stakeholders; 2) accurate analysis and interpretation of results; 3) utility of the various assessment techniques; and 4) utilization of the decision-support tool to prioritize and guide management actions.

Section 12.1 Collaboration with Local Stakeholders

One of the greatest achievements of this investigation was the extremely high level of cooperation and collaboration with local stakeholders. The approach used in this project fostered collaboration throughout all levels of the project: 1) collecting background information; 2) performing one-on-one intensive interviews with key local entities; 3) conducting a project workshop ("Maps on the Table"); 4) validating the conclusions from Phase I through a field reconnaissance ("Walk the WBID") exercise; 5) facilitating and ongoing investigations with individual entities to help hone in on specific sources in identified "hot spots"; and 6) documenting all of the findings of the detailed explorations conducted in each of the basins.

Local stakeholder participation and cooperation helped this project to operate on schedule and under budget.

Section 12.2 Accurate Analysis and Interpretation of Results

As demand for MST services grows, there is a recognized necessity for: 1) utilizing additional laboratories to effectively expand the analytical capacity within Florida for these projects; and 2) validating the performance of selected assays in order to reduce the need for application of multiple analytical methods for a given fecal source within a study. This need arises from incomplete knowledge about the performance of the individual methods in the many complex environmental scenarios that are encountered in most studies of water quality.

Microbial source tracking is the term applied to a group of methods that seek to identify the source(s) of fecal contamination in surface waters. These methods are currently used or are proposed for use in a variety of scenarios, including aiding in the assessment of human health risk at beaches and as a means to identify point source and non-point source contamination of surface waters for TMDL plans. The probable usefulness of these methods for assessing and potentially improving surface water quality has resulted in an increased demand for such services in Florida. PBS&J's MST experience in conjunction with Dr. Harwood's laboratory at the USF in tributaries of the Lower St. Johns and Hillsborough Rivers has demonstrated the need to build on existing experiences in order to further validate MST methods for individual performance (i.e.,

accuracy, precision, sensitivity, specificity, and robustness) against a broad array of known contamination sources.

Section 12.2.1 Co-occurrence of MST Markers

Although the MST markers used in this proposal have been used in scientific investigations for several years, much is unknown about their fate, transport, and survival characteristics with respect to each other, and in relation to indicator bacteria and pathogens. It is therefore important to utilize multiple markers for a given fecal source, whenever possible, to increase confidence in the results. It is also important to use control sites where contamination is expected (e.g. ruminant at BW2) and where it is not anticipated (e.g. non-human markers at HR1). The use of multiple human markers provided very high confidence in the assessment of human source contamination at several Hillsborough River sites, including HR3 and BK1. In contrast, little evidence for human or ruminant contamination was found throughout the Spartman Branch sites, suggesting that the bacterial loading there is primarily from stormwater and natural sources. Continued exploration of the relationship between MST markers, indicator bacteria, and pathogens will increase their usefulness and ease of interpretation as the field advances.

Section 12.2.2 Correlation between Indicator Organisms and the Detection of MST Markers

The use of indicator bacteria to assess the impacts of fecal contamination on water dates to the end of the 18th century. The ideal indicator would be found only in feces, would never replicate outside its host, would have transport and survival characteristics that mirror those of the various waterborne pathogens, and would be highly correlated with all types of pathogens (including bacteria, protozoa and viruses). The ideal indicator is not known to exist, and in fact all indicator bacteria currently used for regulatory purposes are know to have failed for each of these criteria under certain (or sometimes most) conditions.

In spite of their drawbacks, epidemiological studies have consistently shown a significant correlation between certain indicators (i.e. *E. coli* and enterococci) and the risk of gastroenteritis in humans that are using the water for recreation, particularly in waters that are known to be contaminated by human sewage. Much less is known about the risk to human health from recreation in waters that are contaminated by feces from other animals and from mixed sources such as stormwater; however high-risk sources of fecal pollution from animals known to frequently carry human pathogens, such as poultry and cattle, are considered high-priority pollutants by the U.S. Environmental Protection Agency (EPA 2007b). Thus, determining the source(s) of indicator bacteria in environmental waters is a major factor in estimating human health risk as well as for developing appropriate remediation and best management practices.

It is not unusual for MST markers to fail to correlate with indicator bacteria concentrations in environmental studies (McQuaig et al. 2006c). Pathogens also

frequently fail to correlate with indicator bacteria (Harwood et al. 2005). This disconnect is due to the highly variable sources, transport and survival characteristics of the different indicator bacteria, MST marker-bearing microorganisms, and pathogens. What is clear is that if reliable markers of human fecal contamination are consistently detected in a water body, a fecal source is present that carries a distinct health risk for humans using the recreational water users.

Variable results were achieved through a combination of multivariate analyses of bacterial community data used in this investigation to help determine the effectiveness of indicator organism concentrations in predicting the presence of source-specific pollution, as detected by MST markers. Relationships were identified between the detection of human and ruminant *Bacteroides* in individual basins; however, these results did not extend to all markers or into all watersheds and the observed associations were relatively weak with Global R values of less than 0.537. In addition, higher *E. coli* concentrations were observed to correlate with the detection of ruminant *Bacteroides*.

A chi-squared goodness of fit test was used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for HPyV, *esp*, human *Bacteroides*, ruminant *Bacteroides*, and horse *Bacteroides* markers). The null hypothesis for each WBID was constructed based on MST concordance with the state-recognized 400 CFU/100mL concentration level. Evidence for this relationship was found for four of 18 tests; however, 17 test combinations (MST versus WBID) had greater than 20% of expected values under five and were subsequently removed from the analysis. Interestingly, and in contrast to the results of the multivariate analyses described above, all of the human-specific markers showed some relation to the 400-limit in at least one of the six basins.

Section 12.2.3 Utilization of Horse Bacteroides Marker

The *Bacteroides* marker for horse feces was adopted directly from a peer-reviewed publication (Dick et al. 2005b), but had not been extensively validated in the USF laboratory. When this marker was detected in WBIDs where a horse source was not plausible, further validation efforts began. The PCR method was modified from a "touchdown" variation to a conventional PCR protocol, and primer concentrations were lowered. Subsequent testing against target and non-target fecal samples showed that the modified PCR assay was sensitive and specific to horses. Use of the modified assay through the remainder of the study yielded no apparent false-positives tests as well as confidence in the accuracy of this horse-specific MST test.

Section 12.3 Utility of Various Assessment Techniques

Section 12.3.1 Fluorometry

The most notable lesson learned concerning the utility of various assessment techniques implemented in this project was the use of fluorometry in detecting wastewater contamination. During this study Dr. Harwood's laboratory and the FDEP Southwest

District Office attempted a variety of techniques to test the utility of this tool. Despite the apparent success of fluorometry in detecting plumes from OSTDS in surface waters in other states (Grant 1998, TAMU 1999), the presence of organic compounds in Florida waters, proved to interfere with the detection of OBs by fluorescing and giving a "false-positive" signal.

In Dr. Harwood's evaluation of UV-degradation rates, it was determined that pure water and Hillsborough River water have similar degradation rates and that an adequate signal was not being obtained from sewage. Furthermore, results from the field station samples also suggested that the levels of OBs at the different sites were all similar and could not be discriminated from one another, despite significant differences in fecal coliform levels.

Two fluorometry sampling strategies were also employed by the FDEP. The first involved the collection of grab samples for concurrent bacteriological and chemical analysis while the second used a real-time flow-through fluorescence system. The prior technique was designed to discriminate optical brighteners from other compounds by utilizing a dual-fluorometer system for analysis in the laboratory. This methodology allowed for the calculation of relative fluorescence using a ratio between fluorescence resulting from CDOM and that by optical brighteners. The second technique implemented by the FDEP utilized an in-water probe that could be used in systems that are not navigable by boat (e.g., Lower Hillsborough River, Lake Thonotosassa).

Chris Anastasiou, FDEP, reported that the methodology that utilizes a dual-fluorometer, flow-through system designed to discriminate OBs from other compounds, appears to be the best methodology. Furthermore, as determined in the Tributary Pollution Assessment Project (PBS&J 2006b), it is very difficult to draw definitive conclusions using techniques that involve using isolated samples from individual locations since the idea is to identify differences on a relative scale. The equipment used for this methodology allows for the calculation of relative fluorescence using a ratio between fluorescence resulting from CDOM and that by OBs. It should be noted, however, that both of these methodologies are limited in their utility by many factors, including when people do their laundry and how long it takes that water to move through the system.

Currently, results from both the USF and FDEP approaches have been largely inconclusive; however, direct comparisons of the various OB-fluorescence methods as well as results from the microbial tests are expected to continue in future months.

Section 12.3.2 MST Markers in Sediments

The potential for sequestration and resuspension of indicator bacteria and microorganisms carrying MST markers in sediments led to an effort to test the sediments for MST markers. The inability to process an adequate sample size because of filtration difficulties led to the decision to end this effort after several months. Future efforts to process sediment samples for MST testing must await tests that are more sensitive and

more resistant to inhibition from interfering compounds so that low target numbers can be detected in smaller sample volumes.

Section 12.4 Utilization of the Decision-Support Tool

The bacteria decision-support tool, developed in the Hillsborough River project for statewide application, combines information on bacteria hotspots with human health risk-based contaminant source surveys to prioritize and guide management actions at the waterbody and station level (Section 10) of impaired recreational (Class III) water bodies. This should significantly decrease the costs associated with identifying sources and implementing restoration activities and allow the state and local stakeholders to focus resources on the impairments that pose the greatest risk to human health.

Integration of the results of the decision-support tool (PBS&J et al. 2008) and the source identification project demonstrated that the MWQA and CSS information generated by the two projects can be combined and evaluated to suggest a BMAP implementation strategy for the six WBIDs. This information was used to prioritze the WBIDs, and the monitoring locations within them, for follow-up action based on the estimated likelihood of public health risk so that appropriate management actions could be recommended to address fecal coliform impairments.

Through this process, some interesting statistical relationships were observed between the three groups of variables that were included in the MST project (IO concentrations, CSS assessment rankings, and presence/absence of MST markers). Future applications of the tool at the onset of the source identification process may produce a more robust dataset increasing the confidence in the sources identified. Testing of the decision-support tool using the MST project data has proven effective in assessing the tool's underlying assumptions and modifying the tool in ways that make it more useful in the resource management process.

Section 12.4.1 Relationships between CSS ratings, IO concentrations and MST marker detections

A number of epidemiological studies have found significant statistical associations between human health risk (e.g., for acute gastroenteritis) and recreational use of water containing elevated IO concentrations, particularly in waters that are known to be contaminated by human sewage. The correlation coefficients reported in those studies are often small, however, and the underlying relationships between risk and IO concentrations are highly variable(WHO 2003, NRC 2004). Less is known about human health risk from waters that are contaminated by feces from non-human sources, or from mixed sources such as stormwater. However, fecal pollution from animals that frequently carry human pathogens, such as cattle, swine and poultry, are considered a priority issue by the U.S. Environmental Protection Agency (EPA 2007a).

The development and addition of MST markers to water quality monitoring programs is a relatively young and rapidly-evolving area of applied microbiological research. By

providing information on the likely sources of fecal contamination, the approach has the potential to generate more accurate estimates of human health risk than are provided by the traditional IO-based methods (NRC 2004). Because MST markers are not yet in widespread use, however, little information is available from field studies regarding statistical relationships that may exist between the markers and the older IOs. Although only limited environmental studies are available, inconsistent relationships have been found between the detection of MST markers, the presence of pathogens, and measured concentrations of indicator bacteria (Harwood et al. 2005, McQuaig et al. 2006b). This appears to be due to the presence of highly variable contributing sources as well as the abilities of the different IOs, MST marker-bearing microorganisms, and pathogens to survive and be transported through the environment.

Regression analyses were used to assess relationships between the three categories of information (CSS ratings, IO concentrations and MST marker detections) collected during the MST project. The CSS variable took on values ranging from 1 (very low estimated likelihood of human health risk from fecal contamination) through 5 (very high estimated likelihood of health risk), based on ratings developed through the on-site CSS surveys conducted as part of the MST project. IO concentrations were expressed as colony forming units (CFU) per 100 mL sample, using the IO monitoring data (concentrations of fecal coliforms, *E. coli*, and enterococci) provided by the MST project. IO concentrations were $\log_e$-transformed prior to analysis for normalization and variance stabilization, and to provide more linear relationships with the other variables.

For the MST markers, binary (1,0) response variables designated MST_h and MST_r were used to record the detection or non-detection of the human-specific and ruminant-specific markers, respectively, that were used in the MST project. For each sampling station and date on which tests for human-specific MST markers were performed, the MST_h variable was given a value of 1 if at least one of the human-specific markers were detected. If no human-specific markers were detected at that station and date, the MST_h variable was given a value of 0. The same 0,1 scoring method was applied to the ruminant-specific (MST_r) response variable.

Because MST_h and MST_r are binary rather than continuous variables, logistic regression was used to examine relationships between the frequency of detection of human-specific or ruminant-specific markers (i.e., cases in which $MST_h = 1$ or $MST_r = 1$) and the concentrations of indicator organisms. In logistic regression the dependent variable is the "logit", or log odds, that a marker will be detected given the observed values of one or more explanatory variables, $X_1,\ldots,X_k$ (which in this case are the CSS category values and the measured concentrations of the bacterial IOs).

The logistic regression model takes the form:

$$\text{logit}\{\Pr(Y = 1|X_1,\ldots,X_k)\} = \log_e\{\Pr(Y = 1| X_1,\ldots,X_k)/1- \Pr(Y = 1| X_1,\ldots,X_k)\}$$

$$= \beta_0 + \beta_1X_1 + \ldots + \beta_kX_k$$

where:

- $log_e\{Pr(Y = 1| X_1,\ldots,X_k)/1- Pr(Y = 1| X_1,\ldots,X_k)\}$ is the log odds that the response variable (MST_h or MST_r) will have a value of 1, given the values of the explanatory variables;
- $X_1,\ldots,X_k$ are the potential explanatory variables (observed concentrations of IOs); and
- β_i is a fitted regression coefficient.

To provide contrasting views of relationships that may exist between the MST markers and the potential explanatory variables, three types of logistic regressions were run, each of which used the detection/non-detection of the MST markers as the response variable:

1) Simple regressions using a single explanatory variable, to provide a direct examination of the relationship between the log odds of detecting the MST markers and each of the potential explanatory variables;

2) Stepwise (backward selection) multiple regressions, in which all the potential explanatory variables were initially included in the regression model, and only those with significant ($p<0.05$) slope coefficients were retained in the final model; and

3) Stepwise (backward selection) multiple regressions in which only the two variables (fecal coliform concentration and CSS rating) that are used in the decision-support tool were included in the initial model, to examine the abilities of these two indicators to predict the frequency of marker detection.

For the human-specific markers each of these analyses was run three times: once using the entire data set; once using only the data from the non-tidal, strictly freshwater stations (i.e., omitting the tidally-influenced stations from the Lower Hillsborough River); and once using only the Lower Hillsborough River stations. This approach was used to provide insights into the potential role of salinity as a confounding factor that may complicate efforts to understand relationships among the variables.

For the ruminant-specific markers these analyses were only applied to data from the inland, non-tidal stations (i.e., omitting stations located in the Lower Hillsborough River). This was done because the primary purpose of the ruminant-specific marker is to detect fecal inputs from cattle and ruminant wildlife (e.g., deer), neither of which are common in the vicinity of the highly-urbanized Lower Hillsborough River.

The results of these analyses are summarized in Tables 44 through 46.

Table 44. Results of simple (single explanatory variable) logistic regressions applied to the MST project data, using detection/non-detection of MST markers as the response variable. Variables whose slope coefficients were significantly ($p<0.05$) different from zero are shown in bold. (Data source: PBS&J)

Station Type	Explanatory Variable	Human-specific markers			Ruminant-specific marker		
		n	Regression Slope Coefficient	Signif. (p)	n	Regression Slope Coefficient	Signif. (p)
Freshwater Stations (LHR Stations Omitted)	**CSS**	125	**0.40**	**<0.05**	78	**0.84**	**<0.01**
	Log_e (fecal coliforms)	178	n.s.	>0.05	79	n.s.	>0.05
	Log_e (enterococci)	112	n.s.	>0.05	79	n.s.	>0.05
	Log_e (*E. coli*)	110	n.s.	>0.05	77	n.s.	>0.05
Tidally-Influenced Stations (LHR Stations Only)	**CSS**	83	**0.48**	**<0.05**			
	Log_e (fecal coliforms)	104	**0.32**	**<0.001**			
	Log_e (enterococci)	57	**0.21**	**<0.01**			
	Log_e (*E. coli*)	57	**0.26**	**<0.01**			
All Stations	**CSS**	208	**0.37**	**<0.01**			
	Log_e (fecal coliforms)	282	**0.19**	**<0.001**			
	Log_e (enterococci)	169	**0.13**	**<0.02**			
	Log_e (*E. coli*)	167	**0.12**	**<0.05**			

Table 45. Results of stepwise (backward selection) logistic regressions applied to the MST project data. All potential explanatory variables were included in the initial model. Variables whose slope coefficients were significantly ($p<0.05$) different from zero, and were therefore retained in the final model, are shown in bold. (Data source: PBS&J)

Station Type	Explanatory Variable	Human-specific markers			Ruminant-specific marker		
		n	Regression Slope Coefficient	Signif. (p)	n	Regression Slope Coefficient	Signif. (p)
Freshwater Stations (LHR Stations Omitted)	**CSS**	109	**0.43**	**<0.05**	76	**0.86**	**<0.01**
	Log_e (fecal coliforms)		n.s.	>0.05		n.s.	>0.05
	Log_e (enterococci)		n.s.	>0.05		n.s.	>0.05
	Log_e (*E. coli*)		n.s.	>0.05		n.s.	>0.05
Tidally-Influenced Stations (LHR Stations Only)	CSS	56	n.s.	>0.05			
	Log_e (fecal coliforms)		**0.30**	**<0.01**			
	Log_e (enterococci)		n.s.	>0.05			
	Log_e (*E. coli*)		n.s.	>0.05			
All Stations	**CSS**	165	**0.52**	**<0.001**			
	Log_e (fecal coliforms)		n.s.	>0.05			
	Log_e (enterococci)		n.s.	>0.05			
	Log_e (*E. coli*)		n.s.	>0.05			

Table 46. Results of stepwise (backward selection) logistic regressions applied to the MST project data. Only the two potential explanatory variables used in the decision-support tool (fecal coliform concentrations and CSS scores) were included in the initial model. Cases in which the slope coefficients for one or more of those variables were significantly ($p<0.05$) different from zero are shown in bold. (Data source: PBS&J)

Station Type	Explanatory Variable	Human-specific markers			Ruminant-specific marker		
		n	Regression Slope Coefficient	Signif. (p)	n	Regression Slope Coefficient	Signif. (p)
Freshwater Stations (LHR Stations Omitted)	**CSS**	125	**0.40**	**<0.05**	78	**0.84**	**<0.01**
	Log_e (fecal coliforms)		n.s.	>0.05		n.s.	>0.05
Tidally-Influenced Stations (LHR Stations Only)	CSS	83	n.s.	>0.05			
	Log_e (fecal coliforms)		**0.27**	**<0.01**			
All Stations	**CSS**	208	**0.37**	**<0.01**			
	Log_e (fecal coliforms)		n.s.	>0.05			

Because the sampling that was carried out for the MST project was not designed to evaluate relationships among this group of variables, and given the large differences in the sample sizes that are available for the regression analyses shown in Tables 44 through 46, the results of these analyses should be interpreted with caution. (In the multiple regressions, for example, the number of observations included in a given regression model is determined by the explanatory variable with the smallest sample size, since all samples in which one or more of the explanatory variables have missing values are omitted from the analysis.) With those caveats in mind, however, the following conclusions appear reasonable based on the data generated by the project:

- CSS category was the most consistent predictor of the odds of MST marker detection in this group of samples. CSS category was a significant predictor in each of the simple regressions models (Table 44), in three of the four multiple regression models in which all three of the monitored IOs were available as potential explanatory variables (Table 45), and in three of the four multiple regression models in which only the CSS category and the log-transformed fecal coliform concentration were available as potential explanatory variables (Table 46). This suggests that CSS category rankings, if carefully and consistently applied, may prove to be useful as alternatives to MST analysis in situations where management actions can be initiated without the need for MST data, or where financial considerations or other factors limit the number of MST analyses that can be performed.

- Concentrations of fecal coliforms and the other IOs (enterococci and *E. coli*) sampled in the study were significant predictors of MST marker detections at the tidally-influenced stations located in the Lower Hillsborough River WBID, but not in the inland, strictly freshwater stations located in the other WBIDs (Table 44). The causes of this pattern are unknown, but given the fact that several of the MST markers are bacterially-based it may be that both the marker organisms and the bacterial IOs were affected in similar ways by the salinity regimes that were present in the Lower Hillsborough River during the sampling period. If that was the case, the statistical associations between the IOs and the MST markers observed in this study may have been due, at least in part, to similar die-off rates of the different bacterial taxa in estuarine waters. This possibility could be framed as a hypothesis and tested in subsequent monitoring projects.

Section 12.4.2 Salinity as a complicating factor

As previously noted, salinity has a differential effect on the die-off rates of bacteria taxa which may influence the interpretation of the IO concentrations. Although this was not a factor identified in the five freshwater watersheds (Spartman Branch, Baker Creek, Flint Creek, Blackwater Creek and New River), salinity is a potentially confounding factor in the MST data set for the Lower Hillsborough River. This waterbody is a tidally-affected river reach whose salinity levels have fluctuated (both spatially and temporally) between fresh (<0.5 ppt) and estuarine (>20 ppt) in recent years. Elevated salinities have been particularly pronounced in the Lower Hillsborough River during extended periods of low rainfall, when anthropogenic withdrawals from the Hillsborough River Reservoir (located

immediately upstream) have reduced or eliminated freshwater inflows to the river's tidal reach.

In the field, survival times of *E. coli* and other fecal coliform bacteria are often inversely correlated with salinity (Goyal et al. 1977, Harwood et al. 1999, NRC 2004). In the Lower Hillsborough River, long-term information on relationships between fecal coliform concentrations and salinity are available from four EPCHC monitoring stations, which were sampled on a monthly basis during the years 2001 through 2007. Data from those stations are summarized in Table 47. Significant inverse associations (Kendall's tau) between salinity and fecal coliform concentrations were observed at three of the four stations. The exception was the most upstream location (EPCHC station 105), which exhibited a lower salinity range and lower maximum salinity levels than were observed at the more downstream monitoring sites (Table 47).

Table 47. Salinity ranges and associations between salinity and fecal coliform concentrations at four EPCHC monitoring stations on the Lower Hillsborough River, 2001 – 2007. Stations are arranged in upstream to downstream order. (Data source: EPCHC).

EPCHC Sta. No.	Location	No. observations	Salinity range (PSU)	Association Between Fecal Coliform Concentration and Salinity (Kendall's Tau)	Significance Level (*p*)
105	Rowlett Park Drive	81	0.1 – 16.8	n.s.	>0.10
152	Sligh Avenue	84	0.1 – 23.2	- 0.33	<0.0001
137	Columbus Drive	84	0.1 – 28.0	- 0.38	<0.0001
2	Platt Street	84	0.4 – 32.1	- 0.48	<0.0001

Given the associations shown in Table 47, it appears possible that monitoring stations located in estuarine or marine waters may exhibit different relationships between fecal coliform concentrations and the frequency of detection of human or ruminant fecal markers than are observed at fresh water stations. Therefore, when analyzing the IO and fecal marker data provided by the MST project, the analyses were segmented in the following three ways to provide information on the potential confounding effects of varying salinity levels:

1) Each analysis was carried out using the entire data set, including both fresh water and estuarine salinity conditions

2) The analysis was also carried out using data from the fresh water stations alone, omitting all observations from the Lower Hillsborough River WBID

3) The analysis was also carried out using data from the Lower Hillsborough River alone, omitting all observations from the strictly freshwater WBIDs.

Section 12.4.3 Identification of Potential Next Steps

Proposed priority rankings of WBIDs and MST sampling locations for further monitoring or management action, based on updated CSS and MWQA categories and frequencies of MST marker detection, are provided in Section 10. For those instances where the most probable sources have been identified, it is suggested that the next steps include evaluation of the sufficiency of the projects included in the Hillsborough River BMAP to address those sources. In many cases, the results of the decision-support tool should be used to focus additional field reconnaissance and sampling efforts to further target the identification of sources. This may include utilization of current and new MST assays (e.g., quantitative PCR techniques) or more directed field reconnaissance in specific areas of interest. For example, for those stations with elevated MWQA scores and relatively low CSS ratings, wildlife surveys may be helpful in confirming a potential wildlife source.

The decision-support tool can also be employed on a statewide level to identify priority watersheds and stations for additional sampling and remediation. It is strongly recommended that the Department continue to validate source identification methods and provide leadership, and possibly cost-sharing opportunities, to local governments to identify and remove sources. The progress of these efforts can then be tracked using the decision-support tool through the development of annual reports. Workshops can be conducted throughout the state, and especially in priority communities, to educate and encourage local stakeholders in the identification of sources and the remediation of water quality.

Section 13.0 Conclusion

The approach that was developed and documented in the lower Hillsborough River watershed builds on the work carried out in Duval County. It provides a useful, cost-effective tool for guiding the implementation of fecal coliform BMAPs. Sources are defined to a level at which remedial action can be defined and successfully implemented, while also considering cost. Lower-cost, more basic methods are used first, followed by higher-cost, more sophisticated methods.

In Phase I of the project, Initial Screening, relevant documents, historical data, and local knowledge were compiled, reviewed, and used to guide field reconnaissance and sampling. The sampling methodology was used to characterize the upstream, midstream, and downstream areas of each WBID. In Phase II, Implementation, indicator bacteria from the sampling were enumerated and used to assess microbial pollution and distribution in the water column and sediments. MST methods were then used to help identify specific sources. In the final step, sources identified in the testing process were linked to the potential sources observed in the field to confirm the results. The decision tree used in Phase II builds on the results of Phase I, placing more weight on the positive or negative result of multiple methods (i.e., the weight-of-evidence approach).

In the lower Hillsborough River project, by developing detailed knowledge of sources, the accurate level and potential sources of impairment in six tributary WBIDs were determined and specifically targeted to individual human (wastewater and stormwater), animal, and natural sources. Determining contributing sources is particularly important with fecal coliform contamination, because pathogens from human sources present the highest potential for infection. Thus accurate source identification is critical to implementing management actions to improve water quality and protect human health.

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Appendix A

University of South Florida Methods for Laboratory Analyses

University of South Florida Methods for Laboratory Analyses

Fluorometry. From June to August, water samples from fixed sites were tested for the presence of optical brightners, associated with human sewage (Poiger et al. 1998, Boving et al. 2004), using the Aquaflor handheld fluorometer (Turner Designs) by the method described by Hartel (2007). Briefly, optical density of a water sample is taken using a 445nm emission filter, the sample is exposed to UV light (365nm) for 5 minutes, and the optical density was taken again. Samples were considered positive if the optical density of the sample after UV exposure was at least 15% lower than the original optical density.

Enumeration of Indicator Bacteria. Water and sediment samples were processed by membrane filtration (0.45µm pore-size, 47mm diameter) using a vacuum pump to enumerate *Escherichia coli*, fecal coliforms, and enterococci. Prior to filtration, sediment samples were diluted 1:10 in sterile buffered water and sonicated (Anderson et al. 2005) to release bacteria attached to sediment particles. Fecal coliforms were enumerated on mFC agar after 24 h incubation at 44.5°C (American Public Health Association 1995); enterococci were enumerated on mEI agar at 41°C after 24h incubation (United States Environmental Protection Agency 2002a); *E. coli* was enumerated on mTEC media at 35°C for 2h, followed by 22h incubation at 44.5°C (United States Environmental Protection Agency 2002b). Colonies on plates were counted and concentrations were reported as CFU · 100 ml^{-1} or CFU · 100 g^{-1} (wet weight) for water and sediment samples, respectively.

Microbial Source Tracking (MST).

***esp* gene.** The assay for detection of the human-associated *esp* gene of *Ent. faecium* by polymerase chain reaction (PCR) was performed as described previously (Scott et al. 2005). Three hundred ml of water or 25ml of sediment sample (diluted as described above) was filtered through a 0.45µm pore-size membrane and incubated on mEI agar at 41ºC for 48h. Filters were than transferred to 5ml azide dextrose broth (Difco) and incubated at 41ºC with shaking for 3h. A DNA extraction was then performed on 2ml of this culture using the QIAamp DNA Stool Mini Kit according to the manufacturer's instructions (Qiagen). DNA was then used as PCR template using conditions described previously (Scott et al. 2005). The PCR product (680bp) was visualized by agarose gel electrophoresis (1% agarose gel) and captured using the FOTO/Analyst Archiver (FotoDyne). To test for the possibility of matrix inhibition of the PCR reaction, cells of the positive control strain *Ent. faecium* C68 were spiked into environmental water samples and processed in the same way. Sterile buffered water was also filtered and processed as described above as a method blank to ensure that false-positives did not occur as a result of contamination. A negative control for the PCR reaction alone was also included for each sample event.

Human Polyomavirus. Presence of human polyomavirus (HPyV) was detected using a method previously described using 600ml of sample (McQuaig et al. 2006). PCR products (172bp) were visualized by agarose gel electrophoresis (2% agarose gel) and captured as described above. This procedure was repeated four times or until HPyV was detected for each sample to account for possible heterogeneity in the DNA template. Viral particles of the BK strain of the virus were spiked into

environmental waters to test for matrix inhibition. Buffered water was also processed as a method blank, and a PCR negative control was run for each sample event.

Bacteroides. The procedure and PCR conditions used for *Bacteroides* assays was changed during the sampling period. From May through July, PCR was performed on whole cell templates using a modified version of Layton's method (Layton et al. 2006). Briefly, 60ml of sample was pushed through a 0.2μm pore-sized syringe filter and back-flushed into a 2ml microcentrifuge tube with 1ml of AE Buffer (Qiagen). The sample was then centrifuged at 10,000 rpm for 10min, after which 0.9ml of the buffer was removed and the pellet resuspended in the remaining 0.1ml. The resuspension was used as template for PCR reactions using primers described previously for human- (Bernhard & Field 2000), ruminant- (Bernhard & Field 2000), and horse- (Dick et al. 2005) associated *Bacteroides*. In August, 100ml of sample was processed by membrane filtration as described for enumeration of indicator bacteria and DNA was extracted from the filter using a MoBio UltraClean DNA kit following the manufacturer's instructions (MoBio). For the rest of the sampling period, 500ml of sample was processed by membrane filtration and DNA was extracted using a MoBio Power Soil DNA kit following the manufacturer's instructions (MoBio). DNA was used as PCR template beginning in August.

From May through July, PCR products were re-amplified – PCR product from an initial round of PCR was used as template for an identical second round to increase sensitivity of the assay using an initial whole cell template (Roux 1995). From May through August, PCR reactions were prepared using JumpStart Taq Ready Mix (Sigma) and a touchdown PCR program (Don et al. 1991) for all primer sets. The touchdown PCR program was maintained for the human and ruminant assays throughout the study and through the September sampling event for the horse assay. From September through the end of the study, PCR reactions were prepared using GoTaq Green Master Mix (Promega). Also beginning in September through the end of the study, primer concentrations for all *Bacteroides* assays was reduced from 0.5μM to 0.4μM. Beginning in October, the horse assay was conducted using a standard PCR program with an annealing temperature of 55º. Results were visualized by agarose gel electrophoresis (2% agarose) as described above.

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Appendix B

GIS Data Acquisition Timeline

Hillsborough BMAP

GIS Data Acquisition Timeline

Prepared 2. February 2007.
Last Modified 7. May 2007

Received	Data	Origin	Format	Contact	Agency
In House	Florida County Boundaries (FDEP)	FDEP	Arcview Shapefile	PBSJ	PBSJ
In House	Florida City Boundaries (FDEP)	FDEP	Arcview Shapefile	PBSJ	PBSJ
In House	Hydrology Line (FDEP)	FDEP	Arcview Shapefile	PBSJ	PBSJ
In House	Hydrology Line (FDEP)	FDEP	Arcview Shapefile	PBSJ	PBSJ
In House	Aerial Photography (2004)	FDEP / LABINS	ArcView MrSID Raster	PBSJ	PBSJ
12.08.2006	List of Exisiting BMAP Datasets: In-House and Still-Needed	N/A	Excel File (2)	Holly Greening	TBEP
12.15.2006	Parcel Boundary Centroids for Suspected OSTDS (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	EPCHC AWQM Stations	HCEPC WQ	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Wastewater Facilities (WACS; FDEP)	FDEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Wastewater Facilities (WAFR; FDEP)	FDEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Landfill Areas (WACS; FDEP)	FDEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	NPL and State Funded Waste Cleanup Sites (FDEP)	FDEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Permit Compliance Enforcement Data (FDEP)	FDEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Permit Applications (FDEP)	FDEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Node Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Net Junctions (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Sediment Control Structure Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Mitered End Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Headwall Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Inlet Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Manhole Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Control Structure Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Pump Station Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Major Point Sources (>100,000 gal/day; 1999-2003; TBEP)	TBEP	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Management Permit Locations (HC Records and Data Dep)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Potable Water Wells (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Manhole Inventory (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Miscellaneous Inventory (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Node Inventory (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Lift Station Inventory (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Inlet Inventory (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Water Supply Restoration Program Private Drinking Well Contamination Database (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Box Culvert Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Pipe Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Underdrain Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Ditch Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Major Stormwater Conveyance System (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Main Inventory (HC EPCHC)	EPCHC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Inventory of Channels/Ditches (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Stormwater Pond Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	EPCHC Historic and Existing Landfill Site Inventory (EPCHC)	EPCHC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Red Line Property Inventory (COT)	COT	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Developments of Region Impact Projects (HC - Unincorporated)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Land Use (SWFWMD)	SWFWMD	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Environmental Land Acquisition Protection Program (HC)	HC	ArcView Geodatabase	Gerold Morrison	EPCHC
12.15.2006	Potential Septic Parcel Locations (USF)	USF	ArcView Geodatabase	Gerold Morrison	EPCHC
12.27.2006	CIP Completed (HC)	HC	Arcview Shapefile	Jodi Pracht	
1.23.2007	STORET Stations	EPA	Arcview Shapefile	PBSJ	PBSJ
1.23.2007	Legacy STORET Stations	EPA	Arcview Shapefile	PBSJ	PBSJ
1.23.2007	USGS NWIS Stations	USGS (NWIS)	Arcview Shapefile	PBSJ	PBSJ
1.23.2007	Water Quality Data	USGS (NWIS) / EPA / HCEPC WQ / Stream Waterwatch WQ	Excel File	WaterAtlas Webpage	N/A
1.25.2007	OSTDS Parcels (as of 012506; HC)	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Wastewater Node Inventory (COT)	COT	ArcView Geodatabase	Ed Sherwood	EPCHC
1.25.2007	Wastewater Gravity Main Inventory (HC)	HC	ArcView Geodatabase	Ed Sherwood	EPCHC
1.25.2007	Wastewater Lateral Line Inventory (HC)	HC	ArcView Geodatabase	Ed Sherwood	EPCHC
1.25.2007	Wastewater Pressurized Main Inventory (HC)	HC	ArcView Geodatabase	Ed Sherwood	EPCHC

Hillsborough BMAP

GIS Data Acquisition Timeline

Prepared 2. February 2007.
Last Modified 7. May 2007

Received	Data	Origin	Format	Contact	Agency
1.25.2007	Utility Expansion Maps	DOH	PDF	Mike	DOH
1.25.2007	Septage Spreading Areas	DOH	Arcview Shapefile	Ed Sherwood	EPCHC
1.31.2007	Dairy Farm GPS Information	COT Stormwater Department	Email	Jennifer Lana	EPCHC
1.31.2007	SSO Data (2001 - 2007 COT)	COT	Arcview Shapefile	Carrie Jones	COT
2.07.2007	Water Treatment Facilities (FDEP)	FDEP	Arcview Shapefile	PBSJ	PBSJ
2.07.2007	Sewage Treatment Facilities (FDEP)	FDEP	Arcview Shapefile	PBSJ	PBSJ
1.25.2007	Alafia River sub_basin_st83	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Bullfrog basin83.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Curiosity Creek sub_basins_st83.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Cypress sub_basin_st83.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Delaney basin.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Duck Pond sub_basins_st83.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	East Lake basin.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	HCSTORMARCS.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	hills_sub_basin_state_83.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Hillsborough_TMDL_subbasins.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
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1.25.2007	STL subbasin.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	tbc_subbasin_st83.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Hillsborough Major Stormwater Conveyance and Inventory Data (DUPLICATE)	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Hill_BMAP_COMET.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Hill_BMAP_PermittingApplications.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Hill_BMAP_WACS.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_COMET.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_PermittingApplications.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_WACS.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Solid Waste Treatment Facilities (FDEP)	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_BrownfieldAreas.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_BulkPetroleumStorageFacilities.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_DryCleaningProgramSites.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_GroundwaterContaminationAreas.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_Landfills.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_LargeQuantityGenerators.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_NPLStateFundedWasteCleanupSites.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_PetroleumContaminationMonitoringfromSTCM.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
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1.25.2007	SW_StorageTankContaminationMonitoring.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_ToxicReleaseInventory.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_Wastewater_Facs_from_WAFR.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	SW_WastewaterSites_FromWAFR.shp	DEP	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Septic Tank Study 2006	HC	PDF / PPT / DOC	Ed Sherwood	EPCHC
1.25.2007	Buffer_of_GravityMain_WW.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Buffer_of_LateralLine_PW.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Buffer_of_LateralLine_WW.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
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1.25.2007	cnty_pap_not_ww_cust_not_srvcarea_possible_GT100ftGrav.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	cntypap.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC

Hillsborough BMAP

GIS Data Acquisition Timeline

Prepared 2. February 2007.
Last Modified 7. May 2007

Received	Data	Origin	Format	Contact	Agency
1.25.2007	cntypap_notcot_or_cntywwcust_or_cntysrvcarea_reduced.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	cntypapcentroids.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	COT_NO_WW.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	COT_NO_WW_NOT_CTY_.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	COT_NO_WW_NOT_CTY_POSSIBLE.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	COT_septic_Centroids.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	COT_YES_WW.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	HCWD_customers.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	OSTDS_Parcels_012506.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Septic_Tanks_HCWRS.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Working_Possible.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Working_Possible_noprvtplnt.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Working_Possible_noprvtplnt_FAR.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Working_Possible_noprvtplnt_MEDIUM.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Working_Possible_noprvtplnt_NEAR.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	Working_Possible_noprvtplnt_TOOFAR.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	CAFO_permit.shp	HC	Arcview Shapefile	Ed Sherwood	EPCHC
1.25.2007	PW, RW, WW, WD_Misc Infrastructure (All_1.mdb)	HC	ArcView Geodatabase	Ed Sherwood	EPCHC
4.17.2007	Wastewater Control Valve Inventory	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater Fitting Inventory	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater Manhole Inventory	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater Network Structure	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater System Valve Inventory	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater Line Inventory (Annotated, Point, and Line Files)	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater Force Main Inventory	Polk County	Acrview Shapefiles	Carl Metz	Polk County
4.17.2007	Wastewater Gravity Main Inventory	Polk County	Acrview Shapefiles	Carl Metz	Polk County
5.1.2007	Wastewater Manhole Inventory	Pasco County	ArcView Geodatabase	Amy Tracy	Pasco County
5.1.2007	Wastewater Liftstation Inventory	Pasco County	ArcView Geodatabase	Amy Tracy	Pasco County
5.1.2007	Wastewater Lateral Line Inventory	Pasco County	ArcView Geodatabase	Amy Tracy	Pasco County
5.1.2007	Wastewater Force Main Inventory	Pasco County	ArcView Geodatabase	Amy Tracy	Pasco County
5.1.2007	Wastewater Gravity Main Inventory	Pasco County	ArcView Geodatabase	Amy Tracy	Pasco County
5.1.2007	Wastewater Flow Direction Summary	Pasco County	ArcView Geodatabase	Amy Tracy	Pasco County

Appendix C

Detail Description of Statistical Analyses and Associated Results

Section 1.0 Detailed Description of Statistical Analyses and Associated Results

Upon completion of Phase II, Level I sampling, the *Hillsborough River BMAP Fecal Database* contained 241 unique entries consisting of fecal coliform, *E. coli*, and enterococci bacterial concentrations (i.e., log abundance), MST test results, and a host of abiotic parameters (e.g., pH, temperature, conductivity, salinity, turbidity) recorded during 8 sampling events (May-December 2007) at 55 stations throughout 6 WBIDs in west-central Florida. Stations falling within five miles of a daily rainfall gauge (three operated by COT, one by USGS; Map 1) were appended with time-lagged rainfall data (1-day, 2-day, 4-day, 7-day, and 14-day totals) matching the periods of record. All database entries were vetted for missing, duplicate, or erroneous values prior to further analysis. A detailed description of each analysis and the associated findings are provided below.

Section 1.1 Summary Statistics

Each of the six watersheds sampled had sites with indicator organism (IO) concentrations which regularly exceeded the state standard for fecal coliforms (*http://www.dep.state.fl.us/legal/Rules/shared/62-302/62-302.pdf*; Table 1) designated at 400 CFU/100mL as well as the EPA standard for enterococci, designated as 33 CFU/100mL(*http://www.epa.gov/waterscience/criteria/library/ambientwqc/bacteria1986 .pdf*). Geometric mean (+/- SE) and maximum recorded values were calculated for each of the indicator suite organisms (hereafter, 'IO Suite') under flowing conditions and were graphically displayed by station and by WBID (Figures 1 and 2). In most cases, the IO Suite abundance was higher in the sediment than the water samples, with *E. coli* typically showing depressed values compared to the other two IO Suite organisms (*water samples only*). Generally, data coverage was similar amongst five of the six WBIDs, with slightly more fecal coliform samples from Hillsborough River (fecal coliforms; N=100) than the other water bodies (fecal coliforms; New River, N=22; Spartman Branch, N=32; Flint Creek, N=24; Baker Creek, N=24; Blackwater Creek, N=37); other species remained similar among the WBIDs.

Comparison of the mean IO concentrations for each WBID (calculated as the average of counts from all sites with IOs sampled five or more times) using one-way analysis of variance (ANOVA) revealed no significant differences between fecal coliform and *E. coli* concentrations in water samples. In contrast, the mean enterococci concentrations were significantly different between WBIDs ($p < 0.0157$) and enterococci concentrations, on average, were significantly higher in the water samples collected from Spartman Branch than in than those from the Lower Hillsborough River ($p < 0.05$). Differences between mean fecal coliform and *E. coli* levels in the sediments collected from each WBID were not significant when analyzed by ANOVA ($p > 0.05$). In contrast, mean enterococci concentrations in sediments were significantly different between WBIDs ($p = 0.0366$) and were significantly higher in the Lower Hillsborough River than in Flint Creek ($p < 0.05$). All three IO concentrations were significantly positively correlated between

samples collected from water and sediment (Table 1). All post hoc analyses of ANOVA results to determine differences between groups were done by the Tukey post hoc test.

Table 1. Correlation of IO concentrations in water and sediment samples.

IO	r^2	Significance
Fecal Coliforms	0.4905	$p < 0.0001$
E. coli	0.4736	$p < 0.0001$
Enterococci	0.2749	$p = 0.0006$

Only those subsets of data that had adequate coverage, count summaries of IO Suite abundance and concurrence with abiotic, MST, and/or rainfall data, were calculated on a station-specific basis. Only those stations with six or more ($N \geq 6$) observations were used in further study. All stations were considered to be independent and data were not combined for any set of locations.

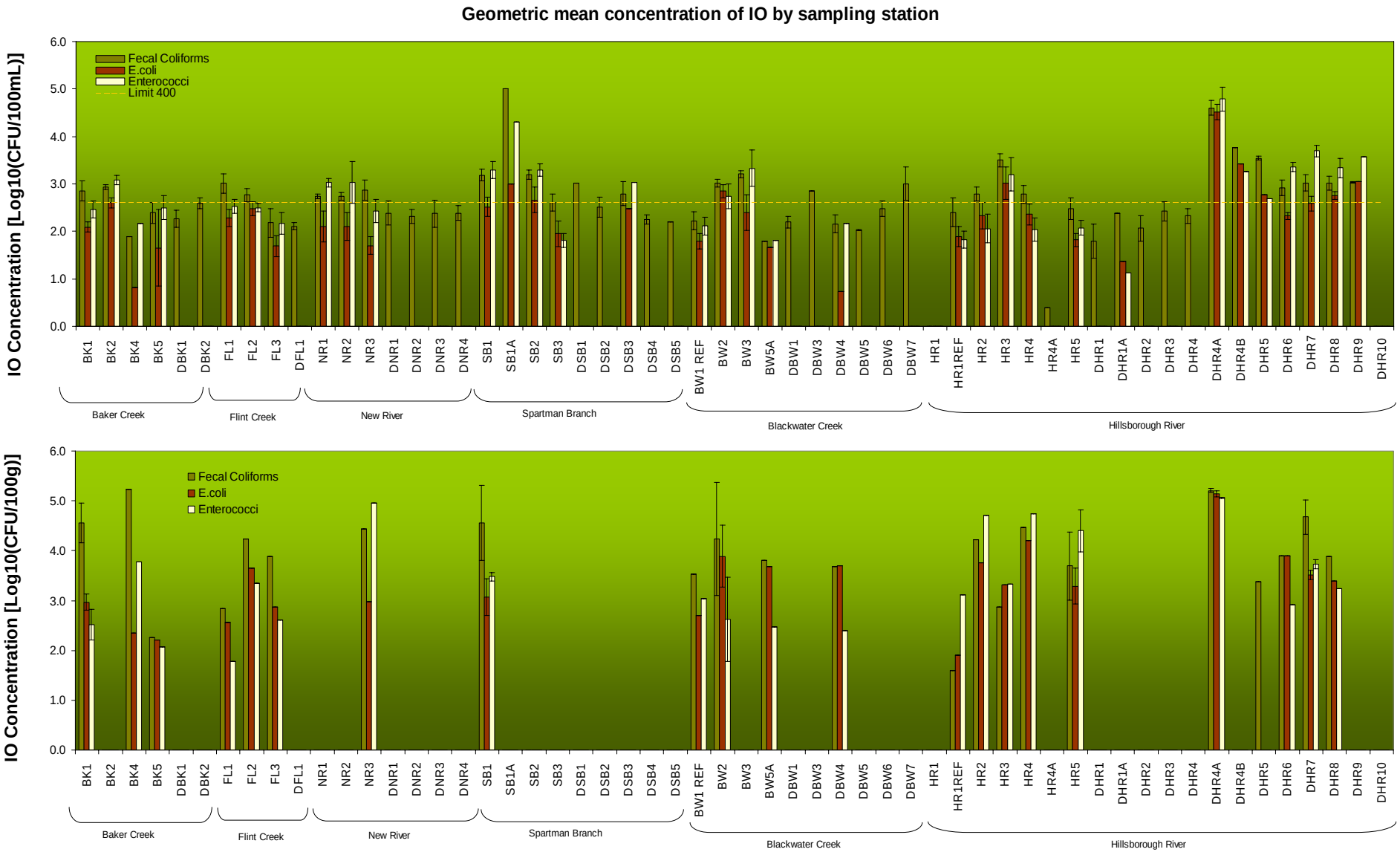


Figure 1. Geometric mean concentration of indicator organisms (IOs) by sampling station in water samples (top graph) and sediment samples (bottom graph). Calculations were performed on all available data collected under flowing conditions.

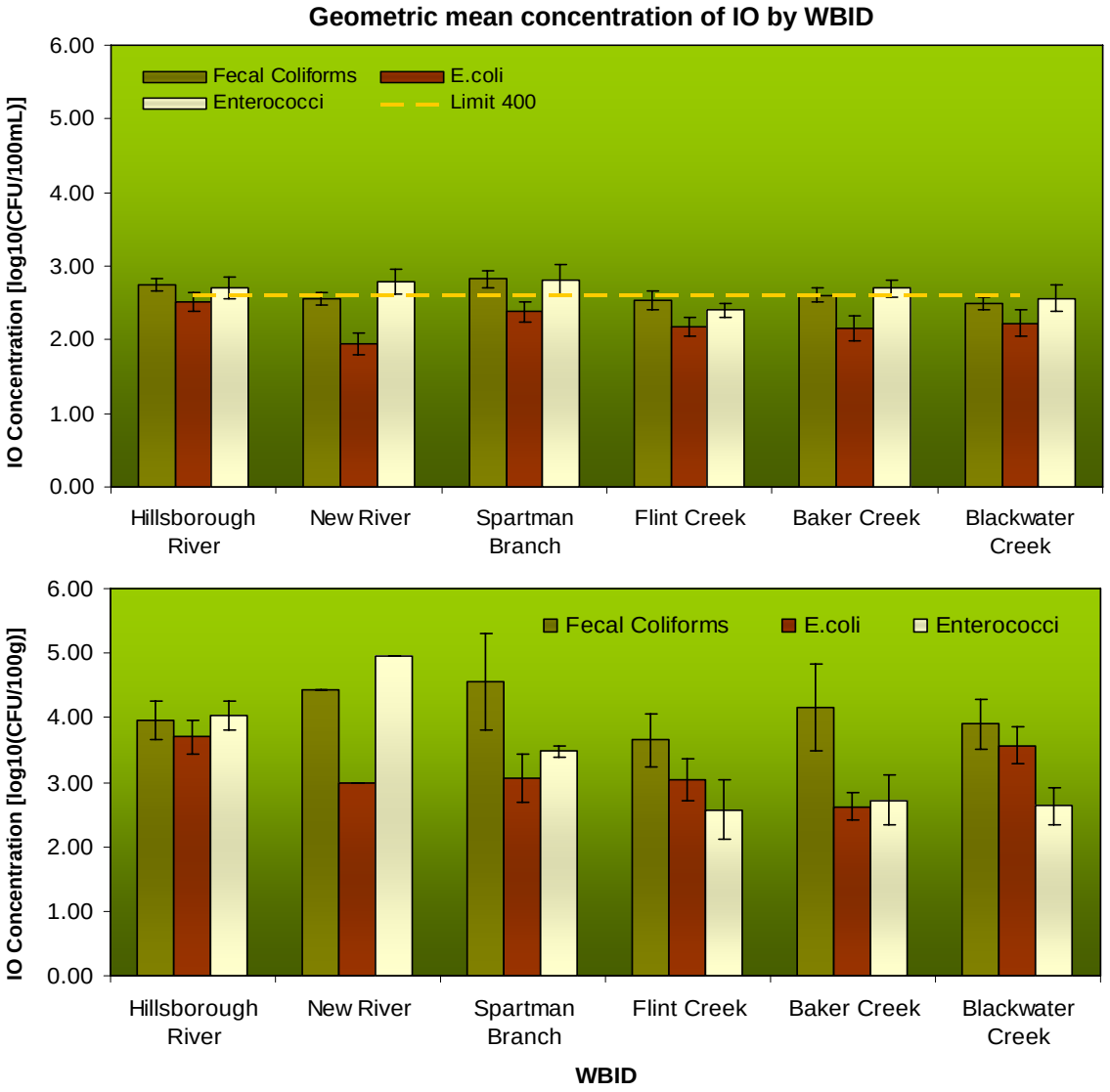


Figure 2. Geometric mean concentration of indicator organisms (IOs) by WBID in water samples (top graph) and sediment samples (bottom graph). Calculations were performed on all available data collected under flowing conditions.

Section 1.2 Indicator Suite Abundance and the Abiotic Environment

IO Suite abundance values from 19 stations representing 5 WBIDs (Blackwater Creek, Flint Creek, Spartman Branch, Baker Creek and Hillsborough River; New River did not meet the $N \geq 6$ criterion for analysis) were then tested for correlations with available water quality parameters; i.e., turbidity, salinity, pH and temperature. The significance of the resulting Pearson Product Moment Correlation Coefficients were independently assessed at an alpha of 0.05; all data were square-root or log-transformed where appropriate. The resulting correlation matrix can be seen in Table 2. Significant linear relationships were found for fecal coliform and *E. coli* abundance with pH at various stations; however, because the correlation itself and the direction of the correlation are not consistent, the detection of significance may merely be the result of chance after numerous tests were performed or the presence of an interacting factor. As expected, fecal coliforms were generally negatively correlated with salinity at several sites within the Lower Hillsborough basin. This is likely due to the high inactivation ("die-off") rate of both fecal coliforms and *E. coli* in saline waters (Anderson et al. 1979, Solic & Krstulovic 1992, Bordalo et al. 2002, Anderson et al. 2005). It should be noted that a small subset of abiotic parameter values were excluded from analysis due to apparent instrument error.

Table 2. Summary of results for the correlative analysis of abiotic parameters with indicator organism concentration for stations within Baker Creek, Flint Creek, Spartman Branch, Blackwater Creek, and the Lower Hillsborough River. Significance was assessed at an alpha of 0.05. Reported values are Pearson correlation coefficients.

Station	Indicator Organism	Temp.	pH	Salinity	Turbidity	Comments
BK1	Fecal Coliforms	-	**-0.808**	-	-	Marginally significant at p=0.052
	E.coli	-	-	-	-	
	Enterococci	X	X	X	X	
BK2	Fecal Coliforms	-	-	-	-	
	E.coli	-	**0.841**	-	-	
	Enterococci	X	X	X	X	
DBK1	Fecal Coliforms	-	-	-	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	

Station	Indicator Organism	Temp.	pH	Salinity	Turbidity	Comments
FL2	Fecal Coliforms	-	**-0.892**	-	-	
	E.coli	-	-	-	-	
	Enterococci	-	-	-	-	
FL3	Fecal Coliforms	-	-	X	n/a	
	E.coli	-	n/a	n/a	n/a	
	Enterococci	-	n/a	n/a	n/a	

Station	Indicator Organism	Temp.	pH	Salinity	Turbidity	Comments
SB3	Fecal Coliforms	-	**0.804**	-	n/a	Marginally significant at p=0.054
	E.coli	-	-	-	n/a	
	Enterococci	-	-	-	n/a	
DSB2	Fecal Coliforms	-	-	X	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	

Station	Indicator Organism	Temp.	pH	Salinity	Turbidity	Comments
BW2	Fecal Coliforms	-	-	-	-	
	E.coli	-	-	-	n/a	
	Enterococci	-	-	-	n/a	
DBW1	Fecal Coliforms	-	-	-	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	
DBW4	Fecal Coliforms	-	**-0.745**	X	n/a	Marginally significant at p=0.054
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	

Station	Indicator Organism	Temp.	pH	Salinity	Turbidity	Comments
HR1REF	Fecal Coliforms	n/a	-	n/a	-	
	E.coli	n/a	-	n/a	-	
	Enterococci	n/a	-	n/a	-	
HR2	Fecal Coliforms	-	-	-	X	
	E.coli	-	-	-	n/a	
	Enterococci	-	-	-	n/a	
HR3	Fecal Coliforms	-	-	**-0.857**	X	
	E.coli	n/a	-	n/a	n/a	
	Enterococci	n/a	-	n/a	n/a	
HR4	Fecal Coliforms	-	-	-	-	
	E.coli	n/a	-	n/a	n/a	
	Enterococci	n/a	-	n/a	n/a	
HR5	Fecal Coliforms	-	X	-	-	
	E.coli	-	X	-	-	
	Enterococci	-	X	-	-	Positive trend with turbidity (r=0.720,p=0.068)
DHR2	Fecal Coliforms	-	-	-	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	
DHR3	Fecal Coliforms	-	X	**-0.742**	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	
DHR4	Fecal Coliforms	-	-	**-0.908**	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	
DHR5	Fecal Coliforms	X	-	**0.853**	n/a	
	E.coli	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	

' - ' = Not significant
X = Not normally distributed
n/a = N<6

Section 1.3 Comparison of Indicator Bacteria

Comparisons among IO concentrations are important as the persistence, as well as the potential for multiplication, *esp*ecially in warm subtropical waters, may differ among species. For example, higher concentrations of enterococccci may be indicative of an older source of pollution (e.g., stormwater runoff) as opposed to a more recent source (e.g., SSO). Although it has not yet been scientifically documented, enterococci appear to demonstrate greater persistence and growth under certain circumstances, such as within enclosures and underground stormwater storage units (personal communication, Dr. Valerie J. Harwood, October 3, 2007).

Correlations amongst IO Suite organisms within each WBID were investigated for all locations with normally-distributed abundance data (log-transformed bacterial counts). In all cases other than New River, bacterial species were positively correlated with each other (Table 3). Pearson Product Moment Correlation Coefficients were particularly high for the Lower Hillsborough River, with greater than 80% of the variation explained by individual correlative pairings.

Table 3. Summary of results for the correlative analysis among IO Suite organism concentrations within each WBID. Significance was assessed at an alpha of 0.05. Reported values are Pearson correlation coefficients.

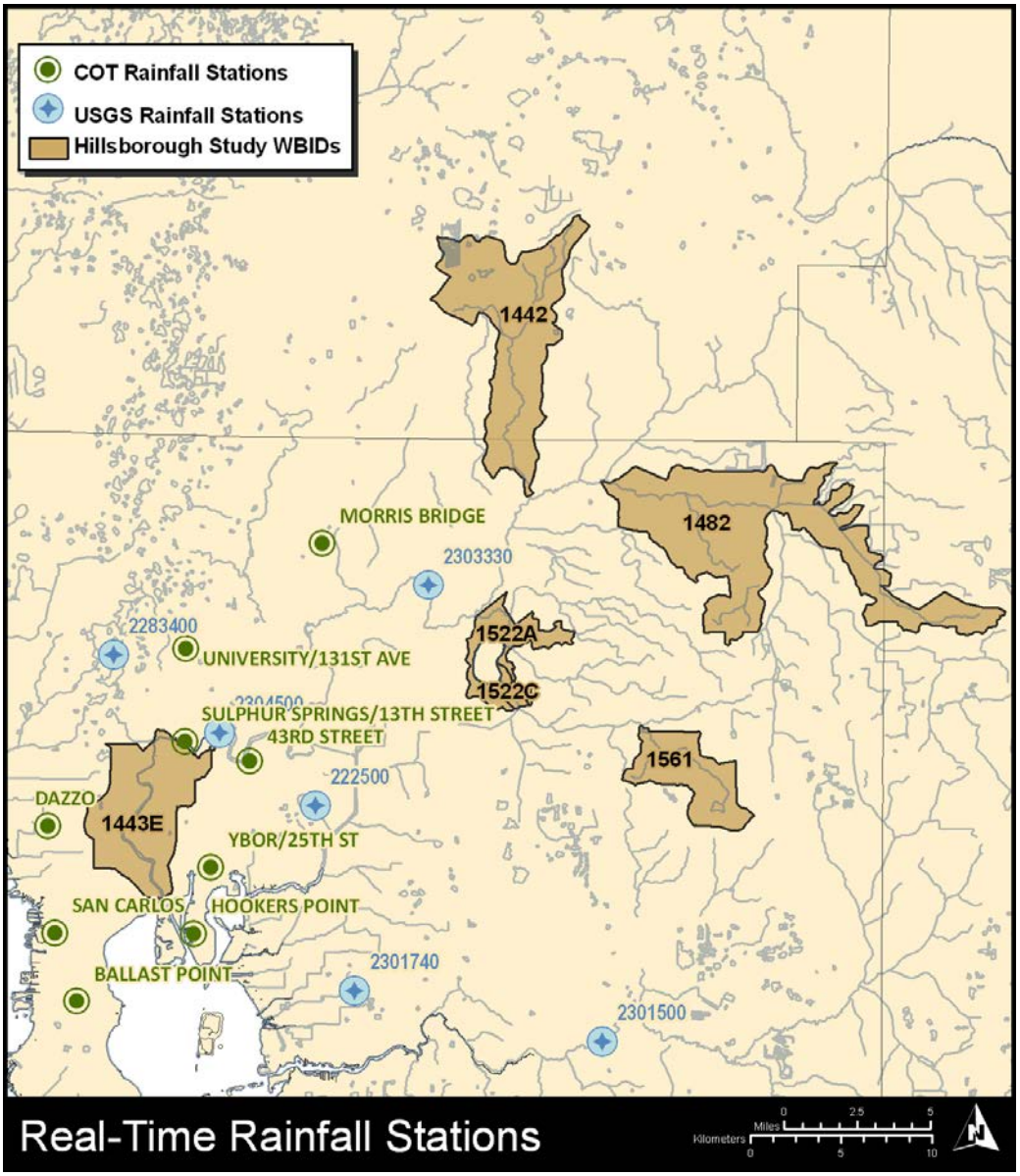
WBID	IO	Fecal Coliforms	E.coli
BK	Fecal Coliforms		
	E.coli	**0.715**	
	Enterococci	-	X (r=0.661,p=0.007)
BW	Fecal Coliforms		
	E.coli	**0.681**	
	Enterococci	**0.745**	**0.622**
FL	Fecal Coliforms		
	E.coli	**0.666**	
	Enterococci	**0.576**	**0.596**
HR	Fecal Coliforms		
	E.coli	**0.903**	
	Enterococci	**0.807**	**0.853**
NR	Fecal Coliforms		
	E.coli	-	
	Enterococci	-	-
SB	Fecal Coliforms		
	E.coli	X	
	Enterococci	X (r=0.747,p=0.001)	**0.743**

' - ' = Not significant
X = Not normally distributed

Section 1.4 Seasonal Evaluation

The occurrence of bacterial numbers with rainfall was investigated for those sampling locations within five miles of an actively-reported rain gauge (Map 1). Nine stations in three WBIDs (Hillsborough River, Flint Creek and Baker Creek) were evaluated using

time-lagged rainfall totals and each of the IO Suite organisms. Data were log-transformed as needed to meet the assumptions of normality; however, low rainfall rates contributed to non-normality in the majority of 1-day to 4-day totals. In many cases, recorded rainfall remained below an inch until week-long blocks were examined. Significant correlations with bacterial abundances were only observed in the 7-day and 14-day totals for stations within the Lower Hillsborough River; these trends were always positive in nature (Table 4). Most commonly, they reflected fecal coliform and enterococci concentrations; however, station HR2 yielded a strong correlation with 14-day rainfall and all three IO Suite organisms (fecal coliform, r=0.838; *E .coli*, r=0.907; enterococci, r=0.900).



Map 1. USGS and COT rainfall stations and Lower Hillsborough River MST project WBIDs.

Table 4. Summary of results for the correlative analysis of rainfall data with indicator organism concentration in Flint Creek, Baker Creek and the Lower Hillsborough River. Significance was assessed at an alpha of 0.05.

Station	Indicator Organism	1d	2d	4d	7d	14d	Comments
HR1REF	Fecal Coliforms	X	X	X	X	-	
	E.coli	X	X	X	X	-	
	Enterococci	X	X	X	**0.712**	-	Despite non-normal 7d rainfall data
HR2	Fecal Coliforms	X	X	-	**0.722**	**0.838**	
	E.coli	X	X	-	-	**0.907**	
	Enterococci	X	X	-	**0.908**	**0.900**	
HR4	Fecal Coliforms	X	X	**-**	**0.698**	-	
	E.coli	X	X	**-**	**-**	-	
	Enterococci	X	X	-	**0.895**	-	
DHR2	Fecal Coliforms	X	X	-	-	-	
	E.coli	n/a	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	n/a	
DHR3	Fecal Coliforms	X	X	-	**0.850**	**0.905**	
	E.coli	n/a	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	n/a	
DHR5	Fecal Coliforms	X	X	-	-	-	
	E.coli	n/a	n/a	n/a	n/a	n/a	
	Enterococci	n/a	n/a	n/a	n/a	n/a	

Note: Low rainfall led to non-normality for 1-day and 2-day totals. Normality was marginal for 4-day totals.

' - ' = Not significant
X = Not normally distributed
n/a = N<6

Station	Indicator Organism	1d	2d	4d	7d	14d	Comments
FL1	Fecal Coliforms	X	X	-	-	-	
	E.coli	X	X	-	-	-	
	Enterococci	X	X	-	-	-	
FL3	Fecal Coliforms	X	X	X	X	-	
	E.coli	X	X	X	X	-	
	Enterococci	X	X	X	X	-	

Note: Low rainfall led to non-normality of data.

' - ' = Not significant
X = Not normally distributed
n/a = N<6

Station	Indicator Organism	1d	2d	4d	7d	14d	Comments
BK1	Fecal Coliforms	X	X	-	-	-	
	E.coli	X	X	-	-	-	
	Enterococci	X	X	X	X	X	

Note: Low rainfall led to non-normality of data.

' - ' = Not significant
X = Not normally distributed
n/a = N<6

Section 1.5 Multivariate Analysis of Indicator Suite Abundance and MST Results

Multivariate analyses of bacterial community data were made using the PRIMER 6 (Windows XP) software for analysis of similarities (ANOSIM) and similarity percentages (SIMPER). The multidimensional scaling (MDS) of sample composition was based on resemblance matrices of Bray-Curtis similarities (bacterial counts were log-transformed). Global R statistics were assessed for significant MST effects (presence or absence by marker type) at $p = 0.05$. All data from both "fixed" and "flexible" stations were utilized after being filtered for missing values. Significant clusters based on MST test results (i.e., 'hits') were further examined for factors contributing to these differences using the SIMPER procedure. These approaches utilize a host of distribution free, primarily permutation-based tests, to quantify differences in multivariate datasets. A lack of restrictive assumptions make them particularly versatile in the assessment of environmental data and provide a unique alternative to more traditional parametric testing, for which many of PRIMER's routines are direct analogs. Therefore, the following analyses ask questions about sample positions in a multivariate space, thereby

incorporating considerably more of the acquired data into the resulting test statistic than would standard univariate approaches.

The effect of MST results on per-sample bacterial communities were found to be significant for human-specific *Bacteroides* (hereafter, 'human *Bacteroides*') at 'all WBIDs' and the Lower Hillsborough River; and for ruminant-specific *Bacteroides* (hereafter, 'ruminant *Bacteroides*') at Blackwater and Baker Creeks (Table 5; Figure 3). These effects were relatively small with Global R values of less than 0.537; however, SIMPER identified *E. coli* concentration as a major driver of group dissimilarity in each of the significant ruminant *Bacteroides* tests (Figure 4). In both cases, higher *E. coli* levels were correlated with the detection of the ruminant marker.

Table 5. Summary of the results showing effect of MST results on per-sample bacterial communities. Global R statistics were assessed for significant MST effects at p = 0.05. All data were filtered for missing values and grouped by WBID prior to analysis. HPyV = human polyomavirus. *esp* = enterococcal surface protein.

WBID	HPyV	Human Bacteroides	*esp*	Ruminant Bacteroides	Comments
All	-	**0.145 (A)**	-	-	
HR	-	**0.115 (B)**	-	-	
BW	-	-	-	**0.537 (C)**	SIMPER dissimilarity driven by E.coli (46.81%)
NR	-	n/a	n/a	-	
FL	-	-	-	n/a	
BK	-	-	-	**0.354 (D)**	SIMPER dissimilarity driven by E.coli (45.54%)
SB	-	n/a	-	-	

Significant global R values (ANOSIM) presented in bold; letter denotes MDS plot
' - ' = Not significant
'n/a' = no positive hits

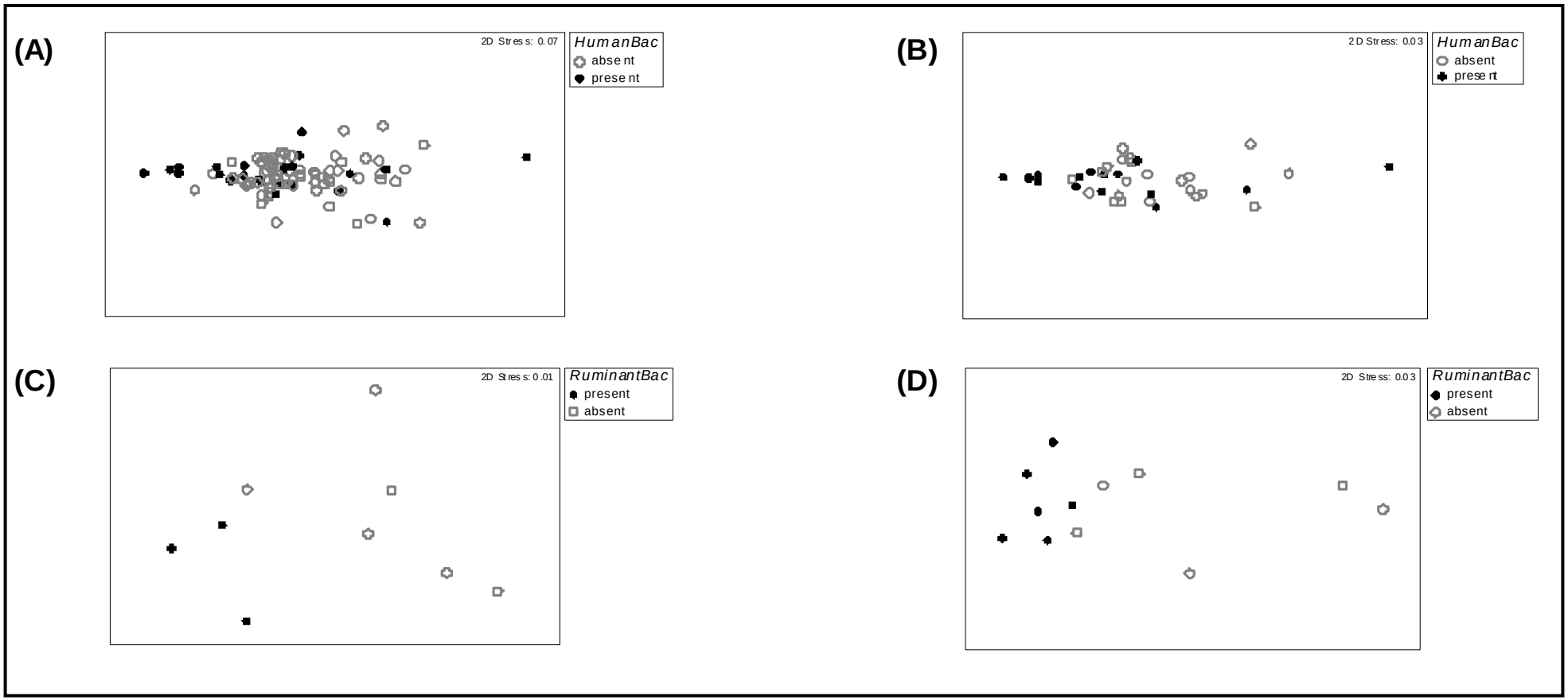
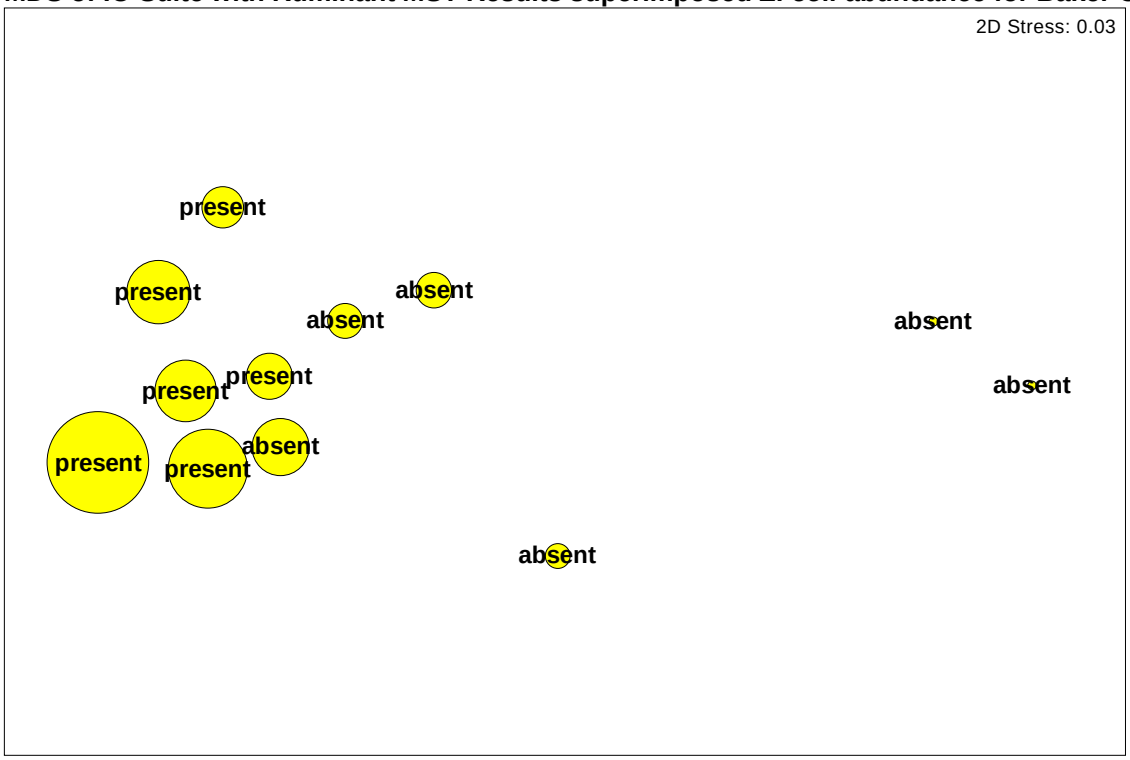


Figure 3. The effect of MST results on per-sample bacterial communities were found to be significant for human *Bacteroides* at 'all WBIDs' (A) and the Lower Hillsborough River (B); and for ruminant *Bacteroides* at Blackwater (C) and Baker (D) Creeks. Global R statistics were assessed for significant MST effects at p = 0.05. All data were filtered for missing values and grouped by WBID prior to analysis.

(A) **MDS of IO Suite with Ruminant MST Results superimposed *E. coli* abundance for Baker Creek**



(B) **MDS of IO Suite with Ruminant MST Results superimposed *E. coli* abundance for Blackwater Creek**

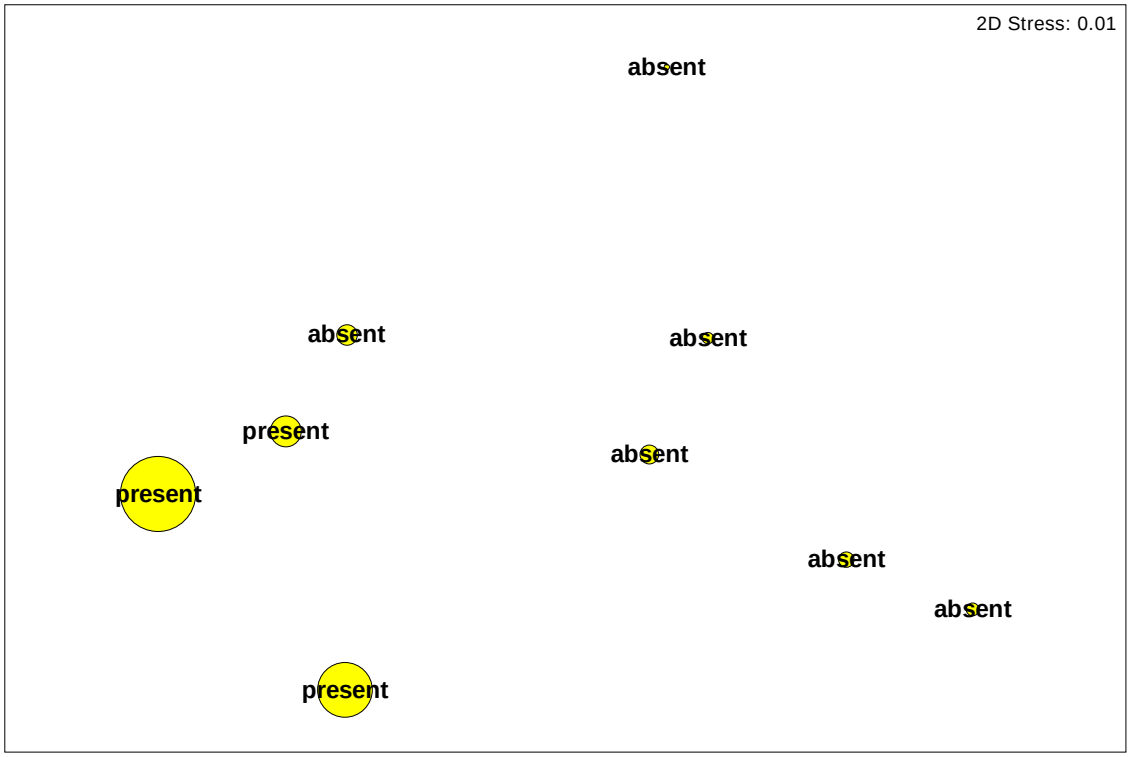


Figure 4. The effect of MST results (presence or absence by marker type) on per-sample bacterial communities was found to be significant for ruminant *Bacteroides* at (A) Baker Creek and (B) Blackwater Creek. SIMPER identified *E. coli* concentration as a major driver of group dissimilarity in both instances where higher concentrations of bacteria correlated with the detection of the ruminant marker.

A chi-squared goodness of fit test was used to further investigate the relationship between fecal coliform concentration and the MST test results (i.e., presence or absence for human polyomavirus (HPyV), *esp*, human *Bacteroides*, ruminant *Bacteroides*, and horse *Bacteroides* markers). Theoretical ratios of positive 'hits' were calculated from the number of MST sampling events with greater than 400 CFU/100mL and were recorded on a per WBID basis. These ratios were then applied to the number of MST samples (n) to produce the expected counts for positive marker occurrence. Since the null hypothesis for each WBID was constructed based on MST concordance with the state-recognized 400 CFU/100mL concentration level, a significant chi-squared test statistic (df=1, $P<0.05$) indicated a departure from this relationship. Thus, chi-squared sums less than the critical value (alpha = 0.05) for a distribution with 1-df signified that MST hits occurred in the same ratios as did samples breaching the 400 CFU/100mL limit.

Evidence for this relationship was found for four of 18 tests; however, 17 test combinations (MST versus WBID) had greater than 20% of expected values under five and were subsequently removed from the analysis. Those MST tests that showed some relation to the 400-limit were HPyV at Flint Creek ($X^2=2.79$, df=1, $P=0.094$), human *Bacteroides* at Blackwater Creek ($X^2=1.33$, df=1, $P=0.249$), and *esp* at Blackwater Creek ($X^2=3.00$, df=1, $P=0.083$) and Flint Creek ($X^2=2.65$, df=1, $P=0.105$). No other individual WBIDs, or all WBIDs combined, showed a significant relationship with the 400-limit.

Section 1.6 Co-occurrence of MST Markers

The use of several MST markers for human contamination is one means of increasing the confidence in results. Because the markers are different in terms of sensitivity, specificity, and fate in the environment (e.g. *esp* does not survive well in septic systems; viruses are smaller and more mobile in surbsurface flow than bacteria), their results in the same sample frequently differ. The confidence with which one can conclude that "microorganisms from human sources are present" (or are likely to be absent) at a given site is greatly increased when multiple markers are observed across more than one sample event.

The horse-specific *Bacteroides* marker produced some false-positive results at the beginning of the study (notably at HR sites) due to the formation of PCR artifacts. Further testing showed that the spurious PCR products were due to the formation of primer dimers. The protocol was altered to lower the primer concentration and remove the "touchdown" component of the cycle. Subsequent testing against target and non-target feces and water samples showed the reaction to be sensitive and specific for horse feces. The new protocol was used from the October sampling event on, and produced no results that appeared to be false-positives (all positives were consistent with land use).

Percentages of MST marker co-occurrence were calculated over all "fixed" sites sampled as well as stations DHR4A-10 in the Lower Hillsborough River (Table 6). Differences in average marker frequency over the sites analyzed were significantly different from one another ($p < 0.0001$). Human *Bacteroides* was detected significantly more frequently than any other marker over the study period ($p < 0.001$), possibly due to higher

concentrations in water than either *esp* or the HPyV. Although a complete understanding of the performance of the individual methods in the variety of complex environmental scenarios encountered in this study is still required, the relatively high level of detection of the human *Bacteroides* marker is also possibly due to a greater level of sensitivity of this assay as compared to the other human-specific markers (the HPyV marker is likely the least sensitive of the assays utilized in this study). In addition, occurrence of this marker was detected in 42.1% of the samples where the ruminant *Bacteroides* marker was identified, the largest percentage of co-occurrence for the project.

It is also important to note that some of the lowest levels of co-occurrence were found between *esp* and the other human-specific markers [Probability (P)(*esp*|HPyV) = 11.8%; P(*esp*|H) = 12.1%]. Although the *esp* gene is commonly found in human sewage resulting from sanitary sewer systems, it less frequently survives through the storage tanks and associated drainfields of septic systems [personal communication, Dr. Valerie J. Harwood, September 24, 2007; (Whitman et al. 2007)]. This disparity has not been observed for human *Bacteroides*. The lack of positive *esp* indicators, together with the presence of human *Bacteroides* and HPyV markers, lends support to the idea that OSTDS are a probable source. As a result, the low level of co-occurrence between *esp* and the other human-specific markers would be expected if some of the sources identified in the project WBIDs include OSTDS.

Table 6. Percentages of MST marker co-occurrence. H = human *Bacteroides*, *esp* = enterococcal surface protein, HPyV = human polyomavirus, R = ruminant *Bacteroides*. P = Probability. The percentage indicates the chance of finding the marker listed first given the detection of that shown second.

Markers Compared	Raw Data	Percentage Observed
P(H|*esp*)	4/20	20.0%
P(HPyV|*esp*)	2/20	10.0%
P(H|HPyV)	3/17	17.6%
P(*esp*|HPyV)	2/17	11.8%
P(*esp*|H)	4/33	12.1%
P(HPyV|H)	3/33	9.1%
P(H|R)	8/19	42.1%
P(*esp*|R)	2/19	10.5%
P(HPyV|R)	3/19	15.8%

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Appendix D

Photographs

PHOTOGRAPHS
New River

Photograph 1. New housing developments, serviced by central sewer, dominate the headwaters of the western branch of the New River north of SR-54.

Photographs 2 and 3. Pasturelands characterize the headwaters of the eastern branch of the New River north of SR-54.

Photographs 4 and 5. The New Beginning RV Park, located immediately south of SR-54, utilizes a septic drainfield located within 20 m of the New River.

Photograph 6. Trash was commonly observed in the main channel of the New River during the May 8, 2007 field effort. This property is directly adjacent to the channel.

Photograph 7. A slight sewage odor was detected in this area during the "Walk the WBIDs" effort on May 8, 2007.

Photograph 8. A manmade dam located between Brisk Drive and Chancey Road just upstream of a wetland that may help to mediate impacts from potential upstream sources of fecal pollution.

Photograph 9. Secondary sources of fecal pollution just south of SR-54 include wildlife and domesticated animals.

Photograph 10. Field observations indicated that there was no flow in the New River on May 7, 2007.

Flint and Baker Creeks

Photograph 11. Lake Thonotosassa control structure at the headwater of Flint Creek.

Photograph 12. New housing developments are common along the shoreline of Lake Thonotosassa.

Photographs 13 and 14. Livestock were observed standing alongside creek beds and associated wetlands during both preliminary field reconnaissance efforts (February and May 2007).

Photograph 15. Trash was observed, apparently dumped from the road, in the dry bed of Baker Creek along Kingsway Road during the May 8, 2007 field visit.

Photograph 16. Station DBK1 was characterized by highly vegetated water resulting in a greater potential for persistence and growth of bacteria.

Spartman Branch

Photograph 17. The headwater of Spartman Branch is Walden Lake, located in a large residential golf community.

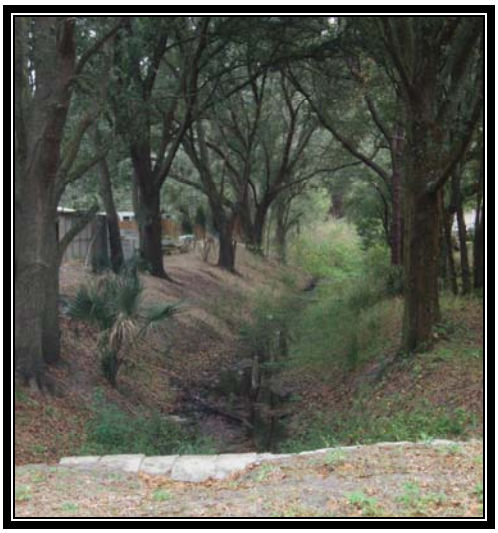
Photographs 18 and 19. City of Plant City lift station and manhole located on Airport Road in the industrial area of Spartman Branch.

Photograph 20. A discolored stormwater pond was identified at the junction of Sammonds Road and Woodrow Wilson Street during the May 10, 2007 site visit.

Photographs 21 and 22. Pasturelands and natural landscapes are characteristic of areas in Spartman Branch, especially in transition areas such as around Turkey Creek Road.

Photograph 23. Livestock are a potential source of fecal pollution in Spartman Branch, especially in the northern reaches of the WBID.

Blackwater Creek



Photograph 24. The upstream portion of the WBID is predominantly characterized by low- to medium-density residential communities.

Photograph 25. Chicken houses, such as this one on Rushing Road, are located in the upstream section of Blackwater Creek.

Photograph 26. Cone Ranch, an active cattle ranch, is the primary land use in the middle section of Blackwater Creek.

Photographs 27 and 28. Briarwood Estates is a mobile home park located in the upstream portion of Blackwater Creek and has its own wastewater treatment plant and pond system.

Photograph 29. The downstream section of Blackwater Creek includes large areas of native range, including Two Rivers Ranch.

Lower Hillsborough River

Photograph 30. Rowlett Park and associated dam structure are located just upstream of the Lower Hillsborough River northern WBID boundary.

Photograph 31. Thick algal mats are common in the Lower Hillsborough River in the Rowlett Park area.

Photograph 32. There are several significant stormwater outfalls, such as this double culvert, that line the main channel of the Lower Hillsborough River. These conveyance systems are potential sources of fecal pollution to the tributary.

Photograph 33. Homeless populations, such as this one in Riverfront Park, are common along the Lower Hillsborough River. Although these communities are probable contributors of fecal contamination, their overall impact is likely to be relatively minor and insignificant.

Photograph 34. Live-aboard vessels docked at marinas scattered throughout the Lower Hillsborough River possibly contribute relatively minor levels of fecal pollution to the tributary.

Photograph 35. This stormwater grate in the Fountain Bridge Apartment complex parking lot was consistently surcharged. Elevated bacteria concentrations and the human Bacteroides marker were detected in the water when sampled in December 2007 (station DHR10).

Appendix E

Examples of Site Classification Methods Used in Decision-Support Tool

Examples of Site Classification Methods Used in Decision-Support Tool

Step-by-step examples of the site classification methods used in this report are illustrated here using a case-study approach.

E-1 ASSIGNING INITIAL MWQA CATEGORIES

Multi-year (2001-2007) EPCHC fecal coliform data were used to assign MWQA categories to long-term monitoring stations. The category assignments are based on the percentage of samples that exceed the State's 400 CFU/100 mL fecal coliform criterion. For example, EPCHC station 152 in the Lower Hillsborough River (WBID 1443E) was assigned to MWQA category B because the percentage of samples exceeding the criterion fell between 10% and 30%.

Table E-1. Initial MWQA category for EPCHC Station 152

EPCHC Station Number	Sample Size (n)	MWQA Category Based on 2001 – 2007 EPCHC Data
152	84	B

E-2 ASSIGNING UPDATED MWQA CATEGORIES

Although a minimum of 30 fecal coliform measurements collected over a five-year period is ideal, shorter-term, between-site comparisons can be made using smaller data sets. Updated MWQA categories were assigned to stations monitored as part of the MST project. For example, EPCHC station 152 (station HR4 in the MST study) in the Lower Hillsborough River (WBID 1443E) was assigned to an updated MWQA category C because the percentage of samples exceeding the 400 CFU/100 mL criterion from that station during the MST project fell between 30% and 50%.

Table E-2. Updated MWQA category using MST project data

EPCHC Station Number	EPCHC Sample Size (n)	MWQA Category Based on 2001 – 2007 EPCHC Data	MST Project Station Number	MST Project Sample Size (n)	MWQA Category Based on MST Project Data
152	84	B	HR4	8	C

E-3 ASSIGNING INITIAL CSS CATEGORIES

Initial (Phase 2) CSS categories were assigned to the EPCHC monitoring locations, based on the evaluation of long-term data sets, GIS information, and observations from site visits made by the members of the project team and local partners during a "walk the WBID" exercise that was conducted during May 7-10, 2007. EPCHC station 152 in the Lower Hillsborough River (WBID 1443E) was assigned to a CSS category 4 (indicating a high estimated likelihood that fecal sources present at the site could pose human health risks) because chronic SSOs (five reported to have reached surface waters between 2001-2007) ranging in volume from 50 to 19,000 gallons have occurred in this area. In addition, the site is located in a high density residential area where major stormwater outfalls are present at and in close proximity the monitoring location.

E-4 ASSIGNING UPDATED CSS CATEGORIES

CSS ratings, based on more detailed site assessments and MST analysis, were assigned to each of the monitoring locations used in the MST project. In the case of EPCHC station 152 (station HR4 in the MST study) in the Lower Hillsborough River (WBID 1443E), the CSS category did not change with the inclusion of updated information.

Table E-3. Updated CSS category using more detailed site assessments

EPCHC Station Number	Original (Phase 2) CSS Category	MST Project Station Number	Updated (Phase 3) CSS Category
152	4 (High)	HR4	4 (High)

E-5 ASSIGNING CLASSIFICATION MATRIX OUTCOMES

Classification matrix outcomes, based on a combination of fecal coliform measurements (represented by the updated MWQA group) and Phase 3 CSS information (determined through detailed site assessments), were established for each of the monitoring locations used in the MST project. EPCHC station 152 (station HR4 in the MST study) in the Lower Hillsborough River (WBID 1443E), was assigned outcome C4.

Table E-4. Assignment of Classification Matrix Outcome

EPCHC Station Number	MST Project Station Number	Updated MWQA Category	Updated (Phase 3) CSS Category	Classification Matrix Outcome
152	HR4	C	4 (High)	C4

E-6 INCORPORATING GENETIC MARKER DETECTION FOR IDENTIFYING PRIORITY SITES FOR MANAGEMENT ACTION

Epidemiological evidence indicates that there is a greater likelihood for human health risk from waters contaminated by human fecal material than waters polluted by non-human sources. As a result, data on the detection of genetic markers, when available, should be used in combination with the classification matrix outcomes to prioritize individual monitoring sites for the implementation of management actions. Those sites with a very poor classification matrix outcome (E5) and a high percentage of samples showing evidence of human-specific contamination would be of the highest priority for remediation. EPC station 152 (MST project station H4) in the Lower Hillsborough River (1443E) would be of moderate priority for management actions due to a classification matrix outcome of C4 and a human marker detection rate of 67%. Overall, among the six WBIDS investigated during the MST project, WBID 1443E would have the highest relative priority for management action due to its classification matrix outcome, the frequency of human marker detections there, and the significant recreational use the WBID receives.

ERDC TN-EMRRP-SI-34
July 2009

Mammalian Survey Techniques for Level II Natural Resource Inventories on Corps of Engineers Projects (Part I)

by Chester O. Martin

PURPOSE: This technical note is a product of the Ecosystem Management and Restoration Research Program (EMRRP) work unit titled "Natural Resource Inventories for Special Status Species on Corps Operating Projects." The objective of this note is to provide information on methods for conducting inventories of mammalian species to satisfy the requirements of Level II Natural Resources Inventories for Corps of Engineers operating projects. General information is provided on survey methodologies for a variety of mammalian species, with emphasis on broad-based methods that can be used to obtain occurrence/non-occurrence data for multiple species within a community (Martin et al. 2006). Selected techniques used to survey ungulates (hoofed mammals), carnivores, lagomorphs (rabbits and hares), squirrels, large aquatic rodents (beavers, muskrats, nutria), ground-dwelling rodents, and insectivores (shrews and moles) are described. Methods to determine presence/absence and/or relative abundance are emphasized. Inventory methods for bats are addressed separately in Part II of Mammalian Survey Techniques (Martin et al., in preparation).

BACKGROUND: Conducting inventories of free-roaming animal populations is often a difficult task that requires careful planning, preparation, and execution (Figure 1). Techniques appropriate for Level II inventories on Corps projects generally provide presence/absence or trend data rather than census data. This is because estimating population size for most mammals is constrained by limitations imposed by the underlying assumptions of census techniques and/or the amount of data required for a reliable sample. Also, mammalian inventory methods used for rigorous scientific study are often prohibitively expensive and time-consuming for routine surveys on Corps lands, and usually can only be justified when there is special concern regarding sensitive species. The survey method selected for a species or species group will be influenced by (1) constraints of time and cost,

Figure 1. Surveys of free-roaming mammals require careful planning, preparation, and execution *(photo courtesy of Mike Watkins)*.

(2) objectives of the survey, (3) desired level of accuracy and precision, (4) reliability and repeatability, (5) time of year, (6) and terrain and habitat features. Although presence-absence surveys are limited in their ability to provide reliable population size data, they are often the only feasible alternative for monitoring large areas when funds are limited (Pollock 2006). Issues and innovations associated with presence-absence sampling are discussed in several recent studies (Vojta 2005, Pollock 2006, Rhodes et al. 2006, Marsh and Trenham 2008).

Mammal surveys on Corps projects have historically been conducted primarily for game and furbearing species. However, the current emphasis on biodiversity and ecosystem management requires districts to have a better understanding of nongame species and their importance to ecosystem diversity on project lands. The following sections provide general guidelines for surveying a variety of mammals that may be present on Corps projects. Emphasis is placed on small mammal and carnivore surveys because inventory methods used for game species are generally conducted in accordance with procedures established through coordination with state wildlife agencies.

OMBIL SPECIES LIST: Mammalian special status species that potentially occur on Corps projects are listed in the Corps Operations and Management Business Information Link (OMBIL) species database. The current list includes six shrews and moles (Order Soricimorpha), 23 bats (Order Chiroptera), 21 carnivores (Order Carnivora), four hares and rabbits (Order Lagomorpha), 13 rodents (Order Rodentia), two ungulates (Order Artiodactyla), one manatee (Order Sirenia), and three cetaceans (Order Cetacea). Although the current OMBIL list is incomplete, it is apparent that bats, carnivores, and small mammals (primarily rodents and shrews) are of greatest concern as special status species on most projects. Therefore, these species groups will be emphasized.

PERMITS AND PRECAUTIONS: Permits are required before sampling mammals in any location. The appropriate state agency (usually the state Game and Fish office or Department of Natural Resources) should be contacted to procure the applicable permit well in advance of the sampling event. Federal permits will be required if there is a likelihood that a federally listed species will be captured. The applicant should be aware that obtaining permits can often take weeks or even months and may require a background check, proof of technical competence regarding knowledge of the species to be collected, and references from professional sources knowledgeable of the permitee's abilities. Some states even require completion of an extensive training program before granting a permit. Trapping and handling of mammals should be conducted in accordance with the Animal Care and Use Committee (1998) of the American Society of Mammalogy. Updated guidelines on marking, trapping, housing, and collecting mammals for research are provided in Gannon et al. (2007).

The project manager must take proper precautions to protect field and laboratory personnel from being exposed to zoonoses (diseases transmittable from animals to humans) that could

potentially affect human health. Diseases of concern include lyme disease, hantavirus, and rabies, briefly described below. Other potential diseases and hazards are discussed in Constantine (1988), Kunz et al. (1996), and Cockrum (1997).

Lyme Disease. Lyme Disease (LD) is a tick-borne bacterial disease that has become a significant health problem in some regions of North America. The spiral-shaped bacterium that causes LD is known as *Borrelia burgdorferi*. The primary vector for LD in eastern and Midwestern states is the deer tick (*Ixodes dammini*) whereas the western black-legged tick (*I. pacificus*) is the major carrier in the West. Small mammals, particularly mice, are the more common hosts of the larval and nymphal stages of the tick's life cycle; deer (*Odocoileus* spp.) are the primary hosts for adults. Natural resources personnel should become aware of the potential for exposure to the disease in their area and take appropriate precautions to minimize contacts with ticks when handling wild animals. An overview of Lyme Disease is provided in Centers for Disease Control (CDC) (2007).

Hantivirus. Hantivirus pulmonary disease is an acute disease caused by viruses in the genus *Hantivirus*, which have been documented from several areas primarily in the Southwest (Mills et al. 1995, Dearing et al. 1998, Biggs et al. 2000); however, several eastern states have documented cases in recent years. The deer mouse (*Peromyscus maniculatus*) is the primary reservoir of Sin Nombre virus and related strains of hantivirus in the United States, but several other species of murid rodents have tested positive for the disease. Dried fecal material is the most likely route of transmission for the disease. Where hantivirus is a concern, personnel handling specimens should be required to wear air-purifying respirators (APRs) as a health and safety precaution. APRs typically worn during field surveys are the half-mask, twin-cartridge type (Andrews 1990). Also, leather gloves should be worn by personnel handling rodents in the field, and latex gloves should be worn when measuring and processing specimens. If traps are used to capture specimens, they should be thoroughly washed before storage or reuse.

Rabies. Rabies (*Lyssavirus*) is an acute viral infection of the central nervous system that occurs mostly in warm-blooded animals (Kunz et al. 1996). Contracting rabies from animal bites is a rare occurrence in the United States, but personnel handling mammals should be inoculated with a preventative rabies vaccine before conducting field work. The vaccine usually consists of three injections over several weeks. Animal handlers should have their titer (concentration of the vaccine in one's system) checked annually to ensure protection. This will require that blood be drawn and submitted to a qualified laboratory for testing. Booster doses are needed when the titer level drops below the acceptable level. If a person is bitten while handling any wild mammal, a physician should be consulted immediately. If possible, the specimen should be captured and submitted for testing.

PRE-SURVEY RECOMMENDATIONS: A variety of factors must be considered before conducting inventories. Objectives of the inventory must first be determined and appropriate

techniques selected to provide the most useful information. It is also critical to determine how the data collected will be stored, analyzed, and used for management purposes. When designing a survey, managers must realize that it is seldom possible to meet all sampling criteria, and methods involving direct sightings often provide incomplete counts of individual animals occupying a study area (Rudran et al. 1996). Some important aspects of inventories are provided below.

Factors that Affect Inventories. A variety of factors affect the accuracy of field surveys and reliability of data collected. Major factors that must be taken into consideration are noted below:

- Time of year (there are often significant seasonal differences in movement patterns, thus detectability, of mammals)
- Time of day (most species are best surveyed during nocturnal or crepuscular periods)
- Weather (in most cases surveys should not be conducted during inclement weather)
- Human disturbance (anthropogenic disturbances on-site and adjacent to the survey area should be taken into consideration)
- Habitat conditions (habitat type and terrain features will influence the ability to conduct inventories for different species)
- Population levels of species being surveyed (species with low population levels in an area will generally be more difficult to survey than more common species)
- Behavioral characteristics (movement patterns will often vary according to sex and age)
- Detectability of species (some species are more easily observed than others)
- Skill and experience of observers/data collectors

Consistency in Sampling. Consistency in sampling procedures is extremely important when conducting mammal surveys because of the limitations of most methods to monitor population trends. The following guidelines should be followed by in-house and/or contract personnel for all field inventories:

- Use the same methods among sample sites from year to year
- Use the same transects/plots from year to year
- Conduct inventories at approximately the same time each year
- Start inventories at the same time each day
- Be consistent with data recording from year to year
- Use standard habitat codes for reporting
- Use standard codes or scientific names instead of common names on all data forms
- Identify sample sites using consistent Global Positioning System (GPS) equipment and methodology
- Ensure that all personnel collecting data are properly trained and have the necessary skills
- If possible, the same personnel should conduct surveys during the life of a study

Species Identification. Persons conducting surveys should be thoroughly familiar with species potentially occurring in the area. If surveys are to be conducted under contract, managers should ensure that contractors have experience working with species in the region. Regional guides and diagnostic keys are available for many areas, but descriptive information may be highly variable. Recent field guides for North American mammals include Kays and Wilson (2002) and Reid (2006). Area and state museums and universities should be checked for the availability of museum mounts that can be used to verify species identification.

TECHNIQUES FOR LARGE AND MEDIUM-SIZED MAMMALS: Methods for conducting inventories of large and medium-sized mammals are highly variable. Direct observation techniques may be used for some species, but the nocturnal and secretive nature of many species require the use of indirect techniques such as scent stations, track-plates, hair-traps, and remote cameras. Techniques commonly used to survey ungulates (hooved mammals) and carnivores are first discussed below. Selected techniques with high potential for use on Corps lands are then described in greater detail. Project managers should consult with local and regional state wildlife biologists before selecting a method for use on Corps projects, especially if game species are to be surveyed.

Deer, elk (*Cervus elaphus*), pronghorn (*Antilocapra americana*), Dall's sheep (*Ovis dalli dalli*), mountain goats (*Oreamnos americanus*) and other hoofed animals are often surveyed using aerial counts from fixed-wing aircraft (Pauley and Crenshaw 2006, Udevitz et al. 2006). Moose (*Alces alces*) counts are also generally made aerially, but their habitat preferences and frequent use of forest cover make them difficult to detect from aircraft, even helicopters. Collared peccary (*Tayassu tajacu*), also referred to as javelina, have been censused using aerial surveys, road censuses, and track counts, but these methods are often not feasible in dense brush country (Boyd et al. 1986). However, Langoria and Weckerly (2007) determined presence/absence of javelina from surveys of sign (tracks and feces) in southern Texas.

Javelina and feral swine (*Sus Scrofa*) (also referred to as wild hogs or European wild boars) were detected at scent stations baited with specially prepared attractants in southern Texas (Campbell and Long 2008). Populations of feral swine have expanded throughout the United States and can result in considerable damage to natural areas by their foraging and rooting behavior (Seward et al. 2004, Wilcox and Van Vuren 2009). Thus, it is especially important that managers conduct routine inventories of feral swine when they are present on project lands.

White-tailed deer (*O. virginianus*) and mule deer (*O. hemionus*) are the most common ungulates surveyed on Corps lands (Figure 2). Census methods available for deer include the Hahn deer cruise, drive count (method in which a crew of observers move methodically through an area and count all individuals of a species detected), spotlight census, track counts, aerial surveys, pellet group counts, mark recapture techniques, harvest surveys, and browse surveys. All of these techniques have been used extensively and each provide certain advantages for estimating deer

populations. However, some methods are labor intensive, and several have limited regional application. Traditional methodologies such as drive counts and mark-recapture techniques can be costly, labor intensive, or limited to areas with high visibility (Lancia et al. 1994, Roberts et al. 2006). The spotlight census, a roadside survey in which deer are detected by shining spotlights on either side of the road from a slow-moving vehicle) is a commonly used technique for estimating white-tailed deer population size and distribution (McCullough 1982, Fafarman and DeYoung 1986, Collier et al. 2007) and has been applied to Corps lands in several regions (Mitchell 1986).

Figure 2. A variety of methods are available to inventory big-game populations *(photo courtesy of Mike Watkins)*.

Although not described in detail below, thermal infrared (TIR) imagery is being increasingly used to inventory and monitor populations of large animals. TIR imagers have shown broad potential for locating warm-blooded animals under a wide range of conditions (Boonstra et al. 1994, Garner et al. 1995, Melton et al. 2005). Collier et al. (2007) used a combination of traditional spotlight methods and thermal imagers to detect white-tailed deer in South Carolina. Roberts et al. (2006) experimented with infrared-triggered cameras in Florida and suggested that population estimates based on their data may provide an alternative to road surveys for estimating white-tailed deer densities. Drawbacks include the expense of TIR equipment and critical conditions that must be met for TIR imagery to be effective (Butler et al. 2006). For example, detection of target animals can be constrained by vegetative cover conditions and poor thermal contrast between biological objects and their background (Havens and Sharp 1998).

Carnivores are often difficult to survey because of their secretive nature, relatively low densities, nonrandom distribution, and the mobility and wariness of most species (Spowart and Samson 1986, Sargeant et al. 2003, Gompper et al. 2006) (Figure 3). Survey methods often used for carnivore inventories include mark-recapture techniques, aerial surveys, bounty and harvest records, road kills, predator calls, and counts of sign. Several mark-recapture procedures have been used, but all are expensive and labor intensive. Furthermore, the results are often biased by short retention times of marks, insufficient sample sizes, non-random sampling, and variability of an individual's susceptibility to capture and/or recapture. Aerial surveys, den surveys, and track counts are generally impractical in densely vegetated areas. Noninvasive methods such as remote cameras, hair snares, and scat surveys are being increasingly used to collect extensive data on carnivore occupancy, distribution, and abundance (Long et al. 2007).

Roadside Counts for Rabbits. Roadside counts are most often used to survey lagomorphs (hares and rabbits) and may be useful for detecting some ungulates and carnivores. When used to survey rabbits, the roadside count generally consists of driving along secondary roads in the evening or early morning and observing the eyes of animals that are reflected from the vehicle's beams or a spotlight. The "eye shine" resulting from the spotlight and subsequent "freeze" of the animal permits easy counting and species identification (Chapman and Willner 1986). The survey should be conducted during the daily peak of rabbit activity because small differences in rabbit numbers may be undetectable when populations are low (Chapman et al. 1982). The method can provide an index to relative abundance and may be used as long as factors such as time of day, time of year, and weather conditions remain constant. Rabbit density estimates can be obtained using mark-recapture techniques or drive counts (Davis and Winstead 1980); however, these methods are labor-intensive and not generally suitable for routine inventories on Corps projects.

Figure 3. Carnivores are difficult to survey for several reasons, including their secretive nature and wariness of humans *(photo courtesy of Mike Watkins)*.

Scat/Fecal Pellet Surveys. Counts of fecal material have traditionally been used in surveys of lagomorphs, carnivores, and some ungulates. Chapman and Willner (1986) found that pellet counts for lagomorphs were more useful for determining habitat preference than for estimating density. Fecal pellet-plot methods have been used extensively in snowshoe hare (*Lepus americanus*) studies, but pellets are subject to variable rates of decomposition (Murray et al. 2005), and snowshoe hare pellet-density relationships may not be constant over large distances and across ecoregions (Homyack et al. 2006). Mills et al. (2005) determined that pellet sampling was more suitable for areas with low hare densities. Also, rabbits and some carnivores often exhibit coprophagic behavior (consumption of feces). Livingston et al. (2005) cautioned that coprophagy and other animal behaviors that result in selective removal of feces may alter findings of population estimates based on fecal analysis. For example, feces may be an important source of food for opossums (*Didelphis virginianus*) and other species (Livingston et al. 2005).

The success of scat surveys to determine presence-absence of carnivores is highly variable dependent on species and habitat conditions. Detector dogs specially trained to locate scat have been used in studies of kit foxes (*Vulpes macrotis*), grizzly bears (*Ursus arctos*), black bears (*U. americanus*), and fishers (*Martes pennanti*) (Harrison 2006). Long et al. (2007) compared the use of cameras, hair snares, and scat detection dogs for detecting black bears, fishers, and bobcats in Vermont, and found that scat detection dogs yielded the highest raw detection rate and

probability of detection for each of the target species. Gallant et al. (2007) advised extreme caution when interpreting data from scat surveys to monitor relative population size for certain species, such as river otters (*Lontra canadensis*) due to behavioral characteristics associated with use of latrine sites.

Scent Station Survey. The scent station survey is an indirect technique used to determine species presence and to obtain an index of relative abundance of carnivores and other furbearers (Johnson and Pelton 1981, Warrick and Harris 2001). The technique offers a standardized, repeatable, cost-effective method for inventorying predator populations on large tracts of land, and has been used in many areas to survey populations of bobcat (*Lynx rufus*), coyotes (*Canis latrans*), foxes, raccoons (*Procyon lotor*), opossums, and other mesocarnivores (Conner et al. 1983, Leberg et al. 1983, Leberg and Kennedy 1987). The method basically consists of a lure and tracking medium, usually composed of soft earth or sand (Linhart and Knowlton 1975) or a track plate (Zielinski and Kucera 1995, Zielinski and Stauffer 1996). Appendices A and B provide examples of a scent station survey form and a worksheet for calculating abundance indices. Measurements of relative abundance obtained through scent station surveys are based on the assumption that a consistent relationship exists between visitation rates at the station and actual population density. However, this relationship will vary from survey to survey due to a variety of factors. In most cases, changes in scent station indices must be documented with several years of data before one can reasonably assume that an increase or decrease in the population of a species has occurred. Sargeant et al. (2003) cautioned against the use of cluster sampling (systematic deployment of closely spaced scent stations in lines to reduce travel time and expedite data collection) because it tends to reduce the precision of estimated visitation rates. Also, all species will not be equally attracted to scent stations; for example, Harrison (2006) found that scent station surveys usually resulted in very low detection rates for bobcats. Crooks et al. (2008) used track and camera surveys (discussed below) to provide baseline information on distribution, activity, and habitat associations of mammalian carnivores in Arizona, and determined that the combination of track and camera data was effective in detecting a variety of species in a range of habitat types.

Remote Cameras and Track Plates. Automatically triggered cameras have been deployed to determine presence and estimate density for a variety of species (Cutler and Swann 1999). For example, Zielinski and Kucera (1995) used a combination of remote cameras and carbon-sooted aluminum track plates to monitor populations of American marten (*Martes americana*), fisher, lynx (*Lynx canadensis*), and wolverine (*Gulo gulo*). Hilty and Merenlender (2004) used unbaited, remotely triggered cameras to determine occurrence and compare habitat use of mammalian predators in northern California. Heilbrun et al. (2006) found that automatically triggered cameras provided reliable data on bobcat abundance not previously available without physical capture and radiotelemetry. However, human activity, scent, and the presence of equipment can potentially alter animal behavior and bias results of species photographed. Larrucea et al. (2007) found that the amount of human activity, location of cameras on roads versus trails, and habitat

type influenced the number of photo-captures of coyotes at unbaited camera stations in California. Zielinski et al. (2006) concluded that although track plates (and cameras) are better at discriminating species that are readily detectable, neither method can achieve the goal of estimating the population size of target species. Thus, these methods are best used to determine the occurrence and distribution of species in an area. Examples of survey data forms for cameras, track plates, and snow tracking are provided in Appendices C and D (after Zielinski and Kucera 1995).

Hair-snares. Hair-snares (scented devices upon which animals deposit hair) are now commonly used to detect and obtain DNA information on a variety of carnivores such as black bears, brown bears, Canada lynx, bobcat, martens, fishers, coyotes, wolves (*Canis lupus*), and mountain lions (*Puma concolor*). Other mammals such as Woodrats (*Neotoma* spp.), red squirrels (*Tamiasciurus hudsonicus*), and beaver (*Castor canadensis*) have also been detected using hair snares (Zielinski et al. 2006). The method usually consists of stations where hair is snagged with barbed wire or glue in open baited sites or traps (Depue and Ben-David 2007). Zielinski et al. (2006) compared wire and glue hair snares for identifying mesocarnivores in California and found that glue snares were more effective at collecting hair from most species. Downey et al. (2007) stated that hair-snare sampling has become a common practice for assessing the distribution and abundance of felids, but reported that marking by gray foxes (*Urocyon cinereoargenteus*) may interfere with the tendency of felids to face-rub at sampling stations. Harrison (2006) reported that hair snares were not as effective as other methods for detecting bobcats. Depue and Ben-David (2007) found hair-snare traps to be more effective than traditional methods for sampling river otter populations. Zielinski et al. (2006) concluded that with future snare development, both glue and wire snares may prove useful for multi-species inventory. DNA obtained from hair snares has been used successfully to determine distribution patterns and estimate population size for many carnivores, especially bears (Boerson et al. 2003, DeYoung and Honeycutt 2005).

Time-Area Count for Squirrels. Squirrels are extremely difficult to survey, and no method has proven effective under all, or even most, conditions. Observational and trap-success methods have most often been used in squirrel surveys, but most techniques are best suited for intensive, local studies. A combination of techniques may be desirable to obtain the best information for a given area. Mark-recapture methods are frequently used for intensive studies, but they are labor-intensive and not practical for routine surveys (Teaford 1986). The squirrel time-area count is a direct time-lapse census method used to census both eastern gray squirrels (*Sciurus carolinensis*) and fox squirrels (*S. niger*). The technique basically consists of observers positioning themselves in forested habitat and recording the number of squirrels seen during a specific time period. The process is repeated at a series of plots located along a predetermined transect. Although time-area counts have been found to underestimate squirrel populations (Flyger 1959, Bouffard and Hein 1978), they do provide acceptable information for management purposes and have relatively low manpower requirements. Temporal differences in squirrel activity and observer bias can influence counts, and the user should be aware of the following limitations to the technique: (a) not all members of a population are active at the same time, (b) not all active individuals are

visible to the observer, and (c) counts made in different cover types, by different observers, or during different seasons may not be comparable (Flyger 1959). Counts of squirrels inhabiting known den sites, artificial nest boxes, and leaf nests are often used to supplement time-area count data.

Surveys of Sign for Aquatic Rodents. Inventories of beaver, muskrats (*Ondatra zibethicus*), and nutria (*Myocaster coypus*) require specialized techniques. The presence of beavers along drainages is generally determined by recording beaver dams, lodges, or cuttings. Beavers that occur in western streams may live in bank dens and not build dams, but their presence should be evident from tree- and shrub-cutting activities. Call (1986) recommended making population estimates of beavers by cruising streams in October or November, counting active colonies (evidenced by food caches and repairs on dams and visible lodges), and multiplying the number by five. Aerial surveys of food caches and lodges may be required in backwater areas and relatively inaccessible drainages. Muskrat surveys often consist of counting the number of houses in a marsh, which can be used as an index to the population. However, muskrats construct different types of lodges (Dozier 1953, MacArthur and Aleksiuk 1979), and surveyors must be able to distinguish between active and inactive houses, and between feeding and dwelling lodges. Detecting the presence of muskrats that live along streams and use bank burrows is more difficult. Their occurrence in these areas is best detected by cuttings of vegetation on which they feed (Call 1986). Trapper surveys are often used to provide information that can help managers obtain a general estimate of beaver and muskrat populations.

The nutria or coypu is a large aquatic rodent native to South America that has been introduced throughout the United States and in many other countries (Carter and Leonard 2002, Bertolino et al. 2005). Nutria cause damage to water control structures, crops, and marshlands, and are considered a disease host (Carter et al. 1999, Carter and Leonard 2002), thus monitoring their populations and activity has become an important part of management and control programs in areas where the species is abundant. Mark-recapture, direct observation, tagging methods, and radiotelemetry have been used to monitor nutria activity (Nolfo and Hammond 2006, Myer 2006), but most monitoring practices are labor intensive and have met with limited success. Nevertheless, project managers should arrange to have routine observations made in wetland areas to detect increases in nutria populations.

TECHNIQUES FOR SMALL MAMMALS: A variety of techniques are used to inventory small mammal populations as part of research projects, but some methods may be too costly and labor intensive for routine inventories on Corps projects. Call (1986) stated that there are few situations that require information other than species occurrence and relative abundance of rodents and insectivores for Federal land management programs. Exceptions include inventories needed for federal or state protected or sensitive species, surveys of species that serve as an important prey base for raptors or carnivores whose numbers are critical or declining in a region,

and population estimates needed for species of economic or social value. Small mammal surveys may also be needed for mitigation purposes or when habitat enhancement is a project objective.

Investigators should use extreme caution when interpreting the results of mammal surveys. All individuals in a project area are rarely captured when surveying small mammals, thus estimates of population size based on capture data may not be reliable (Slade and Blair 2000, Hopkins and Kennedy 2004). Nichols and Conroy (1996) stated that trapping methods can provide satisfactory results for temporal and spatial comparisons of abundance for a single species but may not be reliable for providing comparative information about species richness. However, Hopkins and Kennedy (2004) reported that measures of relative abundance provided patterns of population trends proportional to those derived from estimates of absolute abundance. The manager should make every effort to select appropriate sampling techniques and ensure adequate replication through time to help eliminate bias and provide results that can be used for management decisions.

Trapline Transects. Surveys of most rodents and some insectivores can be accomplished by systematic trapping along transects. Occurrence of species within different vegetative communities can be determined by setting an appropriate number of traps within the interior of each community and along edges. Trapping results will provide information on species presence and relative abundance of species within each habitat type. An estimate of population density may also be possible if certain designs are used and appropriate statistical analyses are factored into the sampling effort. The following guidelines apply generally to small mammal surveys where trapping methods are used.

- Describe vegetation communities and soil types at sample locations. If habitat types are to be compared, an appropriate number of traps should be set in each community.

- Establish transects within each vegetation community. Each transect usually consists of trap stations that are 15 m apart but this may vary with terrain features and target species. The transect length can vary, but 15 stations are usually adequate. Call (1986) recommended establishing a series of grids for sampling; however, this would not be necessary if the objective of the survey is to simply obtain presence/absence information. Figure 4 shows trapline transects established to compare rodent use of upland and riparian habitats on White Sands Missile Range, New Mexico (Martin et al. 2004). Trap placement will depend on a variety of factors, including sample objectives, terrain features, and habitat conditions. For example, Manley et al. (2005) placed extra-long traps 15 m apart and down the center of a sample hexagon for presence-absence monitoring in the central Sierra Nevada region.

- Set traps at each station. It is best to set at least two traps at each station because different species will be active at different times of the night. “Sherman live-traps” are

recommended if specimens are to be released after capture (Jones et al. 1996), but some species are difficult to catch with live traps. Therefore, depending on the objectives of the survey, there may also be a need to use snap traps, such as "museum specials" or "Victor rat traps." Extra large live-traps can be used to increase capture rates of larger-bodied squirrels (Slade et al. 1993).

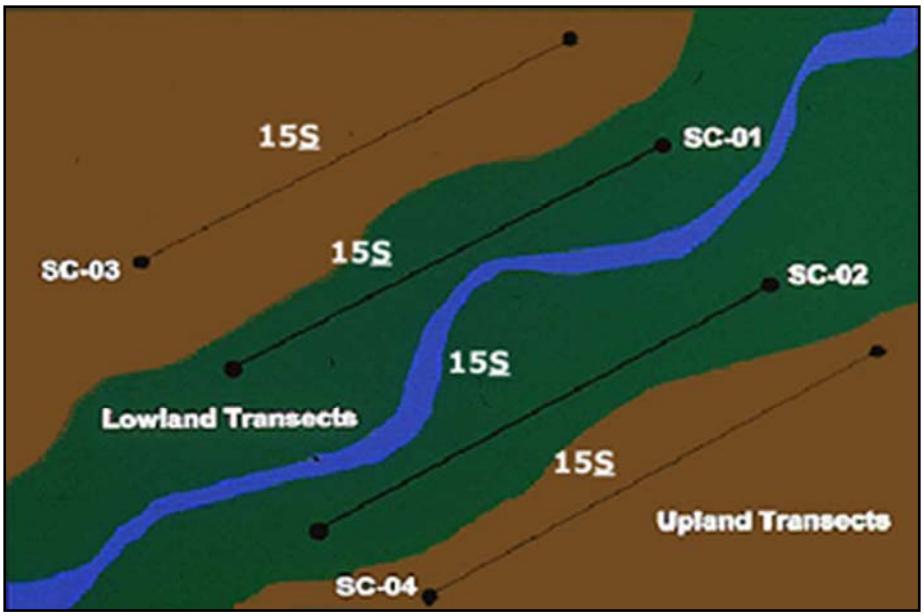


Figure 4. Trapline transects designed to compare rodent use of riparian and adjacent upland habitats.

- Trap baits are variable and some experimentation may be required to determine what works best. Common baits often include rolled oats and peanut butter or grains and seed mixtures. Cotton can be placed in traps to insulate captured animals from cooler temperatures. This is necessary in some areas to prevent mortality.

- Set the traps in early evening and check them every hour until midnight, if possible. This will allow removal of most of the easily caught animals and increase the chance of catching less abundant or more trap-shy animals for the rest of the night. Set the traps again at midnight and leave them until morning. Timing may need to be modified if trap lines are set at remote locations or if they are located far apart. Often it is only possible to open and bait traps in the early evening and check them the following morning. Traps should always be closed during the day unless a survey objective is to trap diurnal rodents.

- Trap-lines should be run for at least two consecutive nights, and preferably three consecutive nights, if possible.

- Tabulate all captures by species, sex, and age (sub-adult or adult). If needed, standard measurements (including total length, tail length, hind foot length, ear length, and weight) can also be obtained and information can be recorded on reproductive conditions. Catches will usually be expressed as numbers of each species per number of trap nights. If the same trapping design is used in different communities, species and relative density can be compared among different community types.

The field biologist should be aware that the above recommendations will often have to be modified because of costs, manpower constraints, and logistical problems. For example, although sampling for three consecutive nights is desirable, it may be more important to sample a greater number of sites than to obtain a third replicate. Extreme care should be taken when interpreting the results of small mammal surveys. The best results will be obtained when surveys can be conducted during several seasons for at least three consecutive years using the same crew. However, this may not be feasible for routine natural resource inventories. Surveys conducted with less intensity are best used only to provide a general estimate of species presence or non-presence within broad habitat types, but it should be realized that absence cannot be absolutely determined without a considerable amount of effort. Mackenzie (2006) and Strickland and McDonald (2006) emphasized that a major concern with using presence-absence data is that an animal may be declared absent from an area because the animal is not detected, not because it is actually absent, and hence could result in erroneous management decisions. Jones et al. (1996) recommended 500 trap nights/habitat as a minimum for determining presence-absence for inventory purposes, but this may vary according to region.

Factors that can affect conclusions drawn from sampling small mammal communities include trap type, trap arrangement and location, trapping method, and type of bait used (Osbourne et al. 2005). There is considerable disagreement in the literature regarding the success of transect versus grid trapping for sampling rodent communities. Pearson and Ruggiero (2003) found that transects resulted in more total captures of small mammals, more individuals of abundant species, and greater species richness compared to grids. Although grids provide better spatial resolution for estimating population density, home ranges, and dispersion, transects provide better information on community composition and habitat relationships (Pearson and Ruggiero 2003). Jones et al. (1996) stated that the easiest way to array traps is along a transect and recommended this procedure for inventory of small terrestrial mammals. However, transect sampling is not suitable for density estimation under most circumstances (Jones et al. 1996).

The Sherman live trap and the Longworth trap are probably the most widely used commercial traps for sampling small mammals (Anthony et al. 2005). The Longworth trap is a two-piece model consisting of a nesting-chamber box attached to a tunnel with a treadle. The Sherman live trap is a simple folding box trap that operates on a door-and-treadle system and is available in several sizes (Figure 5). Trap type and arrangement will depend on specific objectives of the survey. For example, Moore and Swihart (2005) used a combination of Sherman, Fitch, and

Tomahawk live traps set in grid patterns to assess habitat occupancy in forest patches in modified landscapes in Indiana. Tomahawk live traps set in a grid with 50-m spacing between points were installed 1.5 m high on trunks of large trees to sample northern flying squirrels (*Glaucomys sabrinus*) in California (Meyer et al. 2005). Guilfoyle (2006) established grid points and trap lines on Corps project lands to obtain estimates of relative abundance and diversity of small mammals in Habitat Management Units (HMUs) in eastern Washington. Trap lines consisted of four trap stations with five traps set per station. Each HMU was sampled for at least three nights so that a minimum of 500 trap nights per habitat was obtained (Guilfoyle 2006).

Figure 5. Sherman live traps are commonly used to sample small mammals *(photo courtesy of Mandy Like)*.

Pitfall Traps. In some areas shrews and moles are difficult to capture with snap traps or live traps baited with rolled oats or grain. One of the most effective methods for collecting shrews and some rodents is through the use of pitfall traps, commonly used to sample reptile and amphibian populations. The technique basically consists of sinking a series of buckets in the ground in strategic locations, and often includes placement of plastic or metal flashing (referred to as a drift fence) to funnel animals into the buckets. For best results, buckets used for collecting insectivores should hold more than 1 gallon and have a round aperture in the center, which will help reduce the possibility of escape. Setting up a trapping array with either three or four arms (extensions of flashing material, Figure 6) will capture shrews and some other small mammals in most areas. Trapping arrays should be set so that each bucket is connected by a drift fence of wire mesh or tin about 25.4 cm high and set 3-4 cm into the substrate (Call 1986). Various pitfall designs for sampling small mammals are described in Bury and Corn (1987) and Mengak and Guynn (1987).

Bury and Corn (1987) determined that pitfall trapping had several advantages over traditional traplines and that data obtained from pitfalls could be used to assess species presence and relative abundance among forest stands. Osbourne et al. (2005) collected 20 species of mammals in West Virginia using pitfall traps set in upland, edge, and riparian habitats; they concluded that sampling for inventory and monitoring purposes should be stratified by edge and interior locations to provide the best representation of diversity and abundance of small-mammal populations. A major disadvantage of pitfall trapping is that it is labor intensive and some species are not easily captured in pitfall traps; however, once installed they can be run with minimal effort. Another concern is that animals held in pits may become easy meals for snakes and other

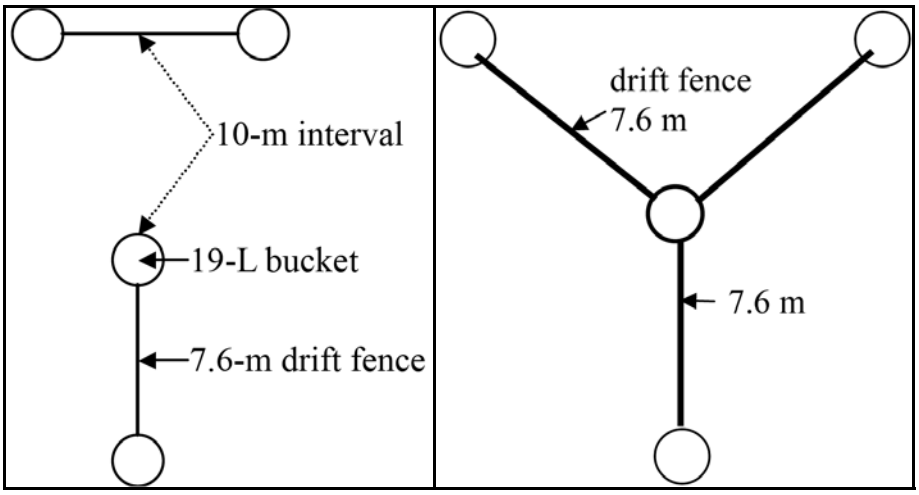


Figure 6. Trapping arrays used for pitfall traps.

predators, but this can be minimized by checking traps frequently during the evening. Ferguson et al. (2008) detected 10 predator species visiting pitfall arrays in south-central Texas, with the raccoon being the most frequently recorded species.

SUMMARY AND CONCLUSIONS: Thorough Level II inventories of mammals on Corps project lands will generally require the application of a variety of methods. As much as possible, techniques should be selected that obtain data for a variety of species (e.g., scent stations, track plates, hair snares, den surveys, transect surveys) rather than single species. Unless there is a need to obtain population data, methods that are most efficient at determining presence/non-presence should be selected. Level II mammal surveys will often need to be contracted out because conducting the surveys will require a commitment of time and labor that may be beyond the capability of project personnel.

Game species are generally surveyed annually by state wildlife biologists, and any method used to inventory these species should be coordinated with the appropriate state agency. Big game animals are often surveyed by state biologists using aerial counts from fixed-wing aircraft. Other traditional methods for large and medium-sized mammals include drive counts, spotlight counts, pellet group counts, mark-recapture techniques, harvest data, and browse surveys. However, many techniques designed to obtain census data are beyond the capability of project personnel and may only be needed for specific situations. Radio-telemetry and TIR imagery are increasingly being used to monitor populations of large mammals. Infrared-triggered cameras (ITCs) are a rapidly developing technology that may provide a viable alternative to wildlife managers because they can be economically used within a random or systematic sampling design (Roberts et al. 2006).

Carnivores are often difficult to inventory because of their secretive nature, relatively low densities, nonrandom distribution of populations, mobility, and wariness of human activity. Survey methods often used for carnivores include mark-recapture techniques, aerial surveys, bounty and harvest records, road kills, predator calls, and counts of sign. Noninvasive methods (e.g., remote cameras, hair snares, track plates, scat surveys) are being increasingly used for carnivore surveys. Each of these methods offers certain advantages, but results may vary from survey to survey due to a variety of factors. Automatically triggered cameras have been used to determine presence and estimate density for a variety of species, but human activity, scent, and the presence of equipment can alter animal behavior and bias results.

Surveys of small mammals can generally be accomplished by systematic trapping along transects. Occurrence of species within different habitat types can be determined by setting an appropriate number of traps within the interior of each community and along edges. Transects usually consist of trap stations 15 m apart, but this may vary with terrain features; transect length can vary, but 15 stations per transect is usually adequate. Sherman live traps are recommended unless there is a need to collect voucher specimens. Trap-lines should be run for at least two consecutive nights, and preferably three consecutive nights, if possible. However, it may be more important to sample a greater number of sights than to obtain a third replicate. The best results will be obtained when surveys can be conducted during several seasons for at least three consecutive years by the same crew. Pitfall traps are necessary for capturing some species, especially shrews. Thus, it may be best to use a combination of standard trap stations and pitfall traps to obtain complete information on the occurrence of small mammals on an area.

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Appendix A
Scent Station Survey Form

Observer(s):_____ Date: _____

Route/Transect:_____ Compartment:_____ Stand: _____

Name of Area (if any): _____

Weather on Night of Operation: _____

Species Identified at Scent Station							
	Red Fox	Gray Fox	Fox sp.	Coyote	Bobcat	Other(s)	Habitat
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							

Instructions: Place an X in the box of each species that can be positively identified at the scent station. If red and gray fox tracks cannot be differentiated, place an X in the Fox sp. column. Write the names of other species in the space provided. Record only species whose tracks you can positively identify. If the station is disturbed by weather or other causes, consider it inoperable and record "IN" next to the station number. Record the dominant habitat types(s) where the scent station is located in the last column. Use the following habitat codes: LS = pine; OGC = bottomland hardwoods; OH = upland hardwoods; OP = mixed pine-hardwoods; TBH = shrub flats; AG = cropland; PA = pasture; OF = old field. More than one habitat type may apply. For example, if the station is on the edge of an old field next to a stand of bottomland hardwoods, record OF/OGC. The first abbreviation should be the habitat type in which the station is actually located.

Appendix B
Worksheet for Calculating Annual Abundance Indices Using Scent Station Data

Project:_____ Date:_____

Compartment:_____ Transect: _____

Prepared by: _____

Species	Total Number of Stations Visited by Species	Total Number of Operable Stations	Total Number of Stations Visited/Total Number of Operable Stations	Abund. Index TNSVx1000 TNOS
Red Fox				
Gray Fox				
Coyote				
Bobcat				
Raccoon				
Opossum				
Rabbit				
Dog				
Armadillo				
Skunk				
Deer				
Other				

Appendix C
Survey Record Form for Camera, Track Plate, and Snow Track Sampling

Survey Type:

CAMERA_____ TRACK PLATE_____ SNOW TRACKING _____

Line Trigger_____ Enclosed_____ Searching for tracks_____

Single Sensor_____ Unenclosed_____ Tracking at bait _____

Dual Sensor_____

Other_____

SAMPLE UNIT NUMBER_____

Number of Stations_____ or Distance searching for tracks _____

State_____ County_____ Landowner_____

Location_____ USGS Quad _____

Legal: T_____ R_____ S_____ , _____ , _____ , _____ .

STATION LOCATIONS: UTM Zone _____

Station ID	UTM N/S	UTM E/W	Elevation (ft/m)

Vegetation type (s) _____

Date installed (or run)_____ Date Terminated _____

Type of bait or scent _____

Name, address, and phone of investigator _____

_____

_____

* After Zielinski and Kucera (1995)

Appendix D
Track Plate Data Worksheet*

Observer_____ Weather[a]_____ Date_____ Page_____ of _____

Location _____

General Comments _____

Station Number	Visit Number	Nights since last visit	Target Species[b]	Other tracks of interest	Comments[c]

[a] Use the following codes: 1 = No precipitation since last visit; 2 = rain, snow, or heavy fog since last visit.

[b] Record the four-letter species code in pencil (e.g. MAAM, for marten) until identity is confirmed.

[c] E.g. box rolled, feces collected, bait removed, bait desiccated

* After Zielinski and Kucera (1995)

WEC144

UF UNIVERSITY of FLORIDA
IFAS Extension

Florida Monitoring Program: Point Count Method to Survey Birds[1]

Mark E. Hostetler and Martin B. Main[2]

Background

Ornithology is the scientific study of birds. It is one of the few fields where information comes not only from trained scientists, but also from the cooperation between students, bird watchers, and scientists. Our general knowledge about birds is in part due to such cooperative efforts. Examples of cooperative efforts include the Audubon Christmas Bird Count, the Breeding Bird Survey, Project Feeder Watch, and the Breeding Bird Atlas. These efforts greatly enhance our ability to conserve birds in North America.

Bird projects usually fall into three categories: inventory, monitoring, and research. People conduct inventory projects to generate a list of species. Birds are identified by visual observation and/or song. Monitoring projects record birds in a region or study site over an extended period of time. Such projects use specific procedures to survey birds in exactly the same way each time. This is critical for comparing information over time. Research is more involved than inventory or monitoring projects, but inventory or monitoring techniques typically are employed in research projects. Research begins by formalizing a question into a hypothesis that can be tested with a study. For example, a hypothesis may be "variation in woodpecker abundance is in part due to variation in tree density." He or she then designs a study, collects and analyzes data, and discusses the results in terms of whether tree density affected woodpecker density.

In terms of monitoring birds, the point count method is used in all types of bird projects. Point counts are used to record a variety of birds, including those species that may not visit a feeder. It is a simple method that provides a uniform way of counting birds over time or across locations. In large areas, randomly allocated point counts can be used as representative samples for the area. Point counts are visited over a period of several days or longer to assess how many and what types of birds are in an area. To increase accuracy, one increases the quantity of point counts and the number of days a point count is repeated.

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Generally, point counts are used to compare bird differences between sites. They can be used to monitor changes in bird populations when an area is changed. They also can be used to study seasonal and annual fluctuations in bird populations. Point counts often are utilized in areas where transects are impractical. A transect is a survey method where a researcher walks a route of a determined length and counts birds on either side of the route. Transects are not practical if it is difficult to walk through a landscape or the area of interest is too small.

Monitoring projects may not begin with explicit questions about the systems being studied. However, the results are often used to generate questions that are answered with additional research. In fact, point count surveys can be used in many educational programs. Participants can look at results and develop hypotheses about why differences occur between the sites of interest. For example, one might find that woodpeckers visit Homeowner A's yard but not Homeowner B's yard. One can visit each of the homeowner's yards and look for habitat differences between the two yards. Unique hypotheses could be developed and tested. This may lead to recommendations for ways to improve Homeowner B's yard to attract woodpeckers. This comparative approach is an effective way to evaluate the impact of landscape changes on bird populations.

Further, one can evaluate the success of wildlife habitat improvements that have been made on a property. One conducts a point count before habitat changes have occurred. Then, one conducts point counts periodically over a number of years. By comparing the initial number of bird species to future numbers, one can get an idea of how changes in the landscape affected bird species on a given piece of property.

The important thing to remember is that the point count method is a standardized method of surveying birds. To insure the reliability of any comparisons, each person should conduct a point count in *exactly* the same way.

Florida Monitoring Program

The objective of the Florida Monitoring Program is to develop a database that is linked to a Web site where people can enter and view collected environmental and ecological data. Homeowners and participants from various natural resource, Cooperative Extension, and state education programs are encouraged to participate. The initial focus will be on birds. However, this initial effort will be used as a pilot to expand to other variables (such as insects, mammals, water quality, vegetation, etc.). One potential benefit of this project may be the production of a database that could be used in a state-wide monitoring effort.

The idea behind this program is to create a Web site for participants to share, view, and display data. This will allow people to interact, pose questions, compare results, discuss, and develop suggestions of how to improve their local environmental condition. For people who have gone through various extension and educational programs, this program will promote the continuation of critical thinking and learning. Participants will be able to compare their results with others. Such data comparisons lead to the development of hypotheses, alternative strategies, and solutions. For example, the University of Florida's **Backyard Wildlife Habitat** (http://www.wec.ufl.edu/extension/fblw/index.htm) and **Florida Yards & Neighborhoods** (http://hort.ufl.edu/fyn/) programs teach people ways to ecologically and environmentally improve the design and maintenance of their yards. A collection component, where data are displayed on a Web site, will promote interest and excitement among the participants. The “fruits” of their labor could be monitored and displayed, allowing a community of like individuals to interact.

Point Count Protocol

Point Counts: A point count consists of standing in a specific location and counting birds. One counts the number of individual birds (of each species) within a circle of a certain radius. In most cases, especially when gathering data to compare one point count to the next, radius size should be consistent. But what radius to choose? The radius

should be as large as possible to maximize information gathering, but not so large that birds cannot be seen or heard throughout the survey area. Also, landscapes are very different from one survey site to the next. It is difficult to select a radius that works for every situation. For this reason and based on our experiences, we suggest participants use a radius of 20 meters (65.5 ft.) for most situations. Keeping the surveyed areas the same makes comparing different point counts that much easier in the long run (Figure 1).

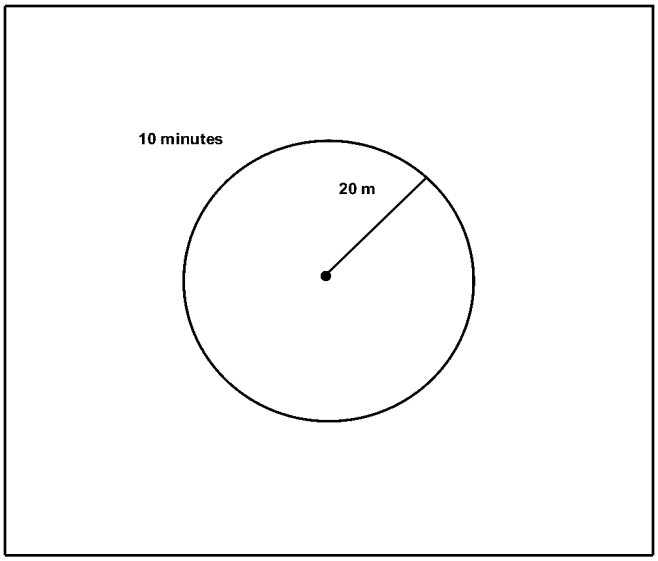

Figure 1. A typical 20 m (m=meter) radius point count where one person counts all the birds seen or heard within a 10 minute period.

In some cases, due to obstacles, the entire circle may not be possible to survey. Try to find a location where you can survey 100% of the circle. If this is not possible (such as a backyard with high walls where birds cannot be seen on the other side), just report the percentage of the circle that could be surveyed. Alternatively, various geometric areas could be surveyed (e.g., rectangles, squares, or triangles). If not using a circle, try to survey an area that equals the area of the 20 m radius circle (1256 m^2).

As mentioned above, situations are different from one area to the next. Although we encourage participants to conduct a circle point count with a 20 meter radius, one can survey birds using point counts of different sizes and shapes. Certain landscapes and situations may warrant utilizing a larger or smaller radius. There are ways to account for different point count sizes when attempting to make comparisons across different sites. Thus, any point count size and shape will work, especially if one is primarily interested in tracking birds over time at one site. The important point is to keep the size of the point count the same from the start. Changing the size of the point count in the middle of your monitoring effort would bias the data. This would have to be accounted for in final analyses.

Important Note: If you would like to do a point count with a *larger* radius, here is what we suggest. Record all birds that are seen or heard within the normal 20 meter radius. At the same time, record birds that are heard or seen within a selected distance outside this 20 meter radius. The birds counted within the 20 meter radius would be marked in the "Number of Birds" column of the data sheet (see Table 3). Birds seen or heard outside the 20 meter radius (within a specified distance) are marked in the "Outside" column of the data sheet.

For example, let us assume that a person wants to record birds within a 40 meter radius. The observer could separately record birds seen or heard within the 20 meter radius and within the 20 to 40 meter band that is outside the 20 meter radius circle. Be sure that the same bird is not counted twice during the survey. A bird that moves from the 0 - 20 meter area to the 20 - 40 meter area should not be counted twice. Count it where it first appeared.

This is a more difficult survey because one is counting birds within two different areas at once. One is counting birds within a 20 meter radius and also within a circular band outside this 20 meter radius. The purpose of surveying birds in this manner is that it allows one to compare across sites. A 40 meter radius count that contains birds seen within 20 meter radius can be compared to other 20 meter counts.

If you choose to do this, please fill-in the distance that you consistently count birds outside the 20 meter radius. This will be marked in the Site Registration section under "Optional, enter survey distance beyond 20 meters" (see Table 1). Write in the distance beyond the 20 meter radius.

Time of Counts: As with the survey area, the time devoted to point counts must be consistent. For the Florida Monitoring Project, point counts will last 10 minutes. We suggest conducting 1-3 counts per month, but one can do more if desired. Any number of months could be counted during a year. Counts should be done within three hours after sunrise. This is when birds are most active. One can also do nighttime surveys to count nocturnal species (done within three hours after sunset). During point counts, record all birds seen and heard within the survey area.

Counting the Birds: Once the survey has started, record all birds that are seen or heard within the point count. You can use your own codes to mark down the birds during the survey as long as you transcribe your codes to the full common name. One can also record, in the Outside Column (see Table 3), birds heard or seen during the count beyond the boundaries of the survey area. This is done on a casual basis. It does not represent a consistent survey of birds at a certain distance outside of the 20 meter radius (see Important Note above). This is especially useful for birds with loud calls that carry long distances, such as hawks or owls. After a count is completed, attempts should be made to identify all birds whose identity was in question.

Starting the Count: Approach your survey location quietly. Once you are at the center of the survey circle, wait for 2 minutes before you start recording birds. This allows you to get oriented, and it allows the birds to acclimate to your presence. Upon entering your point count, record on your data sheet all birds that where flushed from the survey area.

Counting FT (fly-thrus): All birds that fly through a point count area (below the tallest structure in a census area) but do not land on any structure should be counted as FT. However, if you are sure that the flying bird came from somewhere in the point count, do not count it as an FT. Record the number of birds that are FT in the FT Column of the data sheet.

Counting FO (fly-overs): All higher-flying birds (above the tallest structure in a census area) should also be noted if they are within the boundaries of the point count area. Record the number of birds that are FO in the FO Column of the data sheet.

Counting Birds Outside of Survey Area: Only birds seen or heard within the point count area should be recorded (if a bird is 1 meter outside the survey area - do not count the bird). Remember, to make results comparable, each person needs to survey birds exactly the same way. However, if it is an unusual bird or a rather vocal bird, count it in the Outside column of the data sheet. You can also note how far away this bird was heard or seen on the Additional Notes section of the data sheet. Note: If you have reserved the Outside column for birds counted within a certain distance beyond the 20 meter radius (see Important Note above), then birds heard outside the total point count area can only be recorded on the Additional Notes section. For example, birds seen or heard within a 20 - 40 meter band would be tallied in the Outside column. Birds heard beyond 40 meters would be recorded in the Additional Notes section.

Estimating Abundance: When multiple sightings of a species occur within a point count, only include multiple entries for a species if you are reasonably certain they are different individuals. Only count different individuals of a given species. All recorded species in the data sheets are assumed to be separate individuals (example: 5 house sparrows means that 5 different house sparrows were sighted). Provide estimates for large flocks of birds (e.g. blackbirds, grackles, etc.). Be sure to note that they are estimates in the Additional Notes section.

Unidentified Birds: Unidentified birds are listed as such with the closest taxonomic affiliation that can be determined, for example, Unidentified Warbler or Unidentified Sparrow. It should be emphasized that this type of recorded data is very important and can be used to estimate which type of birds are found in the area (mark on the data sheet as Unidentified _____ (fill-in closest taxonomic affiliation of the bird). Avoid counting each unidentified bird more than once. For example, recording two Unidentified Warblers means two different, warbler-like individuals entered your point count area. This is helped by noting (mentally or otherwise) anything you can about the bird (e.g., size, direction last seen, any behaviors, etc). Also, if you record an unidentified warbler, but also saw, for example a yellow-rumped warbler, notes on general coloration

or behavior of the unidentified bird could justify naming the unidentified warbler as a yellow-rumped.

Weather: When conducting the point count, record general climatic conditions. Record wind intensity (estimate its strength: no wind, slight, gusty, strong wind), temperature (Fahrenheit), and estimate percent of cloud cover (e.g., 50 % cloud cover). This is important because climatic variables are known to affect bird activity. Avoid counting birds if it is raining or if it is extremely windy.

Additional Notes: The Additional Notes section at the bottom of the data sheet is there to record anything unusual or interesting. Record bird behaviors, dramatic changes in the habitat, etc. Notes will not be entered into data through the Web site but it is there for your own use.

Clothing Color: Clothes worn should be drab and non-colorful. Bright colors may attract curious birds, or warn others away.

Avoiding Artificial Densities: Do not use sounds that can attract birds to your site. No "spishing", "squeaking", recorded calls, or any other methods that encourage birds to show themselves or to investigate the observer. This would result in artificial densities of birds.

Set-up and Practice: Before conducting the point count, mark the boundaries of the area with some flagging or use some identifiable object (e.g., corner of building, a large tree, etc.). Also, mark the spot where you stand and observe birds. One should return to this exact place each time. Do several practice point counts. This will help you know the boundaries of your area, and you can identify any potential problems with the area that you will be surveying.

To Participate in the Florida Monitoring Program: You will need to get a User ID and a Point Count Code for your point count. Before starting the point count, please contact Dr. Mark Hostetler at 352-846-0568 or hostetlerm@wec.ufl.edu and indicate whether you are connected with an Extension program, a school, a private or public organization, or just on your own. Also, please include your phone number and E-mail address. After contacting us, we will send you a User ID and Code.

To enter survey data, you must use a fairly recent version of **Netscape Navigator** as your browser. You can download the most recent version for free from the Netscape website: http://www.netscape.com/.

For each Point Count, you need to fill out who established the site, where the site is, contact information (E-mail and phone number), and a general landscape description. Please fill out (completely) the forms on the website (see example in Tables 1 and 2). This is done only once, *before* entering the actual bird count data. The site description will allow people to evaluate habitat differences between sites and also will help people know where the survey was conducted.

Please Note: You can start the surveys *before* obtaining the User ID and Code (just save the data sheets). An example data sheet is provided in Table 3. A blank data sheet is provided in Table 4. You just need the ID and Code to enter the data online: **http://bird.ifas.ufl.edu**. Before surveying the birds, please read all of the information above.

For More Information

For more publications on wildlife and other topics, go to the UF/IFAS **EDIS** website at http://edis.ifas.ufl.edu.

Table 1. Site Registration

User ID _____ (use the same ID if you have multiple surveys)
Enter Site Code _____
Name _____
Email _____
Phone (optional) _____

TYPE OF POINT COUNT:

If Circle, enter radius of circle _____m
Enter percentage of circle actually surveyed _____

If Other, enter approximate shape of point count _____
Enter total area actually surveyed _____m^2

Optional, enter survey distance beyond 20 meters _____ m

Associated with a University of Florida Extension Program?
If yes, enter program name (e.g., Master gardener): _____

Associated with a school?
If yes, enter name of the school: _____

Associated with any other private or public organization?
If yes, enter name of organization: _____

Table 2. Habitat description within the point count

The below is to obtain a rough description of vertical habitat structure. Within your site, estimate the percentage of structure at each of the three vertical layers.

0 – 0.15 m
_____% gravel or bare soil,
_____% pavement or building
_____% lawn
_____% other vegetation (e.g., ivy)

0.15 – 2.0 m

_____% shrubs, small trees or other vegetation

> 2.0 m

_____% tree canopy

Table 2. Habitat description within the point count

Human-made bird structures:

Number of Hanging or Post-mounted Seed Feeders _____
Number of Hummingbird Feeders _____
Number of Suet/Peanut Butter Feeders _____
Number of Platform Feeders _____
Number of Bird Baths _____
Number of Bird Boxes _____

Landuse:

Enter the overall landuse designation in which the site is located
(e.g., single family, multi-family, school, park, industrial, etc.)
_____

Table 3. A sample data sheet

Date: 4/7/99 **Point Count Code:** AAMG-MH1
Weather (wind intensity, temp, and cloud cover): gusty, 50 F, no clouds
Primary Observer's Name: Mark Hostetler

TIME START: 7:50 AM				
TIME END: 8:00 AM				
SPECIES	Number of Birds	FT	FO	Outside
House Sparrow	15	1		
Mourning Dove	8			
House Finch	10		1	
Red-bellied Woodpecker				1

Additional Notes: Red-bellied woodpecker heard 20 meters outside point count area on a large oak

Table 4. Blank data sheet

Date: _____ **Point Count Code:** _____
Weather (wind intensity, temp, and cloud cover): _____
Primary Observer's Name: _____

TIME START:				
TIME END:				
SPECIES	Number of Birds	FT	FO	Outside

Additional Notes: _____