Tributary Pollution Assessment Summary Report

FINAL JULY 2006

A report prepared for:

The
Tributary Assessment Team

Jacksonville, Florida

Prepared by

PBS&J

7406 Fullerton Street, Suite 350
Jacksonville, Florida 32256

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List of Acronyms

BMAP	Basin Management Action Plan
COJ PWD	City of Jacksonville Public Works Department
COJ EQD	City of Jacksonville Environmental Quality Division
DCHD	Duval County Health Department
DEP	Department of Environmental Protection
EPA	United Stated Environmental Protection Agency
ESP	Enterococcal Surface Protein
FOG	Fats, Oils, and Grease
HOA	Home Owner's Association
JEA	Jacksonville Electric Authority
MST	Microbial Source Tracking
PCR	Polymerase Chain Reaction
rep-PCR	Repetitive Extragenic Palindromic Polymerase Chain Reaction
RA	Reasonable Assurance
SRJWMD	St. Johns River Water Management District
SSO	Sanitary Sewer Overflow
TAT	Tributary Assessment Team
USF	University of South Florida
WBID	Water Body Identification
WSEA	Water and Sewer Expansion Authority

Section 1. Introduction

The State of Florida has identified 194 tributary Water Body IDs (WBIDs) in Duval County; i.e., small to large tributaries of the St. Johns River. Fifty-one (51) of these WBIDs do not meet State of Florida water quality standards for fecal coliform contamination and are listed as impaired waters. The Tributary Pollution Assessment Project is a continuation of the effort started under the Reasonable Assurance (RA) Plan. The RA Plan participants have been brought together to form the Tributary Assessment Team (TAT).

PBS&J was contracted to work in cooperation with the Tributary Assessment Team (TAT) toward the common goal of sufficiently reducing coliform levels in impaired waterbody segments to restore their recreational use status. The TAT is comprised of representatives from JEA, the Florida Department of Environmental Protection (DEP; Northeast District Office), the City of Jacksonville Environmental Quality Division (COJ EQD), the City of Jacksonville Public Works Department (COJ PWD), the Duval County Health Department (DCHD), the current and former St. Johns River Keepers, the Water and Sewer Expansion Authority (WSEA) and the University of South Florida (USF).

The project objective was to develop and evaluate an approach for assessing the source(s) of fecal pollution in the WBIDs of Duval County and provide a manual for conducting site assessments and evaluating potential sources of contamination. Microbial source tracking is an emerging field of microbiology which is focused on determining the dominant sources of fecal pollution and/or indicator bacteria to water bodies (Harwood et al., 2000; Harwood et al., 2003; Whitlock et al., 2002). A decision-tree or tiered approach relying on a “toolbox” of microbial source tracking (MST) methods provided the data needed for a weight-of-evidence assessment of the contribution of various potential sources to indicator organism concentrations within these waters. MST methods that rely upon genotypic typing of indicator organisms and differences in genetic/DNA sequences were utilized in this study. The TAT tested and field-verified the methodology through site assessments of seven selected tributary water bodies (including a control tributary utilized to determine the accuracy of the assessment techniques) and revised the *Tributary Pollution Assessment Manual* (hereafter referred to as the Manual) based on lessons learned from this process. In addition to designing a blueprint for conducting site assessments, the TAT identified appropriate corrective actions that could be implemented to reduce fecal coliform levels in an attempt to restore the WBIDs to their designated recreational use. Since most of these

WBIDs will have a total maximum daily load (TMDL) for fecal coliforms established by the State, it is likely that the Manual will serve as an integral part of the Basin Management Action Plan (BMAP) for these WBIDs.

Section 2. Methodology

The following was the strategy for developing and evaluating a methodology for conducting tributary pollution assessments for listed water bodies in the Jacksonville, Florida (Duval County) area as referenced in the RA Plan. The approach was divided into six major phases including:

1) Pre-planning;
2) Planning;
3) Development of Tributary Pollution Assessment Manual;
4) Evaluation of Methodology/Manual by Conducting Sanitary Surveys;
5) Summary Report; and
6) Public Workshop.

The Pre-Planning phase (Phase I) entailed four main goals: 1) to obtain and review all documents included in the RA Plan; 2) to develop categories for tributary classification and categorize the 51 priority WBIDs; 3) to overlay each WBID onto land use, infrastructure, and historical sampling maps to begin assessing probable sources and migration pathways; and 4) to develop the Draft *Work Plan*. The Planning phase (Phase II) included the organization and initial meeting of the TAT and the finalization of the *Work Plan*.

The Development of the Manual phase (Phase III) primarily involved the formulation of the assessment methodology for each tributary type described in the Pre-Planning phase, the design of a decision-tree to determine how the assessment methodology best corresponded to each of the selected WBIDs, and the establishment of a model monitoring plan for each tributary category.

The next phase, Evaluation of Methodology/Manual by Conducting Sanitary Surveys (Phase IV), entailed field-verification of the methodology described in the Draft Manual (for the selected water bodies for each category) and application of the results to recommend generic corrective actions and revise the methodology as necessary. The outcome of this phase was the final version of the Manual.

The final two phases, Summary Report (Phase V) and Public Workshop (Phase VI), provide a summary of the results of the tributary pollution assessments, including a discussion of lessons learned, identification of site-specific corrective actions for each tributary investigated, and communication of the results and the Manual to the public. The final phase follows the completion of this report and will also include a written summary of public input received at the workshop.

2.1 PHASE I: Pre-Planning

As noted above, the Pre-Planning phase (Phase I) entailed four main goals: 1) to obtain and review all documents included in the RA Plan; 2) to develop categories for tributary classification and categorize the 51 priority WBIDs; 3) to overlay each WBID onto land use, infrastructure, and historical sampling maps to begin assessing probable fecal coliform bacteria sources and migration pathways; and 4) to develop the Draft *Work Plan*.

Individual meetings were held with each of the TAT members to gain an understanding of their previous involvement in the RA Plan, the intended level of involvement with the Tributary Pollution Assessment Project, any existing databases of relevant information (particularly GIS databases), knowledge of probable sources and priorities (of pollution to the WBIDs), and any particular concerns of their agency regarding the project. In addition, each entity updated Attachment 14 of the RA Plan (Specific Actions for Priority WBIDs). Meeting summaries (including action items) were generated as a result of each of these individual meetings. Table 2.1 provides details on each TAT member's past involvement in the RA Plan and the current or anticipated role and contributions at the time of their interview. Existing databases were retrieved (to the maximum extent practicable at the time) from the various agencies and were used in the initial development of categories and eventual categorization of the 51 priority WBIDs. The consolidated database was continually updated throughout the project. A summary of compiled information and the corresponding source is shown in Table 2.2.

Table 2.1 TAT Member Involvement (Previous and Intended)

Agency	Representative	Past Involvement in RA Plan	Intended Level of Involvement with Tributary Pollution Assessment Project	Current Data Contributions
JEA	Brad Russell	Involved since inception Maintenance of SSO database	Proponent Analytical and informational SSO data - SSO historical data shows no preponderance of individual spots	Existing GIS database including JEA infrastructure data
COJ EQD	Dana Morton	Responsible for majority of data in RA Plan	Be kept informed of decisions regarding sanitary surveys Possible participation in initial field sampling events	Four quarters of fecal data on COJ EQD website COJ sampling station coordinates
COJ PWD	Kehinde Adeshile	Supplied information regarding COJ drainage projects and NPDES programs	Attend TAT meetings and be actively involved in project Provide assistance / requested information	COJ drainage project GIS information
DCHD	Scott Turner	DOH has been working with Surface Water Improvement and Management program (SWIM) since ~1998 on septic tank phase-outs SWIM program provides quarterly reports to DEP	Attend TAT meetings and possibly provide additional information regarding specific sites	Septic Tank Failure and Nuisance Area GIS data

Table 2.1 TAT Member Involvement (Previous and Intended)

Agency	Representative	Past Involvement in RA Plan	Intended Level of Involvement with Tributary Pollution Assessment Project	Current Data Contributions
DEP	Tom Kallemeyn	Involved since inception: - Original categorization of the WBIDs - Development of initial sampling protocol - Assignment of specific actions for each participating agency - Sample collection and analysis	Willing to re-initiate samples and fecal analysis for this project	DEP sampling station coordinates Fecal monitoring data
St. Johns Riverkeepers	Neil Armingeon	Involved since inception: - Assisted in drafting RA Plan - Driving force behind the city posting health advisories for 9 creeks in Duval County	Reliant on COJ and reliant DEP for data Meeting participation and input	
WSEA	Fred Odom, Sherry Hall	No previous involvement	No independent data generated Participation in TAT meetings Anticipates an information-sharing relationship	GIS information for septic nuisance and failure areas WSEA Phase I Conceptual Plan map

Table 2.2 GIS Data		
Data	**Description**	**Source**
SSO	Sanitary sewer overflow	JEA
Cave-Ins	Pipe cave-ins	JEA
Drainage Projects	City drainage projects	COJ PWD
Agricultural Zones	Parcels zoned agricultural	JEA
Proposed Pipe Repairs	Proposed pipe repairs and line extensions	JEA
Sewer Footprints	Footprints of pipe bursting project areas	JEA
Sewer Infrastructure	Sewer mains, lift stations, and manholes	JEA
Pipe-Bursting Projects	Completed Pip-Bursting Projects	JEA
Historical Sampling Data	Tributary sampling stations and historical fecal data (1985 to 2005)	COJ
Stormwater Infrastructure	Stormwater Infrastructure	COJ PWD
Septic Tank Repair Permits	Onsite Sewer Treatment and Disposal System (OSTDS) repair permits issued by date	DOH
Contamination Sites	Sites with contamination problems	DEP
Septic Tank Failure Areas	Location of septic tank failure and nuisance areas	DOH
Landfills and Dumpsites	Duval County landfills and dumpsites	DOH/DEP/COJ
Illicit Connections	Illicit Connections	COJ PWD
Land Use / Land Cover	Land use and land cover classifications as of 2000	SJRWMD
Hydrologic Features	Water features, 1:24,000	SJRWMD/COJ PWD
WBIDs	WBID boundaries	DEP

Land use classifications obtained from the St. Johns River Water Management District (SJRWMD) were used to develop categories of WBIDs. These categories were intended to focus source assessment efforts and essentially constitute a pre-assessment based on the best available existing data. The land use classifications were aggregated into three primary categories: Urban, Suburban, and Rural. Land use data (percent land use for each classification) were used to categorize each WBID, based on the highest proportion of aggregated use (Urban, Suburban, or Rural). The data obtained as part of this effort were used to determine if each WBID had a putative source of fecal coliform contamination. A ‘putative’ source included at least one of the following: 1) Repetitive, recent and localized Sanitary Sewer Overflows (SSOs) that intersected surface waters, 2) a septic nuisance and/or failure area adjacent to, or intersecting, a waterbody, or 3) targeted land use (e.g., cattle farms, aquaculture, marinas). Although the sampling methodology was the same for all WBID categories (discussed in detail in the *Tributary Pollution Assessment Manual*), this categorization scheme provided validation that the sampling methods were useful and appropriate in a variety of land use/pollution source scenarios. Therefore, the WBID categories were as follows:

1a. Urban – Putative Source

1b. Urban – No Putative Source

2a. Suburban – Putative Source

2b. Suburban – No Putative Source

3a. Rural – Putative Source

3b. Rural – No Putative Source

The resultant categorizations of the 51 priority WBIDs are included in Table 2.3, and shown on Figure 2-1.

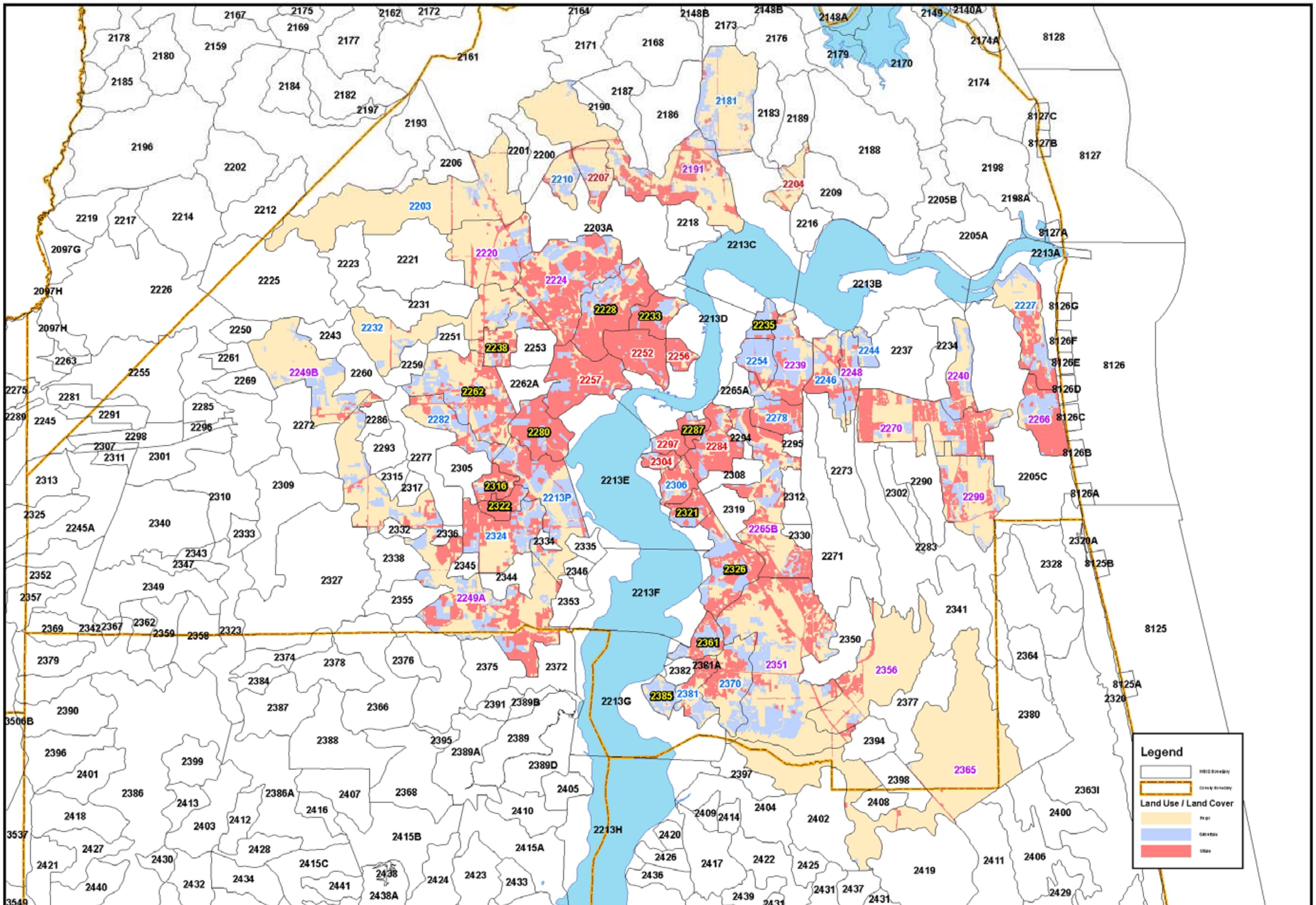
One WBID from each category was selected for evaluation during Phase IV of the project (ground-truthing of the draft *Tributary Pollution Assessment Manual*). The selection process was based on an effort to include those WBIDs that: 1) posed a significant public health issue; 2) geographically represented all of Duval County; and 3) were not undergoing reconstruction that could have impacted the assessment. Thomas Creek was selected as a control WBID (relatively unimpacted by human activities) and was used to verify that analytical methods did not yield false-positive indication of specific pollution sources. The selected WBIDs are shown in color in Table 2.3 which is based on the revised rankings of the WBIDs for 2000-2004 data. The priority numbers are from the original ranking (1998-2002 data) to show how they have been revised over time. The ranking system is based on the combined percent exceedance of each tributary for three separate thresholds (800, 5000, 10000 CFU/100 ml) over a five-year period. For example, each WBID is ranked individually based on the percent exceedance for each of the three thresholds. The sum of the three rankings is used to determine a combined rank for each WBID, utilized for this table. This method allows for the discrimination of those WBIDs that not only demonstrate a high occurrence of exceedances, but also by the highest levels of fecal coliform contamination.

These activities were completed in preparation for Phase II, and were used in the remaining Phases, as described below.

Table 2.3 WBID Categorization and Selection based on Revised Rankings for 2000-2004 Data

Unit	Name	WBID	Original Priority #	Category	Reason
NMS	Craig Creek	2297	1	1a	Failure area
NMS	Deer Creek	2256	1	1b	
NMS	**Miramar Creek**	**2304**	**1**	**1a**	**Failure area**
NMS	Miller Creek	2287	2	1b	
NMS	**Terrapin Creek**	**2204**	**1**	**3a**	**Agriculture**
NMS	**New Castle Creek**	**2235**	**2**	**2b**	
NMS	Hogan Creek	2252	1	1a	SSO
OR	Big Fishwier Creek	2280	2	1a	Nuisance area
NMS	Gin House Creek	2248	4	2b	
SMS	Mandarin Drain	2385	2	2b	
NMS	Little Pottsburg Creek	2284	1	1a	Failure area
NMS	Christopher Creek	2321	2	1b	
TR	Moncrief Creek	2228	2	1a	Nuisance and Failure areas
NMS	McCoy Creek	2257	1	1a	Failure area
TR	**Blockhouse Creek**	**2207**	**1**	**3b**	
OR	**Butcher Pen Creek**	**2322**	**2**	**1b**	
SMS	**Deep Bottom Creek**	**2361**	**2**	**2a**	**Nuisance area**
OR	Williamson Creek	2316	2	1a	SSO
NMS	Cow Head Creek	2244	3	2b	
NMS	Long Branch	2233	2	1b	
NMS	Goodbys Creek	2326	2	1a	Failure area; Marina/Fish Camp
TR	Little Sixmile Creek	2238	2	3a	Failure area
TR	West Branch	2210	3	3b	
OR	Wills Branch	2282	3	3a	SSO
NMS	Silversmith Creek	2278	3	1a	Nuisance areas
IC	Hopkins Creek	2266	4	1b	
IC	Sherman Creek	2227	3	3b	
JC	Cormorant Branch	2381	3	1b	
NMS	Red Bay Branch	2254	3	2a	Failure area
NMS	Jones Creek	2246	3	1a	Aquaculture
NMS	Dunn Creek	2181	3	3b	
NMS	Broward River	2191	4	3a	Failure area
OR	Fishing Creek	2324	3	1a	Nuisance area; Agriculture
NMS	Strawberry Creek	2239	4	2a	Nuisance area; SSO
TR	Trout River	2203	3	3a	Agriculture; Marina/Fish Camp
IC	Open Creek	2299	4	3b	

Table 2.3 WBID Categorization and Selection based on Revised Rankings for 2000-2004 Data					
Unit	**Name**	**WBID**	**Original Priority #**	**Category**	**Reason**
OR	Cedar River	2262	2	3a	Marina/Fish Camp
TR	Sixmile Reach	2232	3	3a	Agriculture
OR	Ortega River P	2213	3	3a	Failure areas; SSO; Agriculture
NMS	Pottsburg Creek B	2265	4	1a	Nuisance and Failure areas; SSO; Agriculture
NMS	New Rose Creek	2306	3	1b	
JC	Oldfield Creek	2370	3	3a	Failure area; SSO
OR	McGirts Creek B	2249	4	3a	Agriculture
IC	Hogpen Creek	2270	4	2a	SSO; Marina/Fish Camp
TR	Ribault River	2224	4	1a	Nuisance and Failure areas; SSO
JC	Durbin Creek	2365	4	3b	
OR	Ortega River A	2249	4	3a	Failure area; SSO; Agriculture
TR	Nine Mile Creek	2220	4	3b	
JC	Big Davis Creek	2356	4	3b	
JC	Julington Creek	2351	4	3a	Failure area
NMS	Greenfield Creek	2240	4	3b	
Category					
1a	Urban, Putative Source				
1b	Urban, No Putative Source				
2a	Suburban, Putative Source				
2b	Suburban, No Putative Source				
3a	Rural, Putative Source				
3b	Rural, No Putative Source				
Possible Reasons for Categorization:					
-- Water body intersects or is adjacent to nuisance/failure area					
-- At least two SSO events-most recent (2004 or later) with at least one recorded surface water impact					
-- Agricultural area, marina, fish camp or aquaculture adjacent/intersecting water body					
-- Selected for evaluation (Phase IV) NMS = North Main Stem SMS = South Main Stem OR = Ortega River TR = Trout River IC = Intracostal JC = Julington Creek					



7406 Fullerton Street, Suite 350
Jacksonville, Florida 32256
(904)363-6100 Fax (904)363-8811

WBID Land Use / Land Cover Classifications

Figure 2-1

File path : I:\Projects\JEA_Duval\wbids_landuse.mxd
Source: St. Johns River Water Management District

2.2 PHASE II: Planning

Phase II of the Tributary Pollution Assessment Project included the Kick-off Meeting of the TAT, presentation and discussion of the *Work Plan* (including discussion of activities conducted under Phase I), and finalization of the *Work Plan* based on TAT recommendations. The final *Work Plan* was used to guide the completion of the Tributary Pollution Assessment Project.

The following phases are explained in Task format, and were completed according to the schedule presented in Figure 2-2.

2.3 PHASE III: Development of the Tributary Pollution Assessment Manual

Task 1: The assessment methodology described below was originally developed for each category and later revised according to results obtained during Phase IV (field-verification of the Manual).

A. Probable sources and migration pathways (e.g., identification of sites with possible direct inputs such as septic systems with old drainfields [identified by high repair rate], wastewater outfalls, fields with cattle) were further assessed. Information obtained during Phase I and Phase II was used as an initial screening tool.

B. Potential assessment techniques were identified and included the following:

1. Suite of indicators to better characterize microbial pollution in sediments and water column at all sampling stations:
    - fecal coliforms
    - *Escherichia coli* (*E. coli*, a member of the fecal coliforms)
    - Enterococci (*Enterococcus* species)

2. Targeted sampling of *Enterococcus faecalis* to locate probable sewage inputs
    - *Enterococcus faecalis* is strongly associated with sewage. *E. faecalis* and *E. faecium* are consistently identified as the dominant *Enterococcus* spp. in human feces (Chenoweth and Schaberg 1990; Gelsomino et al., 2003; Ruoff et al., 1990) and sewage (Harwood et al., 2004; Manero et al. 2002). *E. faecalis* is not

unique to human feces and sewage (Harwood et al., 2004), but has been shown to be uncommon or absent in feces of cows, horses, deer, Canada geese, pigs, rabbits and sheep (Kuntz et al., 2004; Pourcher et al., 1991; Wheeler et al., 2002). McDonald et al. (2006) recently suggested that the identification of at least 30% *E. faecalis* from a water sample coupled with detection of a human-associated marker such as the *esp* gene is indicative of human fecal contamination.

- Targeted sampling for this bacterium at several points upstream and downstream of each intensively sampled site helped to locate pollutant inputs from wastewater.

3. Fluorometry
    - Sensitive fluorometers can detect a relatively dilute wastewater signal from optical brighteners used in clothes washing. Waters at targeted sampling sites for *Enterococcus faecalis* were assayed for a wastewater signal by fluorometry.

4. PCR assay for human and/or ruminant signal
    - Water from intensive sites was assayed for human-specific and/or ruminant-specific signals by PCR. Human-specific primers for *Bacteroides* and *Enterococcus faecium* were employed, as well as ruminant-specific primers for *Bacteroides*.

5. Library-based identification of *Enterococci*
    - *Enterococci* was isolated from water and sediment at the intensive sites and the genomic profile of randomly selected isolates was determined by rep-PCR. In some cases, the profiles from sediments were compared to those from overlying surface waters.

C. Cost-analysis of potential techniques was developed.

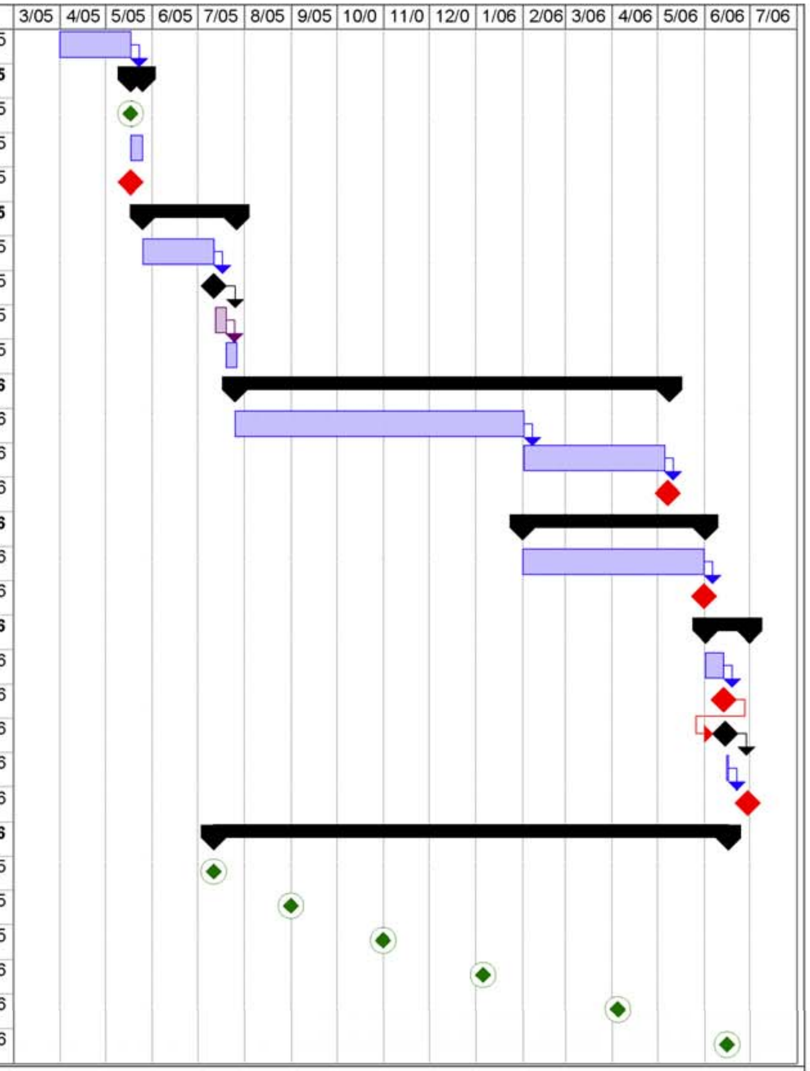
D. Cost-analysis of potential techniques was used to formulate a decision-tree assessment based on water body categories.

Task 2: The decision tree was used to determine an assessment methodology for the selected WBIDs based on available funds and was revised as necessary (i.e., if funds change or have indeterminate results).

Task 3: A model monitoring plan was developed for each tributary category.

Task 4: The Draft *Tributary Pollution Assessment Manual* was submitted to the TAT for review.

Task Name	Start	Finish
Phase I: Planning	Fri 4/1/05	Tue 5/17/05
Phase II: Kick-off Meeting with TAT	**Wed 5/18/05**	**Wed 5/25/05**
Kick-off Meeting with TAT	Wed 5/18/05	Wed 5/18/05
Finalize Work Plan	Wed 5/18/05	Wed 5/25/05
Deliverable 1: Preliminary Consolidated GIS Database	Wed 5/18/05	Wed 5/18/05
Phase III: Develop Tributary Pollution Assessment Manual	**Thu 5/26/05**	**Tue 7/26/05**
Prepare Draft Manual	Thu 5/26/05	Mon 7/11/05
Submit Draft Manual to TAT for review	Tue 7/12/05	Tue 7/12/05
TAT Review of Draft Manual	Wed 7/13/05	Tue 7/19/05
Revise Draft Manual after TAT review	Wed 7/20/05	Tue 7/26/05
Phase IV: Evaluate Tributary Pollution Assessment Manual	**Tue 7/26/05**	**Mon 5/8/06**
Conduct Sanitary Surveys	Tue 7/26/05	Wed 2/1/06
Finalize Manual	Thu 2/2/06	Fri 5/5/06
Deliverable 2: Submit Final Tributary Pollution Assessment Manual	Mon 5/8/06	Mon 5/8/06
Phase V: Summary Report	**Wed 2/1/06**	**Thu 6/1/06**
Prepare Summary Report; use to finalize Manual	Wed 2/1/06	Wed 5/31/06
Deliverable 3: Submit Summary Report	Thu 6/1/06	Thu 6/1/06
Phase VI: Public Workshop	**Fri 6/2/06**	**Fri 6/30/06**
Prepare presentation	Fri 6/2/06	Tue 6/13/06
Deliverable 4: Submit written presentation of results	Wed 6/14/06	Wed 6/14/06
Public Workshop	Thu 6/15/06	Thu 6/15/06
Deliverable 5: Submit written summary of public input	Fri 6/16/06	Fri 6/16/06
Deliverable 1: Submit Finalized GIS Database	Fri 6/30/06	Fri 6/30/06
Meetings with TAT	**Tue 7/12/05**	**Fri 6/16/06**
Meeting 1	Tue 7/12/05	Tue 7/12/05
Meeting 2	Thu 9/1/05	Thu 9/1/05
Meeting 3	Tue 11/1/05	Tue 11/1/05
Meeting 4	Fri 1/6/06	Fri 1/6/06
Meeting 5	Wed 4/5/06	Wed 4/5/06
Meeting 6	Fri 6/16/06	Fri 6/16/06



Project: Proposed Project Timeline
Date: Thu 5/26/05

Figure 2-2. Tributary Pollution Assessment project schedule as proposed in the *Work Plan*.

2.4 PHASE IV: Evaluation of the Methodology/Manual by Conducting Sanitary Surveys

Task 1: A tributary pollution assessment was conducted for one selected WBID for each category using the corresponding methodology described in the Draft *Tributary Pollution Assessment Manual*.

Task 2: The results were evaluated and generic corrective actions were recommended.

Task 3: The methodology was revised to reflect the findings of the tributary pollution assessments.

Task 4: Additional investigations or supplemental samplings were conducted on an as needed basis, provided funds were available.

Task 5: The Draft *Tributary Pollution Assessment Manual* was revised and a final Manual was submitted to JEA.

2.5 Selected WBID Descriptions and Sample Locations

A field site assessment of each selected WBID was performed in order to gain a comprehensive understanding of each tributary. Historical fecal monitoring data and other pertinent information, including GIS data (submitted independently as a consolidated GIS database) provided by various contributing agencies were used as a guide. During the site assessment, site descriptions and observations were recorded, photographs depicting current local conditions were taken (Appendix A), and future sampling stations were determined. Sample station locations were determined using the methods described in Section 4.2.2 of the *Tributary Pollution Assessment Manual* and are shown in Figure 2-3 and briefly described in Table 2.4. These original stations were supplemented throughout the duration of the project by both temporary and permanent stations sampled by the DEP and the COJ WQD (Appendix B; also see consolidated GIS database). The supplemental stations were determined by the DEP in conjunction with the COJ and PBS&J and added to the sampling program on an as-needed basis for a variety of reasons: 1) to represent branches of the tributaries that had not yet been sampled; 2) to focus intensive sampling efforts in areas of suspected sources; 3) to test for specific potential illicit discharges. The Station ID number indicates the relative geographic location of the station with respect to other stations and the St. Johns River. For example, MR1 is the most downstream sampling location for Miramar Creek while MR3 is the most upstream location, with MR2 positioned in the middle.

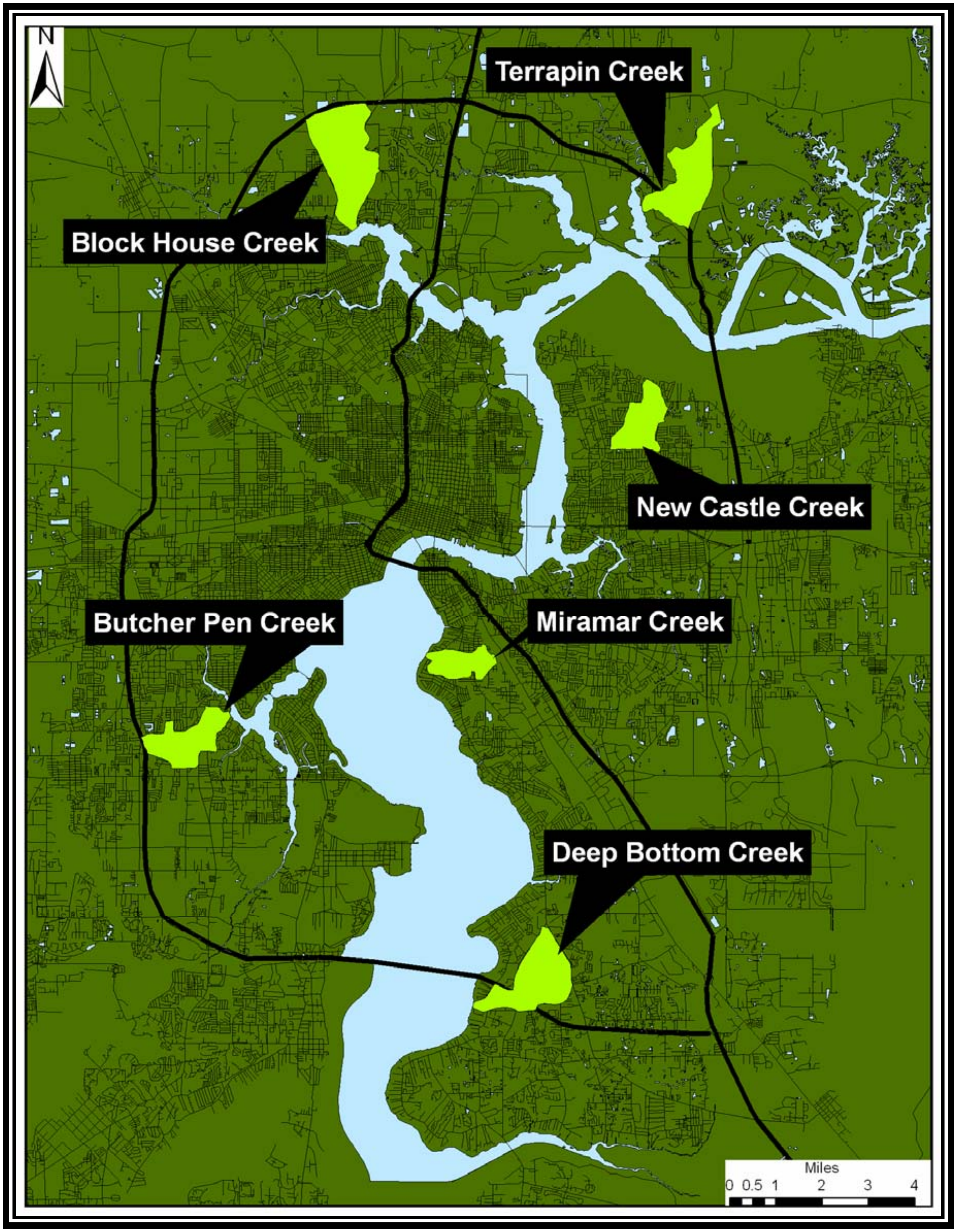


Figure 2-3. Approximate locations of the tributaries used to field-verify the approach described in the Tributary Pollution Assessment Manual.

Table 2.4 Summary of Selected Sampling Stations					
WBID Name	**Station ID**	**Street Location**	**GPS Coordinates**	**Infrastructure-Related Observations**	**Other Observations**
Miramar Creek	MR1	Intersection of San Jose Blvd. and Morvenwood Rd.	-1.656191281 30.284851328	Within septic failure area; pipe (30 inch gravity sewer) crossing over creek; culvert under San Jose Blvd.; old pipe noted along creek bed; spillway enters creek from residential neighborhood	Abundant, turbid/brown water
	MR2	Intersection of Hendricks Ave. and Greenridge Rd.	-81.651281370 30.284106246	Just east of septic failure area; spillway enters creek from Greenridge Rd.; sandbag embankment on north side;	Relatively little water
	MR3	Intersection of Orlando Terrace and Orlando Cir. West	-81.649592469 30.282063518	Well outside both septic failure areas; storm drain (?) pipe entering creek	Turtles, frogs and small fish present; shady; water flow to west
Butcher Pen Creek	BP1	Bridge at intersection of Butcher Pen Creek and Confederate Point	-81.734457406 30.264578281	Residential neighborhood with elementary school on south side; roadway drainage project upstream along Wesconnett; potential illicit connection from adjacent apartment building	Manatee feeding, ducks, and few wading birds present; tannic water; water flow to west; abundant water
	BP2	Bridge at intersection of Blanding Blvd. and Sage Dr.	-81.741393321 30.259446587	Sidewalk and metal fence bordering creek above culvert	Abundant water; grey water; water flow to southwest (tidal?); mix of sun and shade

Table 2.4 Summary of Selected Sampling Stations					
WBID Name	**Station ID**	**Street Location**	**GPS Coordinates**	**Infrastructure-Related Observations**	**Other Observations**
	BP3	Just north of intersection of Randia Dr. and Ducheneau Dr. (down trail at end of cul de sac)	-81.743814544 30.256422202	Adjacent to JEA lift station; sewer pipe extends over creek from lift station; residential area (no septic mounds observed)	Shady; sewage odor; relatively little water; water flow to northeast
Deep Bottom Creek	DB1	Bridge at intersection of Deep Bottom Creek and Scott Mill Rd.	-81.637908990 30.178568392	In Scott Mill Hill septic nuisance area; Potential illicit connection from adjacent home; pipe (12 inch ductile iron water main) over creek; pipe (12 inch ductile iron sanitary force main) running along west side of bridge); pipe (stormwater?) entering creek; stormwater drains on bridge	Abundant water; turbid water; slight water flow to east; sunny on west side of bridge; residential with septic mounds
	DB2	Bridge at intersection of Deep Bottom Creek and San Jose Blvd. just south of Halcyon Ln.	-81.628109207 30.177821380	Partially fenced; concrete canal culvert under San Jose Blvd.; water pumped into canal from retention pond adjacent to I 295; ditch entering creek from adjacent strip mall	Sunny; abundant vegetation; strong flow to west; clear water;
	DB3	Intersection of Hampton Rd. and Las Vegas Rd.	-81.620244754 30.180773915	Gated on west side; roadside ditch feeds into creek	Strong water flow to west; turbid water
New Castle Creek	NC1	Intersection of New Castle Creek and Ft. Caroline Hills Dr.	-81.579708561 30.365749747	Culvert under Ft. Caroline Hills Dr.	Clear water; fish and turtles present; mostly shaded; sulfur odor

Table 2.4 Summary of Selected Sampling Stations					
WBID Name	**Station ID**	**Street Location**	**GPS Coordinates**	**Infrastructure-Related Observations**	**Other Observations**
	NC2	Intersection of New Castle Creek and Millcrest Pl. (down trail into woods)	-81.579707623 30.361895774	Culvert under Millcrest Pl.	Clear water; sulfur odor; water flow to north; shady, wooded area; most water on west side of "V" shape
	NC3	Intersection of New Castle Creek and Greenfern Ln.	-81.579090456 30.356398182	Large concrete canal; culvert under Greenfern Ln.;	Neighboring resident reported that culvert will remain ~half full after big rain and culvert used to carry more rain years ago; relatively little water
Terrapin Creek	TP1	Bridge at intersection of Terrapin Creek and Alta Dr.	-81.580336877 30.428835525		Abundant water; water flow north (tidal?); salt marsh habitat; sunny; residences with personal boat docks
	TP2	Bridge at intersection of Terrapin Creek and Faye Rd.	-81.565655286 30.434411656	Guard rails along bridge; culvert under Faye Rd.	Slight water flow to south; more water on north side of bridge; Hydrocotyl on north side; sunnier on south side of bridge

Table 2.4 Summary of Selected Sampling Stations

WBID Name	Station ID	Street Location	GPS Coordinates	Infrastructure-Related Observations	Other Observations
	TP3	Intersection of Terrapin Creek and road leading into new office park on off New Berlin Rd. South	-81.560385527 30.450761763	Erosion control turbidity barrier at north end of creek	Abundant vegetation, including duckweed, on surface of water; stagnant water; sunny; south of M&M Dairy (cattle and cattle egrets observed to north)
Blockhouse Creek	BH1	Bridge at intersection of Blockhouse Creek and Leonid Rd.	-81.689032424 30.429838559	Pipe (14 inch ductile iron sanitary force main) over creek along bridge; adjacent stormwater retention structure; gas storage tanks adjacent to creek	Water flow to north (tidal?); cattails present; scattered wading birds present
	BH2	West of intersection of Pine Estates Rd. East and Pine Estates Rd. South (down trail at end of cul de sac)	-81.684109358 30.433569431		Abundant water in creek; water flow to north (tidal?); turbid water; several dogs in immediate area

Table 2.4 Summary of Selected Sampling Stations					
WBID Name	**Station ID**	**Street Location**	**GPS Coordinates**	**Infrastructure-Related Observations**	**Other Observations**
	BH3	Intersection of Blockhouse Creek and Irma Rd. just north of Dunn Ave.	-81.689266562 30.439619715	Culvert under Irma Rd.	Turbid water; water flow to southeast; abundant water; shady (sunnier on west side); residential area with septic mounds; construction of new development nearby; light agriculture throughout area
Thomas Creek (control)	TC1	Bridge at intersection of Thomas Creek and New Kings Rd. (US 1)	-81.791620699 30.505280536	Major bridge over Thomas Creek	Abundant water; sunny; abundant vegetation
	TC2	Middle wooden bridge at intersection of Thomas Creek and Thomas Creek Rd. (just southeast of Danson Ln.)	-81.832320713 30.461221629		Abundant water; mostly shady; abundant vegetation; slight water flow to east; light residential area; depth gauge in place adjacent to bridge read 15 feet

Table 2.4 Summary of Selected Sampling Stations					
WBID Name	**Station ID**	**Street Location**	**GPS Coordinates**	**Infrastructure-Related Observations**	**Other Observations**
	TC3	Intersection of Thomas Creek and Ford Rd. (just west of Settler's Ridge Dr.)	-81.884836972 30.436031739		Light agriculture in area; relatively new homes in area with septic tank mounds; cleared land for new developments in area; tannic water; water flow to north; sunny; frogs present

2.6 Sampling Methodology

a. When to Sample?

Each WBID was sampled once at the end of each month beginning in July 2005 and ending in February 2006 to integrate both the local wet and dry seasons. Preliminary analysis of the relationship between local rainfall data and historical fecal data indicated that any correlations would be detected within 3-5 days of a rainfall event. Whenever possible, rain events were captured within the sampling program.

b. What and How to Sample?

The sampling protocol was conducted by two teams; each comprised of personnel from PBS&J and the University of South Florida. Each team was assigned 3-4 WBIDs; every WBID was sampled within the same day. The sampling methodology followed that described in the Standard Methods for the Examination of Water and Wastewater (9060A) and the Tributary Pollution Assessment Manual. Due to financial constraints, one water sample was taken from each sampling station for a total of three samples per WBID, to be used for the bacteriological assays. As mentioned above, additional samples were collected and analyzed for fecal coliform by the DEP and the COJ WQD, as necessary, to assist in the identification of contamination sources. If elevated numbers of fecal coliform were found, more advanced source-tracking techniques (see Figure 2-4) were used to identify a specific source-type (i.e., human or ruminant). One additional water sample from each sampling station was initially collected for analysis of optical brighteners using fluorometry; however, due to the non-navigable nature of the tributaries, flow-through fluorometry was determined to be an ineffective method and was discontinued. Sediment samples were taken at sampling stations to assist in the source-identification process. Additional field data such as salinity, pH, conductivity, temperature, turbidity, and dissolved oxygen, as well as general observations, including weather conditions and general tributary characteristics, were also taken to help determine sources of fecal contamination (available in consolidated GIS database).

c. *Sample Evaluation*

The entire decision-tree described in the *Tributary Pollution Assessment Manual* was implemented, as finances allowed, after each sampling event (Figure 2-4). A brief description of each technique can be found in Section 1.5 of the Manual. To ensure that the full approach could be executed, enough samples were collected from each sampling station to enable the necessary level of analysis. Samples were prepared within hours of collection for use on an as-needed basis throughout the evaluation process. PBS&J, in conjunction with the environmental microbiology laboratory of Dr. Harwood at the University of South Florida (USF), was responsible for the collection of all bacteriological samples. The USF laboratory performed all analyses with the exception of the earlier human-specific *Bacteroides and Enterococcus* PCR and ruminant-specific PCR tests, which were performed by Biological Consulting Services of North Florida. All fluorometry samples were transported to the Mote Marine Laboratory for evaluation.

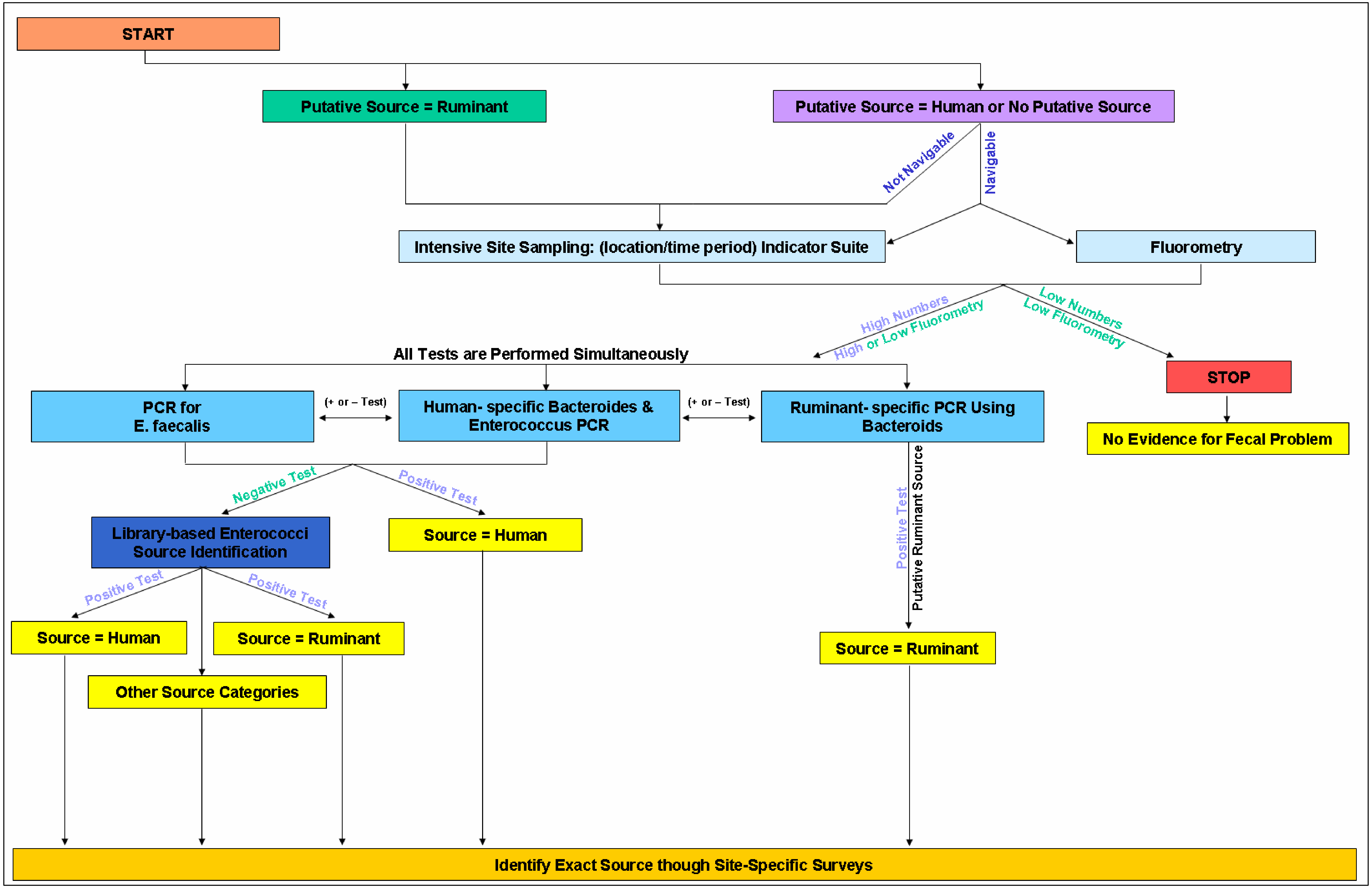


Figure 2-4. Final decision-tree approach implemented after each sampling event.

Section 3. Results and Discussion

3.1 General Results Regarding the Assessment Techniques used in the Decision-Tree

The original decision-tree methodology was revised to reflect what was discovered to be the most useful assessment technique and sequence (Figure 2-4). Fluorometry, included as a preliminary technique in the original matrix, has been proven to 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, the methodology has not been approved by the EPA and presents several logistical constraints when used in shallow, back-water streams (Charlotte Harbor Environmental Center, Inc and Water Resources and Issues, 2003). One of its primary advantages is that it can provide continuous sampling outputs and patterns of fluorescence, particularly when utilized as a flow-through, vessel-mounted instrument; however, this method is restricted to readily navigable waterways, even when using shallow-draft vessels. Furthermore, in an estuarine environment naturally-occurring substances, such as humic acids or tannins also fluoresce, demanding an extensive post-processing effort to separate the various fluorescence signals (Dixon et al., 2005). Due to the non-navigable nature of the tributaries assessed in this project, fluorometry was concluded to be an ineffective method for the investigation of fecal coliform contamination.

Another change in the original tiered approach was the way in which the *Enterococcus* library-based approach of genetic typing was used. Originally, this technique was to be used to match genomic profiles of randomly selected isolates, determined by rep-PCR, to a library of known *Enterococcus* profiles. One such library, developed by Dr. Valerie J. Harwood (University of South Florida) and funded by the FDEP for the Jacksonville region in 2004, included genomic profiles for sources such as sewage, dogs, ducks, wild animals and sediments. Instead of using this technique to identify particular sources, it was used to compare patterns within the genomic profiles of the sediments with those of the water column. Results indicated whether elevated fecal concentrations originated from existing sediment reservoirs or whether they were born from more recent and ongoing sources. The method was particularly useful in examining Terrapin Creek where an old cattle farm, currently disconnected from the tributary, was the putative source of bacterial contamination. Rep-PCR patterns of enterococci isolated from sediment samples collected at Terrapin Creek were more "clonal" (more were identical to each other) than isolates from the corresponding water column, indicating a potential sediment reservoir (Panel A, Figure 3-1). Twelve of the 20 Terrapin Creek sediment isolates were grouped within one clade (or family) in which all patterns were 60% similar or greater. In contrast, when this test was exercised at Butcher Pen, where an ongoing source was suspected, the degree of variability in sediment patterns mirrored those of the surface waters, suggesting that continuous inputs were driving fecal coliform concentrations (Panel C, Figure 3-1). Only four of 19 Butcher Pen sediment isolates fell into the largest clade of >60% similarity. Calculation of the Shannon-Weiner diversity index for these sites, which takes into account the total number of patterns and the frequency of their occurrence, supported this conclusion. A relatively low diversity (many clones) population with a diversity index of 1.34 was sampled from Terrapin Creek sediments, while index of sediment isolates from Butcher Pen 2.36 (relatively high diversity; few clones). Patterns of water column isolates from both sites tended to be much less clonal than those from Terrapin Creek sediments.

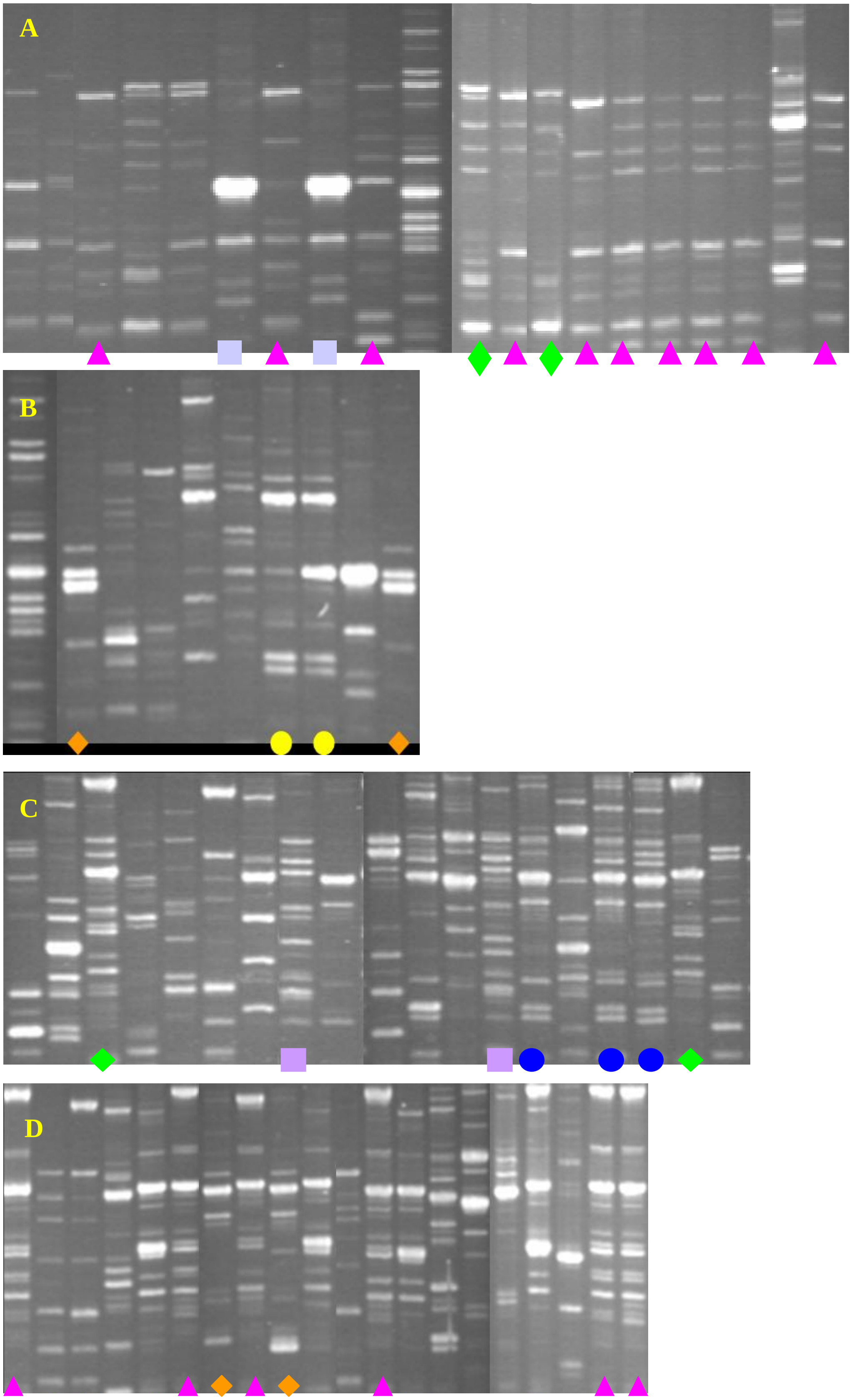


Figure 3-1. Rep-PCR patterns demonstrating differences in similarity between samples taken from (A) Terrapin Creek sediments, (B) Terrapin Creek water column, (C) Butcher Pen sediments, and (D) Butcher Pen water column. Patterns are read vertically. **Within** each sample site (A, B, C, or D) those with matching symbols are >90% similar in terms of the banding pattern, and are considered “clones.”

The results of site assessments over the 8-month study period demonstrated that each of the 6 tributaries commonly exceeded both the EPA standard for Enterococcus and the State standard for fecal coliforms (fecal coliform geomean of 200 CFU/100 ml) (Figure 3-2). Thomas Creek, the tributary designated as the control, was chosen because it was located outside the general influence of sewage and septic systems and was not significantly affected by agriculture. Unfortunately, this tributary was observed to regularly exceed the Florida State standard as well as the EPA standard, though to lesser degree. This is likely a result of stormwater contamination. The geomean is calculated by first transforming each observation in a data series to its $\log_{10}$ value. The mean is taken of the log-transformed data, and this mean is then transformed back to an arithmetic scale by taking the antilog of the calculated value. This strategy is frequently used when microbial measurements are analyzed, in order to reduce the influence of large, outlying observations.

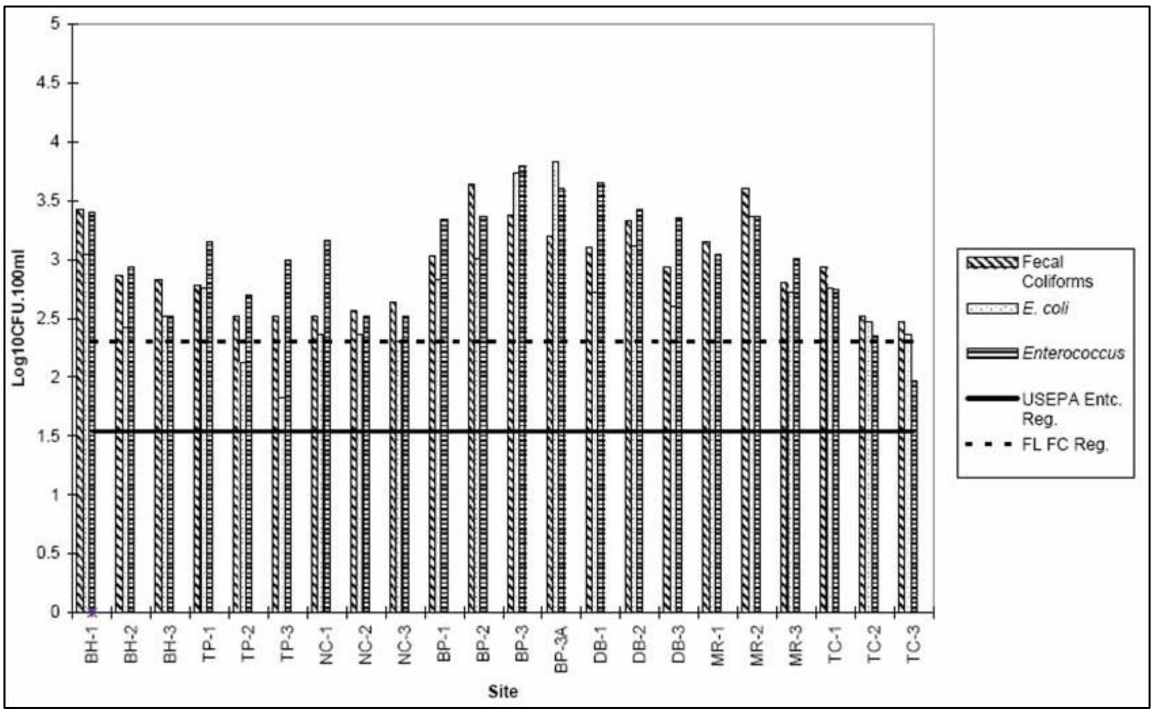


Figure 3-2. Geometric mean concentrations of fecal indicator bacteria in the surface waters of the six experimental tributaries and one control tributary.

The decision-tree primarily incorporates tests which are based on the presence or absence of a particular signal. One exception is the PCR test for *Enterococcus faecalis*. Approximately 50 percent of *Enterococcus* sp. that are isolated from most human sewage samples are *E. faecalis*. As a result, a positive presence of *E. faecalis* in a sample does not necessarily indicate a human source, but can be an indicator of human sewage presence. Using the results of this test from Thomas Creek to set a cut-off value, a human signal was defined as being 40% positive for

E. faecalis for each station at each sampling event. A high percentage of *E. faecalis*, combined with additional supporting evidence (i.e., supplementary human-specific test results or evidence gained through site-specific surveys), was considered indicative of the presence of human sewage.

Given the above explanation of the general results obtained through the use of the tiered approach, the findings, specific to each tributary, are described below. It became increasingly apparent during the program that the necessary assessment techniques were becoming progressively more site-specific and needed to be selected based on individual site characteristics, prior results, and knowledge of remaining funds. This considered, all suspicious surface waters or potential illicit connections were sampled and analyzed accordingly. A graphical representation of indicator species with an overlay of additional positive, source-test results for each of the eight sampling events is provided in Appendix C. In addition to the information described, it must be noted that stormwater is a potential and highly probable conveyance system for each of these more specific sources. As a result, it must be considered as a source unto itself in order to manage it more effectively. Photographs showing the general state of each tributary at each of the eight sampling events are located in Appendix A.

A summary table showing the results of the tiered approach for each tributary is included below. The values in each column indicate the number of positive results over the total number of tests performed for each assessment technique. Note that the criterion for *E. faecalis* is percent positive, as shown in parentheses. A positive test indicating either a human or ruminant source is highlighted in yellow.

3.2 Miramar Creek

Miramar Creek was the WBID evaluated in the Urban, Putative Source category. The results of the assessment techniques utilized as part of the tiered approach strongly indicated that a human source was responsible for the somewhat episodic, but extremely elevated levels of fecal coliform contamination at Miramar Creek. The analyses of human-specific bacteroides and ESP both resulted in multiple positive tests spread out over the three sampling stations; *E. faecalis* was present though not conclusively human (Table 3.1; Appendix C). Due to the urbanized area surrounding Miramar Creek and the low likelihood of significant ruminant sources, the test for ruminant bacteroides was only conducted on one occasion and provided a negative result.

Analysis of the sediments indicated consistently high loads of the three indicator species at stations MR1 and MR2.

Table 3.1 Summary of the results of the assessment techniques utilized for samples collected at Miramar Creek over the eight-month project period.

Site	Bacteroides Human	Bacteroides Ruminant	ESP	*E. faecalis*
Miramar (MR) -1	0/2	0/1	2/2	3/10 (30%)
Miramar (MR) -2	1/1	0/1	0/1	NA
Miramar (MR) -3	1/1	0/1	1/1	0/5 (0%)

In addition to the results of the scheduled sampling and analysis, field reconnaissance conducted by the COJ during the October, 2005 sampling event revealed that a black discharge from a septic system upstream of MR3 was observed. Analysis of water samples collected from the discharge as well as several locations upstream of MR3 verified significantly elevated levels of fecal coliforms. Given the site history obtained from field observations, informal interviews with residents and GIS data, together with the sample results, it was concluded that the principal source of fecal coliform contamination impacting Miramar Creek was septic systems located in the vicinity of MR3 (Figure 3-3.).

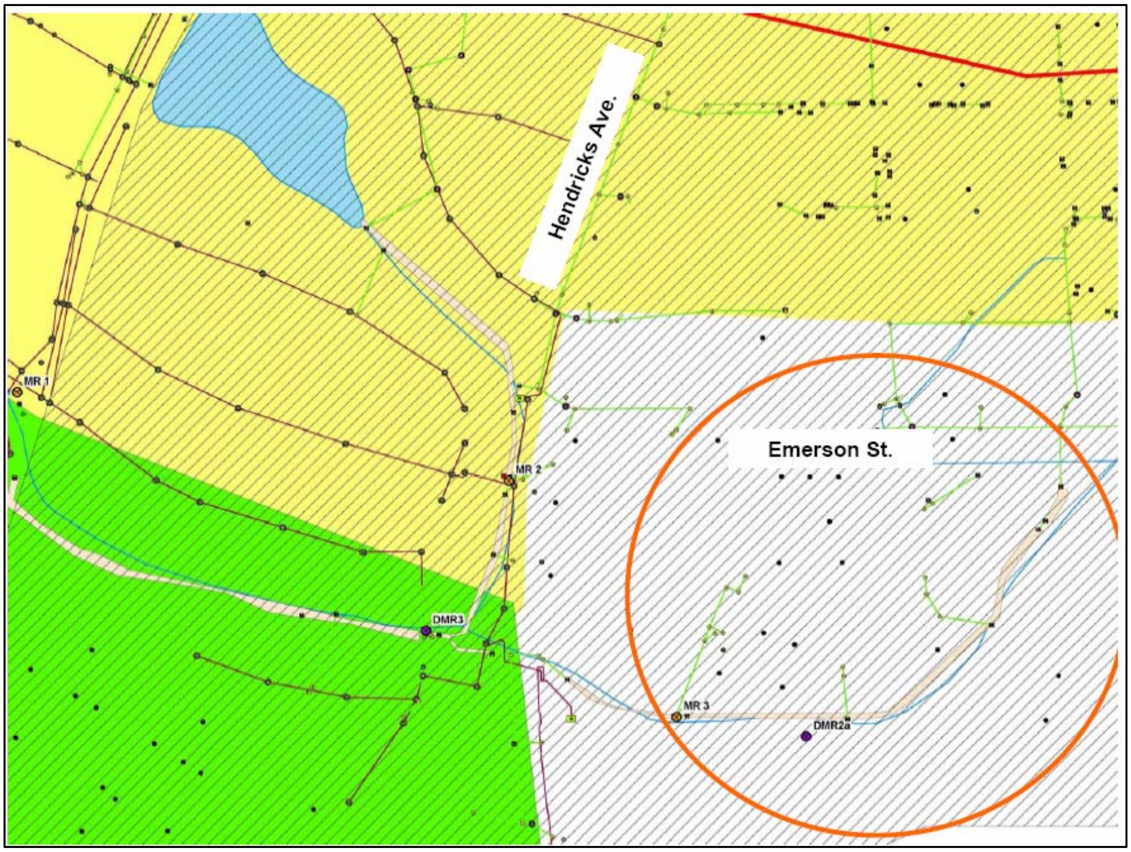


Figure 3-3. Map showing the locations of the sampling stations and GIS data collected for Miramar Creek. The orange circle delineates the approximate area where the most significant contribution of fecal coliform contamination to the tributary originates. A map legend is located in Appendix D.

3.3 Butcher Pen Creek

Butcher Pen was the WBID evaluated in the Urban, No Putative Source category. Similar to Miramar Creek, the results of the assessment techniques used at Butcher Pen Creek indicated that a human source was primarily responsible for the elevated levels of fecal coliform contamination; however, in this case, the extreme levels appeared to be derived from more consistent sources. This notion was confirmed when the genomic patterns of the sediments were compared to those of their overlying surface waters, as described above. PCR for *E. faecalis* consistently revealed a human source at each of five stations sampled while the analyses for human-specific bacteroides and ESP both resulted in positive tests at one station each (Table 3.2; Appendix C). Analysis of the sediments indicated consistently high loads of the three indicator species at each of the stations sampled (BP3, BP3A, and BP3B).

Table 3.2 Summary of the results of the assessment techniques utilized for samples collected at Butcher Pen Creek over the eight-month project period.

Site	Bacteroides Human	Bacteroides Ruminant	ESP	*E. faecalis*
Butcher Pen (BP) -1	0/2	0/2	0/2	4/10 (40%)
Butcher Pen (BP) -2	0/2	1/3	0/2	6/10 (60%)
Butcher Pen (BP) -3	0/4	0/2	0/6	10/14 (71.4%)
Butcher Pen (BP) -3A	1/1	NA	0/2	5/5 (100%)
Butcher Pen (BP) -3B	NA	NA	NA	2/5 (40%)

Although Butcher Pen Creek is in an urban area without significant ruminant sources, the test for ruminant bacteroides yielded positive results at BP2 on one occasion. In order to test the relative significance of this result, the test was repeated at each of the three sites and then again at BP2 for verification. It was determined that the ruminant source was likely deer passing through a small forested area in the vicinity of BP2 and that this source could not account for the consistently elevated levels of fecal coliform contamination throughout most of the upstream area. Instead, due to the preponderance of positive human-indicator tests and the abundance of sewer infrastructure not only present but crossing and directly adjacent to the surface waters throughout the area of elevated levels, it appears most probable, though still not confirmed, that the source of contamination in this area is sewer-related and stems from the area around and upstream of BP3 (Figure 3-4 and Appendix B).

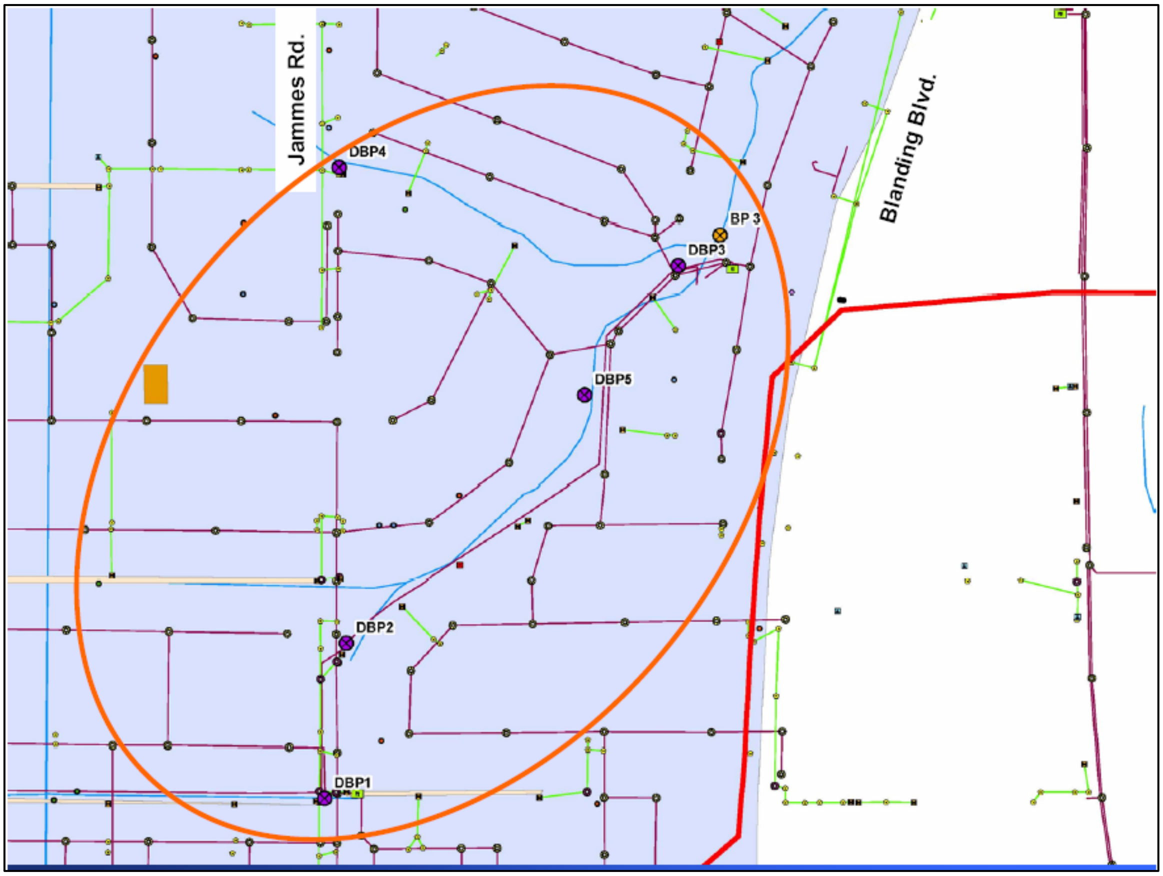


Figure 3-4. Map showing the locations of the sampling stations and GIS data collected for Butcher Pen Creek. The orange circle delineates the approximate area where the most significant contribution of fecal coliform contamination to the tributary originates. A map legend is located in Appendix D.

3.4 Deep Bottom Creek

Deep Bottom Creek represented the Suburban, Putative Source category. Unlike Miramar Creek and Butcher Pen Creek, the results of the assessment techniques used at Deep Bottom Creek did not reveal an obvious source of fecal coliform contamination for this tributary. A human source was indicated by the results of PCR for *E. faecalis* at one of the supplemental sampling stations (DDB2) and one analysis of ESP at DB2; the presence of ruminant bacteroides was not apparent (Table 3.3; Appendix C). Analysis of the sediments within Deep Bottom Creek indicated high loads of all three indicator species at both DB1 and DB3 (there were no sediments at DB2 for testing).

Table 3.3 Summary of the results of the assessment techniques utilized for samples collected at Deep Bottom Creek over the eight-month project period.

Site	Bacteroides Human	Bacteroides Ruminant	ESP	*E. faecalis*
Deep Bottom (DB) -1	0/3	0/1	0/4	1/10 (10%)
Deep Bottom (DB) -2	0/2	0/1	1/3	1/5 (20%)
Deep Bottom (DB) -3	0/3	0/1	0/3	2/10 (20%)
Deep Bottom (DDB) -1	0/1	NA	0/1	0/5 (0%)
Deep Bottom (DDB) -2	0/1	NA	0/1	4/5 (80%)
Deep Bottom (DDB) -3	0/1	NA	0/1	0/5 (0%)

The evidence indicates that human source(s) are primarily responsible for the elevated levels of contamination in this tributary. A handful of individual systems, possibly including both sewer and septic infrastructure, may be responsible. It appears likely that the actual sources are localized but their positions are dispersed throughout the basin (Figure 3-5) and are episodic in nature, making them difficult to identify when sampled monthly. Additional temporal replication will be needed to isolate specific sources. It is suggested that future sampling be continued for this tributary at each of the stations established through this project, with the exception of DB2. Additional field reconnaissance may also be necessary to identify less obvious sources. Due to the apparent episodic nature of the contamination, sampling should occur at least once per week until a pattern has been established or the sources of contamination have been successfully identified.

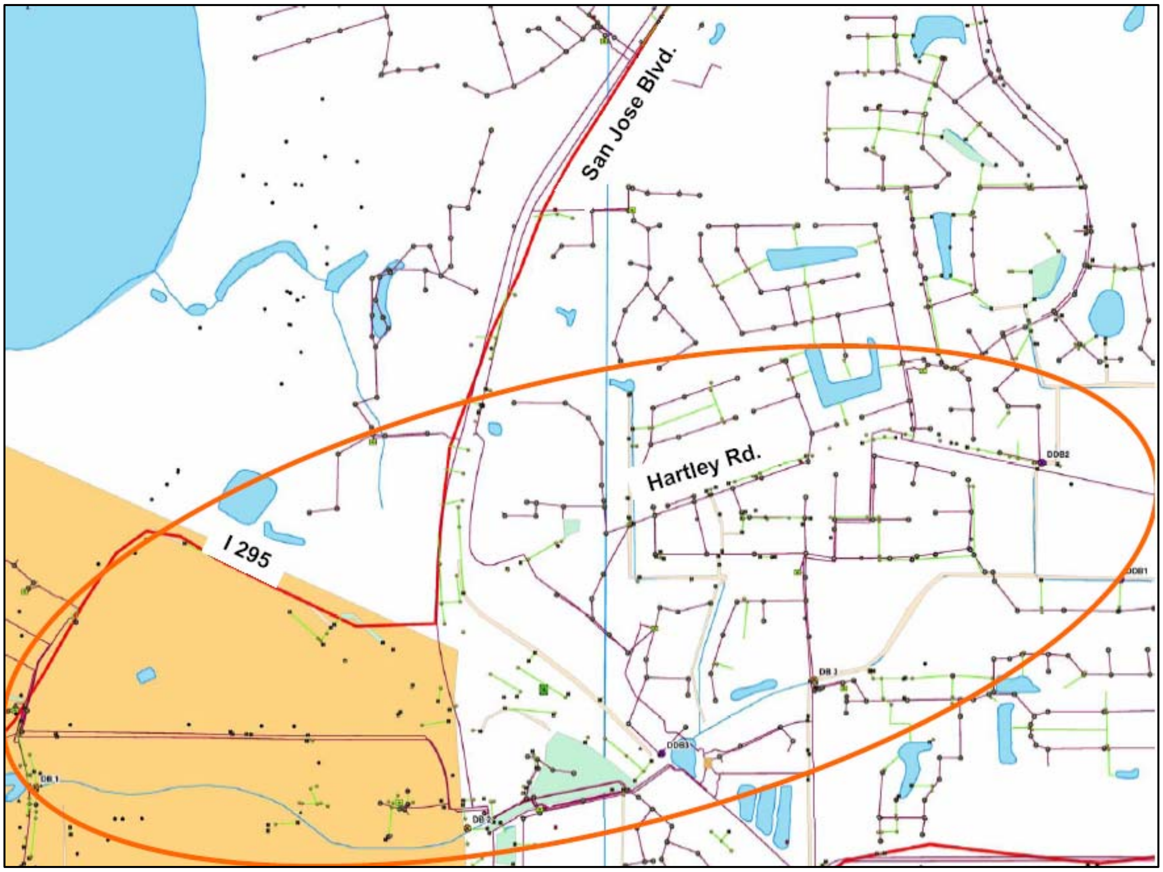


Figure 3-5. Map showing the locations of the sampling stations and GIS data collected for Deep Bottom Creek. The orange circle delineates the approximate area where the most significant contribution of fecal coliform contamination to the tributary originates. A map legend is located in Appendix D.

3.5 New Castle Creek

New Castle Creek was the WBID evaluated in the Suburban, No Putative Source category. It was concluded, using the results of the tiered approach, that a human source was responsible for elevated levels of fecal coliform contamination at New Castle Creek. The test for human *Bacteroides* was positive at all sites sampled, with the exception of NC1, and one positive analysis of ESP at NC3 was obtained. Two positive results for *E. faecalis* (one each at NC2 and DNC2) corroborated these findings (Table 3.4; Appendix C). All tests for ruminant *Bacteroides* were negative. Analysis of sediments within New Castle Creek indicated high loads of the three indicator species at both NC1 and NC2 (there were no sediments at NC3 for testing).

Table 3.4. Summary of the results of the assessment techniques utilized for samples collected at New Castle Creek over the eight-month project period.				
Site	**Bacteroides Human**	**Bacteroides Ruminant**	**ESP**	***E. faecalis***
New Castle (NC) -1	0/2	0/1	0/2	1/5 (20%)
New Castle (NC) -2	1/2	0/1	0/2	2/5 (40%)
New Castle (NC) -3	1/2	0/1	1/2	1/5 (20%)
New Castle (DNC) -2	1/1	NA	0/1	2/5 (40%)

Levels of fecal coliforms were highly variable throughout the sampling regime, indicating that the source at New Castle Creek was episodic in nature; however, it is possible that the tributary was impacted by more constant sources but was filling and discharging at irregular intervals. More importantly, extreme levels of fecal coliforms (over 5,000 CFU/100 ml) were only observed on two occasions, both at station DNC2. It is interesting to note that on two occasions in the eight-month sampling period, this station reported levels of fecal coliforms below 400 CFU/100ml and, on two separate occasions, below 800 CFU/100ml. It appears most probable that the source of contamination is located in the vicinity of DNC2 or further upstream (Figure 3-6). Although relatively little sewer infrastructure exists in this area, it is still possible that the source is sewer-related; however, it must be mentioned that the most southern portion of the New Castle WBID encompasses part of a WSEA/DOH-designated septic tank Failure Area which may contribute to contamination.

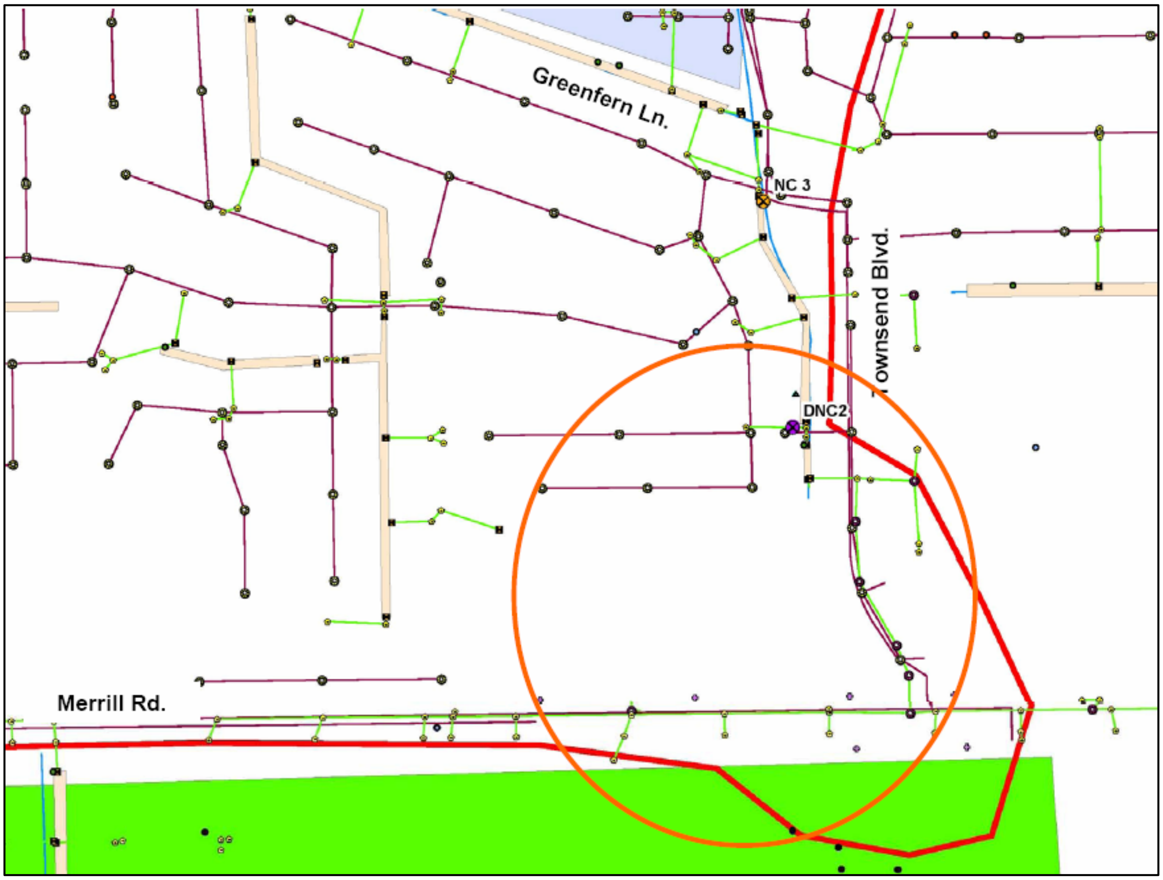


Figure 3-6. Map showing the locations of the sampling stations and GIS data collected for New Castle Creek. The orange circle delineates the approximate area where the most significant contribution of fecal coliform contamination to the tributary originates. A map legend is located in Appendix D.

3.6 Terrapin Creek

The Rural, Putative Source category was represented by Terrapin Creek. Upon commencement of the sampling program, it became apparent that the original putative source for this tributary had been disconnected from the surface waters of Terrapin Creek. The dairy farm, once located in the northern portion of the Terrapin Creek WBID, had been isolated from those surface waters by the construction of a new office park. In doing so, the entire structure of the WBID, and possibly the adjacent WBID (Rushing Branch), had been altered. The system was tested, as originally planned, and the analyses conducted for ruminant *Bacteroides* yielded negative results (Table 3.5; Appendix C). Surprisingly, a single test for human *Bacteroides* was positive; however, this was attributed to error in sampling water from Dunn Creek that had been blown into the lower reaches of Terrapin Creek by wind-driven currents. In addition, the levels of indicator species throughout Terrapin Creek were generally low to moderate, with few exceptions. One notable exception took place at TP1 in January, 2006 when levels exceeded 10,000 CFU/100ml. Once again, given the surroundings at TP1, it appeared that the extreme levels resulted from waters mixed with those from Dunn Creek. It was also obvious that the sediments were harboring high levels of indicator species at each of the three primary sampling stations.

Table 3.5 Summary of the results of the assessment techniques utilized for samples collected at Terrapin Creek over the eight-month project period.

Site	Bacteroides Human	Bacteroides Ruminant	ESP	*E. faecalis*
Terrapin (TP) -1	1/3	0/1	0/2	0/5 (0%)
Terrapin (TP) -2	0/4	0/2	0/2	NA
Terrapin (TP) -3	0/2	0/1	0/1	NA

In order to confirm suspicions that the primary source of contamination at Terrapin Creek was a dairy farm that had since been closed and isolated from the boundaries of the WBID, an analysis comparing the genomic profiles of the sediments to those of the overlying waters was conducted. Results indicated that the sediments contained a reservoir of contamination that had not been recently augmented, supporting the contention that the original source of pollution had been severed (Figure 3-7). Unfortunately, it appears that contamination is still entering the lower portion of this WBID from surface waters associated with Dunn Creek.

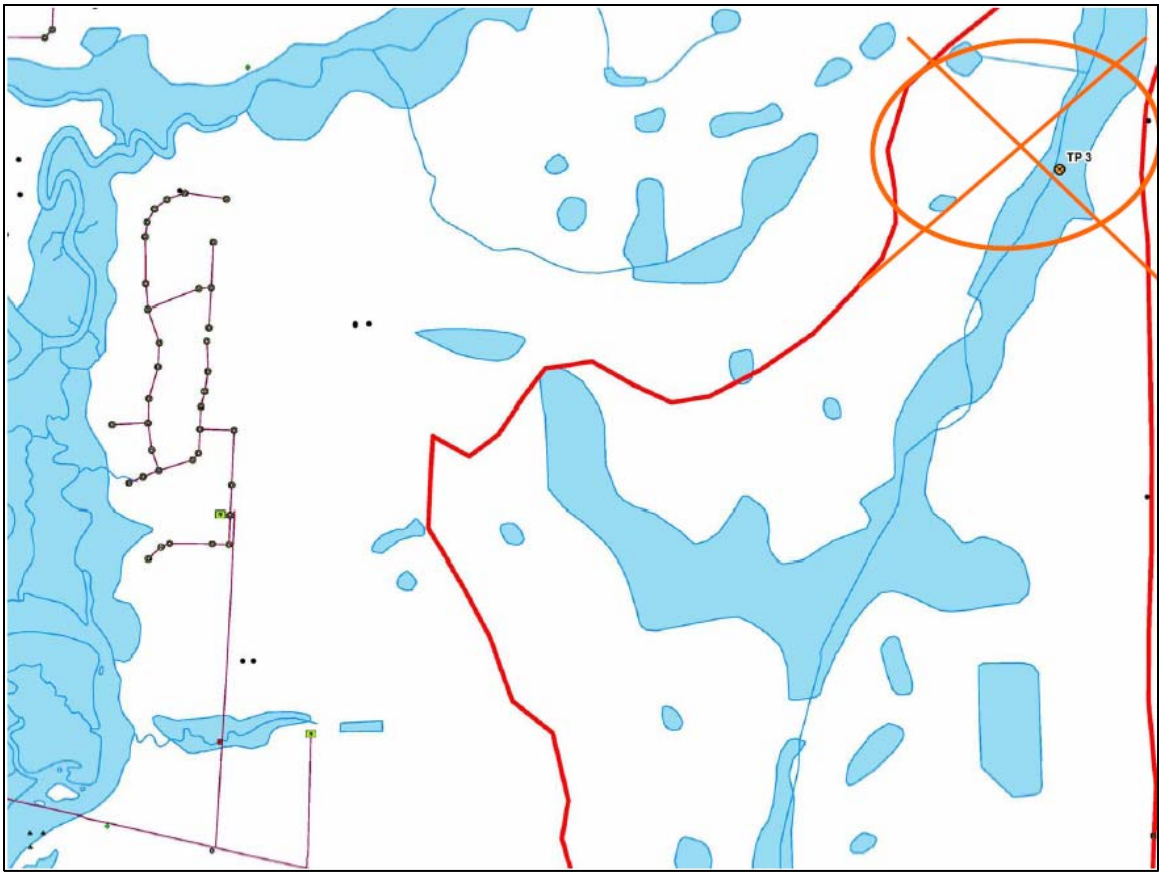


Figure 3-7. Map showing the locations of the sampling stations and GIS data collected for Terrapin Creek. The orange circle delineates the approximate area where the most significant contribution of fecal coliform contamination to the tributary originates. A map legend is located in Appendix D.

3.7 Blockhouse Creek

Blockhouse Creek represented the Rural, No Putative Source category. Unlike the other tributaries described above, the results for Blockhouse Creek identified both human and ruminant sources of contamination, with equal probability for both. Although the analysis of ruminant bacteroides was positive only once, it is likely, given the degree of "light" agriculture throughout much of the area, that these farms were contributing to the contamination of the basin's surface waters. In contrast, a human source was indicated by the results of human bacteroides, ESP, and PCR for *E. faecalis* at BH1 (Table 3.6; Appendix C). The level of indicator species in the surface waters were typically moderate (fecal coliforms generally between 800 and 2500 CFU/100ml) and were never extreme. Analysis of the sediments within Blockhouse Creek indicated high loads of the three indicator species at each of the three primary sampling locations.

Table 3.6. Summary of the results of the assessment techniques utilized for samples collected at Blockhouse Creek over the eight-month project period.				
Site	**Bacteroides Human**	**Bacteroides Ruminant**	**ESP**	***E. faecalis***
Blockhouse (BH) -1	1/3	0/1	1/2	3/5 (60%)
Blockhouse (BH) -2	0/3	0/1	0/2	1/5 (20%)
Blockhouse (BH) -3	0/4	1/2	0/3	3/15 (20%)
Blockhouse (BH) -1Seep	0/1	NA	0/1	NA
Blockhouse (BH) -3Seep	0/1	NA	0/1	NA

During the field reconnaissance portion of this investigation, it became apparent that there were several potential illicit discharges to the surface waters of Blockhouse Creek, each of which were immediately reported to the DCHD for further investigation. Given these observations in conjunction with the sampling results, weight-of-evidence leads to the conclusion that the source of contamination is likely comprised of numerous human-related, small-scale points of contamination (potentially including sewer and/or septic infrastructure) coupled with relatively minor amounts of input from neighboring farms (Figure 3-8). More significant sources, likely episodic in nature, may also be present.

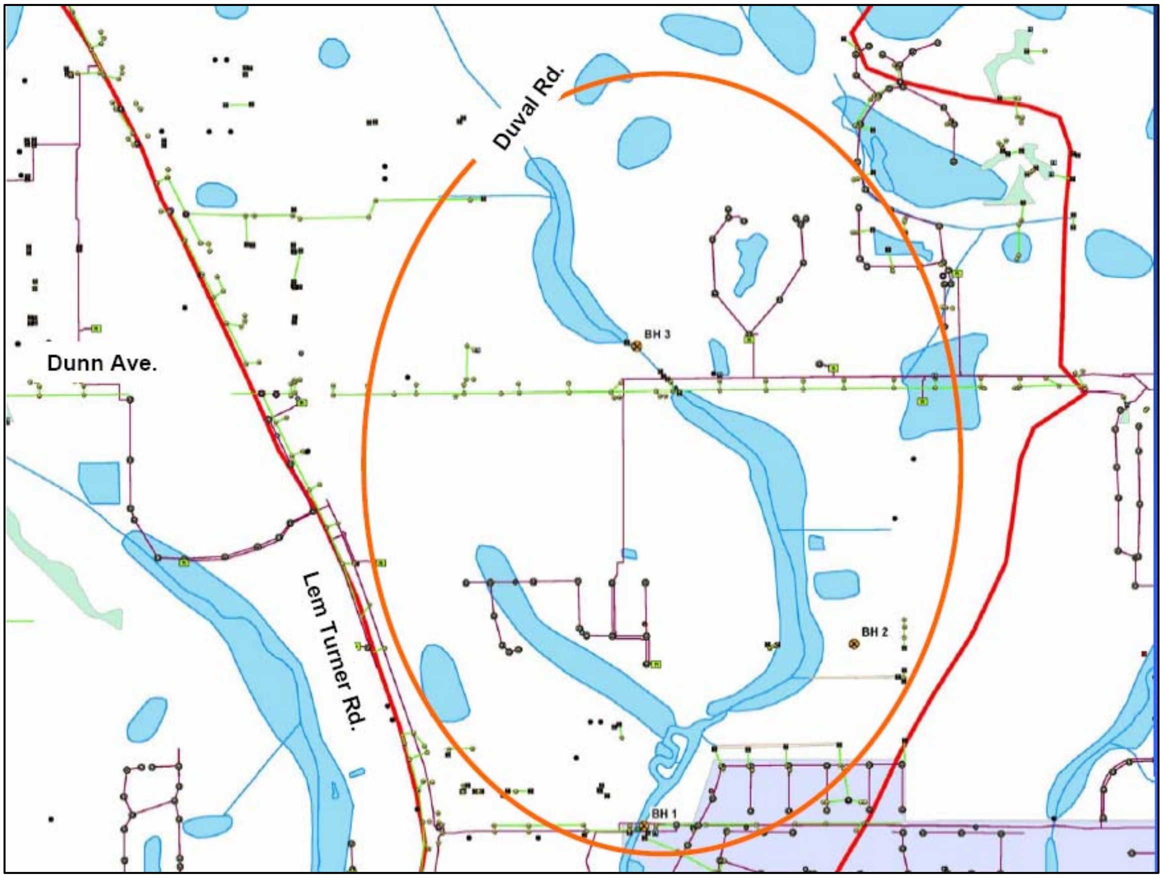


Figure 3-8. Map showing the locations of the sampling stations and GIS data collected for Blockhouse Creek. The orange circle delineates the approximate area where the most significant contribution of fecal coliform contamination to the tributary originates. A map legend is located in Appendix D.

3.8 Thomas Creek

Thomas Creek was selected as a control WBID (relatively unimpacted by human activities) and was used to verify that analytical methods did not yield false-positive indication of specific pollution sources. Although this tributary is not pristine, it was chosen because it was closest to being outside the influence of sewer and septic systems and also not highly impacted by agriculture. The analysis of ruminant bacteroides was positive at all three sites and was not surprising given the degree of "light" agriculture throughout much of the area. In contrast, a human source was not indicated by the results of human bacteroides, ESP, and PCR for *E. faecalis* (Table 3.7; Appendix C). The level of indicator species in the surface waters was typically low (fecal coliforms generally below 200 CFU/100ml with occasional peaks between 800 and 900 CFU/100ml). Analysis of the sediments within Thomas Creek did not indicate a substantial load of the three indicator species.

3.7. Summary of the results of the assessment techniques utilized for samples collected at Thomas Creek over the eight-month project period.				
Site	**Bacteroides Human**	**Bacteroides Ruminant**	**ESP**	***E. faecalis***
Thomas (TC) -1	0/1	1/2	0/2	1/10 (10%)
Thomas (TC) -2	0/3	1/2	0/1	NA
Thomas (TC) -3	0/4	1/2	0/1	NA

Background information and field observations, together with information gained through the sampling results, suggest that Thomas Creek was an acceptable control for this project.

Section 4. Suggested Site-Specific Corrective Actions

Results from testing of the six priority WBIDs indicate that the dominant source of fecal coliform contamination in Duval County is human-related. More specifically, the three main source-types (not listed in order of importance) were: 1) onsite wastewater treatment and disposal systems; 2) existing utility infrastructure; and 3) stormwater (as a conveyance system). One exception to this generalization was Terrapin Creek.

It was determined that the primary source of fecal coliform contamination at Terrapin Creek was the dairy farm that was once located within the northern boundaries of the associated watershed. Given that the hydrology of that basin has changed, such that any remaining livestock have been isolated by new construction of a road and an office park, that source has been disconnected. Further examination of the site would be necessary to determine whether corrective actions should be implemented to remedy the damages already incurred. It should be noted that an influx of contaminated water from Dunn Creek is also a potential source of fecal coliforms in Terrapin Creek. Further study will be needed to determine the necessity of additional measures.

It appears that either onsite wastewater treatment and disposal systems (septic systems) or existing utility infrastructure may be the source of concern regarding fecal coliform contamination at Deep Bottom Creek, New Castle Creek, and Blockhouse Creek. At this stage the dominate source(s) have not been definitively determined. It is suggested that further field reconnaissance of area septic systems and existing sewer infrastructure, be conducted using available techniques such as aerial infrared thermal imaging, groundwater monitoring, dye and smoke testing, and possibly additional sampling, to verify the exact source(s) of the problems within each basin. This additional investigation is beyond the scope of this project.

Implementation of many of the corrective actions discussed below began a few years prior to this project and the impacts have not been fully realized in all impacted tributaries. Nevertheless, it is recommended that all of these programs continue with refinements derived from these and subsequent detailed assessments of the area tributaries. A summary table of suggested corrective actions for each WBID is included at the end of this section (Table 4.1).

4.1 Remediation of Onsite Wastewater Treatment and Disposal Systems

Onsite wastewater treatment and disposal systems, or septic systems, have been identified as a leading source of fecal coliform contamination in Miramar Creek, and possibly to a lesser degree in Deep Bottom Creek, New Castle Creek and Blockhouse Creek. 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, such as those throughout much of the Miramar Creek basin, 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). One program already underway in Duval County is lead by the WSEA, established in 2003 as an independent authority by the City of Jacksonville, to provide a voluntary program through which property owners are given the opportunity to finance water and/or sewer infrastructure in their existing developed neighborhoods (http://www.coj.net/Departments/Independent+Boards+and+Agencies/Water+and+Sewer+Expansion+Authority/default.htm).

Given the high density of degraded septic systems in the Miramar Creek basin, one suggested option is to add the upstream portion of this WBID to the list of WSEA's priority septic tank phase-out areas. Although portions of the Deep Bottom Creek and New Castle Creek basins have already been designated as phase-out areas, there still remains isolated and problematic septic systems outside of these areas that are likely contaminating the neighboring surface waters. Systems such as these, as well as those located within the Blockhouse Creek watershed, may be better served through alternative programs such as:

- a Department of Health-sponsored certification program for haulers, similar to the Fats, Oils and Grease (FOG) program run by JEA;
- a program in which septic tanks must be inspected when home ownership changes;
- a program to execute new maintenance and design requirements that are based on environmental conditions (e.g., location within an impaired watershed, unsuitable soil conditions, proximity to surface water) and are regulated by the City;
- 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) (National Environmental Services Center, 2006); and
- a subsidized septic tank-pumping program that could be used in conjunction with septic-tank phase out programs to help immediately alleviate impacts during the transition period.

In addition to these programs, a broad-ranging septic tank ordinance could be implemented to avoid future water quality impacts. Under this ordinance, a variety of tasks could be performed. For example:

- delineate areas where septic tanks are inappropriate and other options are required;
- determine critical habitat and environmentally sensitive areas where use of septic tanks should be restricted;
- develop and implement a mandatory septic system inspection and tracking program;
- encourage efforts to connect to the existing sanitary sewer system and wastewater treatment facilities; and
- require setbacks and minimum lot sizes for septic tank use.

4.2 Rehabilitation of Existing Utility Infrastructure

Severe and catastrophic utility infrastructure collapses are rare, but as the infrastructure ages, failures become more likely.

It appears that this may be the issue of greatest concern regarding fecal coliform contamination at Butcher Pen Creek, and possibly to a lesser degree, if at all, at Deep Bottom Creek, New Castle Creek, and Blockhouse Creek.

Ongoing rehabilitation of existing utility infrastructure is important in order to help reduce or eliminate sanitary sewer overflows (SSOs). Through proper preventative and predictive maintenance and the identification of problem areas, SSOs can be reduced. Beginning in the late 1990’s the regional utility provider undertook a comprehensive program of pipeline rehabilitation focusing on larger line throughout the service area. To date the utility has expended greater than $500 million in pipeline rehabilitation alone. Since large-scale replacements of existing systems have large 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 1) using remote camera equipment to inspect the lines, 2) cleaning lines, 3) inspecting manholes, and 4) testing pumps at lift stations to ensure proper function. Local utilities should also use “mean time to failure” estimates to replace pumps at lift stations prior to failing as well as warning systems for failures of utilities in order to help minimize impacts when failures occur. These systems have been implemented by the regional utility provider. In addition to performing remedial maintenance on an as-needed basis, the regional utility provider has routinely implemented a variety of corrective actions performed on a prioritized schedule. These include, but are not limited to:

- pipe bursting (to increase the carrying capacity of pipe)
- slip lining (to provide smoother surface within pipe)
- open cut (removal and replacement of pipe)
- fiberglass-line manholes
- replace manholes
- line wet wells
- re-routing
- replace force mains

These measures should be implemented by all area utility providers. Oftentimes, as is the case in Duval County, a single utility is not responsible for all sewer infrastructure throughout the area. Private lift stations, for instance, may be a potential source of fecal coliform contamination to area tributaries. It is suggested that in order to increase the accountability of these facilities, explicit testing and maintenance requirements be employed, a program be implemented to arrange for inspections and repairs by the same entity, with all reports sent to the City.

4.3 Stormwater Treatment

As mentioned above, it is important to consider stormwater as a potential and highly probable conveyance system for a variety of more specific source of bacteria contamination. As a result, it must be considered as a source unto itself in order to manage it more effectively. The following corrective actions would be helpful in the remediation of pollution to each of the six tributaries evaluated in this project.

Corrective actions using stormwater treatment benefit a tributary by reducing the sediment, oil and grease, and nutrient and bacteria loadings discharged into the affected water body. When focusing corrective 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 et al., 1976; LaLiberte and Grimes, 1982; Marino and Gannon, 1991; Davies et al., 1995).

Improvements to the stormwater system, including retrofits and associated programs, may already be underway in the community of interest and could be used to supplement additional, more focused efforts. If these programs are not already in place or are no longer functioning efficiently, implementation or reorganization may be a good place to start. Such programs may include, but are not limited to: 1) active illicit connection programs; 2) public outreach directed specifically towards homeowner's associations (HOAs) that include storm drain stenciling and the distribution of informational door flyers; 3) citizen education programs on reclaimed water; 4) stormwater pond inspection programs aimed at preventing excessive sediment and turbidity in surface waters; 5) construction site inspection programs; and 6) effective street sweeping programs.

Additional stormwater-related corrective actions that should be considered include:

- Additional re-development and retrofit;
- Identification of stormwater utilities responsible for pond maintenance, if unknown;
- Enforcement of zoning and management of proper pond design and creation;
- Use of flocculation via alum injection;
- Use of ultra-violet light disinfection or ozone treatment for small systems;
- Creation of wetland systems;
- Use of individual product-line developments;
- Increased sediment control;
- Increased use of catch basin inlets and coordinating maintenance program;
- Implementation of pet control practices; and
- Application of non-structural BMPs to the affected areas.

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.

Table 4.1 Summary of Suggested Corrective Actions for Each WBID	
Tributary	**Suggested Corrective Actions or Monitoring**
Miramar	1. Add the upstream portion to WSEA's list of priority septic tank phase-out areas. 2. Implementation of programs to address septic systems as sources (e.g., DOH-sponsored certification program for haulers, inspection programs, execution of new maintenance and design requirements, community-based onsite management system program, and/or subsidized pumping program). 3. Implementation of broad-ranging septic tank ordinance. 4. Implementation of stormwater associated programs (e.g., active illicit discharge programs, public outreach, citizen education programs, stormwater pond inspection programs, construction site inspection programs, and/or effective street sweeping programs).
Butcher Pen	1. Conduct further field reconnaissance of sewer infrastructure and possibly additional sampling, as necessary. 2. Increase accountability of any privately-owned sewer infrastructure in area. 3. Implementation of stormwater associated programs (e.g., active illicit discharge programs, public outreach, citizen education programs, stormwater pond inspection programs, construction site inspection programs, and/or effective street sweeping programs).
New Castle	1. Implementation of programs to address septic systems as sources (e.g., DOH-sponsored certification program for haulers, inspection programs, execution of new maintenance and design requirements, community-based onsite management system program, and/or subsidized pumping program). 2. Conduct further field reconnaissance of sewer infrastructure and possibly additional sampling, as necessary. 3. Increase accountability of any privately-owned sewer infrastructure in area. 4. Implementation of stormwater associated programs (e.g., active illicit discharge programs, public outreach, citizen education programs, stormwater pond inspection programs, construction site inspection programs, and/or effective street sweeping programs).
Deep Bottom	1. Implementation of programs to address septic systems as sources (e.g., DOH-sponsored certification program for haulers, inspection programs, execution of new maintenance and design requirements, community-based onsite management system program, and/or subsidized pumping program). 2. Conduct further field reconnaissance of sewer infrastructure and possibly additional sampling, as necessary. 3. Increase accountability of any privately-owned sewer infrastructure in area.

Table 4.1 Summary of Suggested Corrective Actions for Each WBID	
Tributary	**Suggested Corrective Actions or Monitoring**
Terrapin	1. Monthly monitoring of the water column and sediments should be continued to ascertain whether corrective actions are necessary to remedy damages already incurred. 2. The potential impact of Dunn Creek as a potential source of fecal coliform contamination in Terrapin Creek should be investigated further. 3. Implementation of stormwater associated programs (e.g., active illicit discharge programs, public outreach, citizen education programs, stormwater pond inspection programs, construction site inspection programs, and/or effective street sweeping programs).
Blockhouse	1. Implementation of programs to address septic systems as sources (e.g., DOH-sponsored certification program for haulers, inspection programs, execution of new maintenance and design requirements, community-based onsite management system program, and/or subsidized pumping program). 2. Conduct further field reconnaissance of sewer infrastructure and possibly additional sampling, as necessary. 3. Increase accountability of any privately-owned sewer infrastructure in area. 4. Implementation of stormwater associated programs (e.g., active illicit discharge programs, public outreach, citizen education programs, stormwater pond inspection programs, construction site inspection programs, and/or effective street sweeping programs).

Section 5. Monitoring Plan

Once corrective actions are implemented, a monitoring program is needed to determine if the long-term goals of the remediation activities are met. As with any long-term monitoring plan, several factors must be included to effectively establish whether the objectives of remediation have been achieved. The first is the use of ecologically-based hypotheses that would allow for an accurate response to the questions at hand. Another critical component is the need for data acquisition to be standardized and regulated such that all monitoring events are consistent and can be integrated into a continually updated database. The methods described in the Tributary Pollution Assessment Manual should be used to direct future studies of this type in Duval County.

In Duval County, the FDEP and the City of Jacksonville have both implemented individual monitoring programs aimed at tracking fecal contamination levels of specific WBIDs. As a result, a large database of historical data has been developed. This database, in conjunction with the results of studies such as this, should be used to detect signs of long-term trends and establish a baseline of how each system functioned prior to any corrective actions.

It is suggested that, in the future, continual rainfall data be collected at each station sampled for indicators of fecal coliform contamination. Whenever possible, the rain data relevant to the WBID in question should be utilized to perform a linear regression analysis to determine if there is a relationship between fecal concentrations and rainfall events. It is suggested that the antecedent rainfall amount for 1 day, 3 days, and 7 days be examined in this analysis since there is a lag time between when rainfall occurs and when runoff reaches the sampling location. This type of data is beneficial to future assessment efforts because it allows any patterns to be discerned, can help in determining critical sampling times, and could provide predictive capabilities for beach or shellfish harvesting restrictions based on rainfall alone. Unfortunately, rainfall data, available from the SJRWMD (http://arcimspub.sjrwmd.com) or various online weather local and regional services (e.g., intellicast.com) does not always pertain directly to the site in question and must be collected on a more targeted basis. This information could be used in conjunction with sampling results to gain a better understanding of potential source types.

Section 6. Lessons Learned

Several important lessons were learned throughout the duration of this project regarding the practical application of the developed methodology. There are three main categories in which these lessons could be placed: 1) effectiveness of the sampling protocol; 2) utility of the various assessment techniques; and 3) ability to accurately analyze the results and identify specific sources. Finally, it is recommended that the findings of future assessments, continued implementation of corrective action programs and technological developments be used to further refine the guideline assessment manual.

6.1 Effectiveness of the Sampling Protocol

Three significant issues concerning the sampling protocol became immediately apparent. The first was the need for the collection of sediments to be included in the standard sampling procedure. Examination of the sediments not only allowed for the confirmation of residual sources in the benthos but opened the door for the investigation of “old” versus “new” inputs. Another lesson, obvious even prior to commencement of the sampling events but financially unfeasible, was the restriction of collecting one sample per station at each WBID. Like many biological communities, bacteria living in both the water column and sediments are irregularly distributed on most temporospatial scales. As a result, it would be beneficial to collect at least three samples at each site from both the water column and underlying sediments, as necessary, in order to better characterize the biological community. It was discovered that even more limiting, was sampling at only three stations within each WBID. Without firm knowledge of a very specific putative source location, it would have been nearly impossible to identify sources using information collected at only three stations. At a minimum, every branch of each tributary needs to be regularly accounted for in the sampling regime; in some cases, depending on the size of the tributary, multiple stations may be required along each segment. In addition, stations may need to be added, even on a temporary basis, throughout the duration of the investigation in order to better locate or to rule out specific sources.

6.2 Utility of the Various Assessment Techniques

Several lessons were learned concerning the utility of various assessment techniques both included in the original tiered approach and that came to our attention as the project progressed. Most notable was the use of fluorometry in detecting domestic wastewater contamination. It was determined that this technique may prove most beneficial when utilized as a flow-through, vessel-mounted instrument. As a result, this method would be best used in navigable waterways and was not found to be beneficial in the investigation of the six tributaries assessed during this project.

Another example involved the use of rep-PCR. As described in Section 3, this technique was originally envisioned to match genomic profiles of randomly selected isolates to a library of *Enterococcus* profiles from bacteria isolated from known sources in the study region such as sewage, ducks, dogs, wild animals and sediments. Instead, this assessment method was used to compare patterns within the genomic profile of sediments to those of the water column, thereby allowing for the determination of residual or continuous sources.

The potential for two new tools in the investigation of bacterial sources also became apparent. The first, Aerial Infrared Thermal Imaging, can be used to locate potential sources of pollution, including illicit discharges, in and around water bodies, by utilizing an infrared image to detected temperature gradients. An evaluation of the potential for using infrared technology to identify the location of illicit discharges to the surface waters of three tributaries in Duval County was conducted by City of Jacksonville's Environmental Quality Division (EQD). Despite limitations, primarily difficulty in determining signatures and the abundance of permitted discharges identified as potential sources, the results of the study indicated that the technique provides useful information for identifying discharges, and depending on cost, could be a useful tool for detecting pollution sources. An important benefit of Aerial Infrared Thermal Imaging is that it can be used to quickly assess a water body, as hundreds of miles can be scanned in one night under optimum conditions. The second is the monitoring of groundwater wells to help better track microbial contamination, especially from septic systems. It is important to note that in groundwater, bacteria do not tend to travel as far or as fast as viruses. Therefore, if the objective is to focus on potential impacts of septic systems, it would be more useful to focus the efforts of the investigation on bacteriophages rather than on coliforms. Groundwater has also been proven to be a valuable tool for tracking the spread of antibiotic resistance genes (which serve as markers for resistant bacteria) originating from the gastrointestinal tract of production animals. Koike (2004) reported that samples collected from groundwater wells consistently contained antibiotic resistance genes associated with bacteria originating from animal production systems. Not surprisingly, the study also reported that hydro-geological conditions influenced sample results, with a higher frequency of detection in down-gradient wells.

6.3 Ability to Accurately Analyze the Results and Identify a Specific Source

One of the greatest obstacles to this investigation was the reality that the methods and regulatory aspects of source-tracking of microbial populations are still under development, and therefore can be somewhat controversial and remain an inexact, emerging area of science. As a result, it was confirmed that a weight-of-evidence approach, taking the results of several methods as well as background information into account, remains a requirement for this type of endeavor. Despite these methods, in some cases, although a source-type was identified, the physical location of the source input to the WBID was not determined. In these instances, further field investigations are required.

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Web Resources

http://coj.net/Departments/Independent+Boards+and+Agencies/Water+and+Sewer+Expansion+Authority/default.htm

http://arcimspub.sjrwmd.com