Fecal Coliform Bacteria, Fluorescent Whitening Agents, Bacteriological Indicators, and Microbial Source Tracking Studies in Gainesville's Urban Creeks
Microbial "Hot Spots"
June 2004 through August 2007

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Executive Summary

Alachua County Environmental Protection Department (ACEPD) has been monitoring fecal coliform bacteria in Gainesville's creeks for many years and has identified fecal coliform "Hot Spots". These sites are located in the following watersheds: Tumblin Creek, Sweetwater and Rosewood branches, Hogtown and Possum creeks, Rattlesnake Branch, Elizabeth Creek, and Lake Forest and Little Hatchet creeks. In an attempt to determine the source(s) of fecal contamination, ACEPD has utilized numerous fecal indicators and monitoring strategies. Water column samples have been analyzed for fecal coliform, *Escherichia coli, Clostridium perfringens, Enterococcus* spp., fluorescent whitening agents (FWAs), and microbial source tracking (MST) human specific DNA markers. Sediment samples have been analyzed for fecal coliform, *E. coli, Enterococcus* spp, general *Bacteroides* spp., and human specific DNA markers. ACEPD also experimented with a high frequency short duration fecal coliform monitoring strategy and has assessed ambient concentrations of fecal coliform. This report provides a summary of the ambient monitoring data for fecal coliform and the investigations conducted between June 2004 and August 2007 to evaluate sources of the bacterial contamination.

ACEPD recognizes the short comings of the various indicators of fecal pollution, but the studies conducted did provide valuable insight into the possible sources. Additional sampling and watershed analysis would be required to confirm the suspected sources at the "Hot Spots" listed below.

- In Tumblin and Hogtown creeks and Rattlesnake Branch, it is suspected that the main sources of fecal contamination may be wastewater collection systems and waste from domestic and wild animals in the watershed.
- In Sweetwater Branch failing wastewater infrastructure and the transient population camping along its banks are the most likely sources elevating fecal coliform levels.
- Septic tank systems and/or wastewater collection systems are suspected of being the major sources of fecal contamination in Rosewood Branch.
- In Elizabeth Creek failing wastewater collection systems, including possible cross connections with the stormwater collection system, are a likely source.
- The permitted discharge of reclaimed water (treated wastewater effluent) from the Brittany Estates Water Reclamation Facility is likely responsible for the presence of human specific DNA markers in Little Hatchet Creek. However, this conclusion is based on very limited data.
- A probable fecal source in the upper Lake Forest Creek Watershed is the large population of domestic dogs in this area.

Human specific DNA markers provided the most specific source information, but they are also very costly and frequent sampling is needed to develop a statistically valid dataset to evaluate trends. It is important to recognize that MST is an emerging field and that negative results for a human specific DNA marker may only mean that the individual marker tested for was not detected at that time, not that there is no risk of human fecal contamination in the waterbody. The repeated absence of human specific DNA markers is likely indicative of wildlife or domesticated animals as the primary source of fecal

coliform contamination, making it helpful in concluding which sites were likely not influenced by human fecal coliform contamination.

ACEPD found that FWAs were frequently undetectable by the qualitative methods employed and did not correlate well with MST results and fecal coliform counts. FWAs are subject to interference by dissolved organics, sedimentation, surface growth on the sampling media, and photodegradation. It is also likely that the laboratory method used was not sensitive enough to detect FWAs at the concentrations present in the urban creeks. FWAs did not prove to be an effective qualitative screening tool for identifying candidate sites for MST tests or detecting illicit discharges.

Analyzing samples for alternative bacteriological indicators including C. *perfringens* and *Enterococcus* spp. did not provide enough additional source information to justify the use or expense of these indicators. Additionally, there are no state standards applicable to the urban creeks for these indicators. *E. coli* concentrations were found to track fecal coliform colony counts in most instances and will continue to be used to help determine if sources are recent.

High intensity short duration fecal coliform sampling was an effective sampling method for characterizing sites. However, this approach is very time consuming and expensive; and the resulting data did not provide sufficient additional information to warrant the increased costs and staff time. Increases in turbidity and fecal coliform concentrations were observed with rainfall events in several of the studies described in this report.

The results of MST from in-stream sediment samples were not easily interpreted. It was difficult to correlate sediment MST data with elevated fecal coliform concentrations in the water column. Elevated bacterial concentrations related to sediment populations may not be detected in the water column unless sediments are stirred up by high flow conditions or other disturbances. Similar to water column samples, sediment samples are heterogeneous and there may be great spatial variability requiring replicate samples for accuracy.

A partnership between ACEPD, the Alachua County Health Department, the Gainesville Clean Water Partnership (City of Gainesville Public Works Department, Alachua County Public Works Department, and the Florida Department of Transportation), Gainesville Regional Utilities (GRU), and the Florida Department of Environmental Protection (FDEP) has been formed to further explore sources of fecal coliform at the “Hot Spots”. Investigations will include septic tank system surveys and inspections and integrity testing of stormwater collection and wastewater collection systems using TV camera surveys, smoke testing, and other investigative methods. Potential illicit discharges in the “Hot Spots” and other areas will be systematically evaluated using outfall reconnaissance techniques and target sampling for indicators such as fecal coliform, surfactants, ammonia nitrogen, and potassium. The role of wildlife and domestic animals as sources will also be further explored. Additional sampling, which will likely include techniques discussed in this report, will be conducted to narrow in on the sources of contamination in each “Hot Spot.” Quality control measures will be built into all future studies.

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Fecal Coliform Bacteria, Fluorescent Whitening Agents, Bacteriological Indicators, and Microbial Source Tracking Studies in Gainesville's Urban Creeks Microbial "Hot Spots" June 2004 through August 2007

1.0 Introduction

1.1 Purpose

Historically, fecal coliform levels in Gainesville's urban creeks have exceeded state water quality standards (FDEP, 2006). Water bodies impacted by the presence of fecal coliform are present in the three major watersheds in the Gainesville urban area, Hogtown Creek, Paynes Prairie, and Newnans Lake (Figure 1-1). Total Maximum Daily Loads (TMDLs) have been developed for fecal coliform in Hogtown Creek, Sweetwater Branch, and Tumblin Creek. This report presents an analysis of ambient fecal coliform data from 2001 through mid 2007 and a series of studies conducted to better define the sources of elevated fecal coliform bacteria in the creeks. The individual studies were conducted over a three year period from 2005 through early August 2007 and are listed below and presented in detail in the following sections.

- Ambient Fecal Coliform Monitoring and one Stormflow Monitoring Event
- Fluorescent Whitening Agents (FWAs) in Gainesville Creeks, May 2005
- FWAs, Fecal Coliform, Alternative Bacteriological Indicators, and Microbial Source Tracking (MST) at "Hot Spots," April 2006
- FWAs, Fecal Coliform, and MST at "Hot Spots," April 2007
- Intensive High Frequency Short Duration Fecal Coliform Monitoring, Tumblin Creek Pilot Project December 2006
- High Frequency Short Duration Fecal Coliform Monitoring, January and July 2007

1.2 Background and Water Quality Standards

Total and fecal coliform bacteria are fecal pollution indicators used for monitoring water quality. The "indicator concept" is not new and has been used to assess the sanitary condition of drinking water, recreational waters, and waters for shellfish propagation since the early part of the 20th century (Griffin et al., 2001; Scott et al., 2002). Coliform bacteria are commonly divided into two categories based on controlled growth in the laboratory. Total coliform is a large group of bacteria that belong to the family *Enterobacteriaceae* and includes facultative anaerobic, gram-negative, nonspore-forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35^{o}C (APHA, 1998). Some total coliform organisms can live and reproduce in soil and water without a warm-blooded host, so their presence is not necessarily indicative of fecal pollution. Fecal coliform bacteria are a subset of the total coliform group and are distinguished by their ability to grow and ferment lactose under incubation at 44.5 ^{o}C (Griffin et al., 2001; APHA, 1998). Fecal coliform bacteria originate from the digestive track of warm blooded animals; therefore their presence in water is more indicative of fecal pollution than the total coliforms.

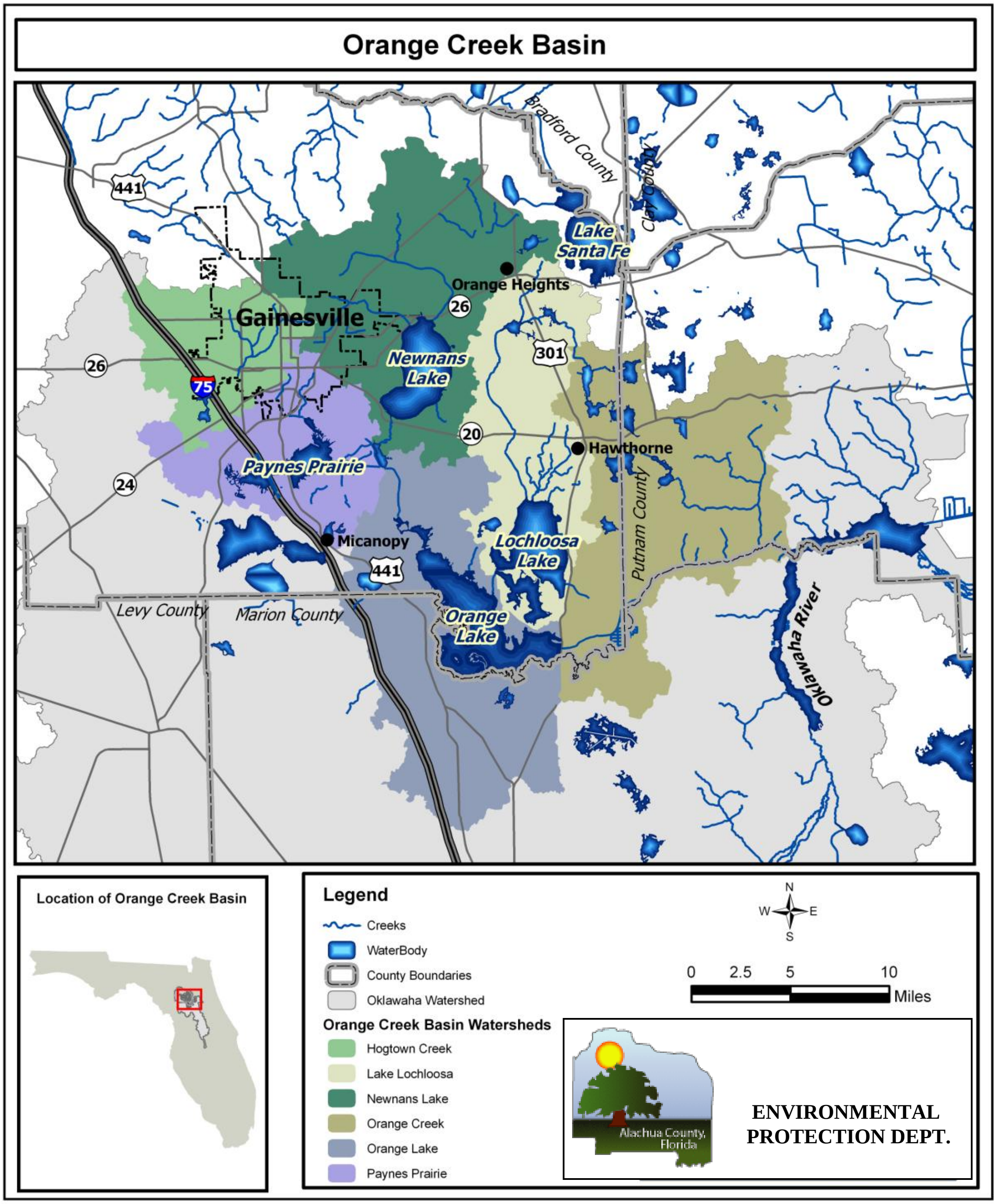


Figure 1-1. Watershed Map for the Gainesville Urban Area

The fecal coliform group has long been used as a microbial indicator of fecal contamination for recreational waters and bathing places (EPA, 1974; EPA, 1986). The Florida Department of Environmental Protection (FDEP) state water quality microbial standards for Class III recreational fresh waters (Table 1-1) are based on fecal coliform bacteria (FDEP, 2006). All of the waterbodies discussed in this report are considered by FDEP to be Class III recreational fresh waters. None of the Gainesville urban creeks are designated as bathing places. In Chapter 62-302 of the Florida Administrative Code (FAC) three coliform criteria are set forth; a single sample maximum, an average value that must not be exceeded in 10% of the samples and a monthly average based on a geometric mean (Table 1-1). For the purpose of this report, we have used the 800 colony forming units per 100 milliliters (CFU/100 mL) single sample maximum as the standard.

Table 1-1. Florida Bacterial Indicator and Water Quality Criteria

Agency	Indicator	Water Type	Water Quality Criteria[a]
US Environmental Protection Agency (EPA)	Fecal Coliform[b]	Marine and Fresh Water	800 CFU/100 mL (SSM) 400 CFU/100 mL in 10% of samples 200 CFU/100 mL (GM) in a monthly average based on a minimum of 20 samples taken over a 30 day period
	Enterococcus[c]	Marine Waters	104 CFU/100 mL (SSM) 35 CFU/100 mL (GM)
		Fresh	61 CFU/100 mL (SSM) 33 CFU/100 mL (GM)
	Escherichia coli[c]	Fresh	235 CFU/100 mL (SSM) 126 CFU/100 mL (GM)
Florida Department of Environmental Protection (FDEP)[e]	Fecal Coliform	Class III Recreational Waters Fresh or Marine	800 CFU/100 mL (SSM) 400 CFU/100 mL in 10% of samples 200 CFU/100 mL (GM) in a monthly average based on a minimum of 20 samples taken over a 30 day period
Florida Department of Health (FDOH)	Fecal Coliform[f]	Designated Bathing Areas Marine and Fresh Water	800 CFU/100 mL (SSM) 400 CFU/100 mL in 10% of samples 200 CFU/100 mL in an average (GM)
	Enterococcus	Designated Bathing Areas Marine Waters	104 CFU/100 mL (SSM)[e] 35 CFU/100 mL (GM)[e]
		Designated Bathing Areas Fresh Water	61 CFU/100 mL (SSM)[f] 33 CFU/100 mL (GM)[f]
FDOH – Proposed	*Escherichia coli* [f]	Designated Bathing Areas Marine and Fresh Water	235 CFU/100 mL (SSM) 126 CFU/100 mL (GM)

a. SSM is the Single Sample Maximum and GM is the Geometric Mean
b. EPA Water Quality Criteria Document (EPA, 1974)
c. EPA Ambient Water Quality Criteria (EPA, 1986) and Water Quality Standards for Coastal and Great Lakes Recreation Waters (EPA, 2004)
d. Surface Water Criteria Chapter 62-302 Florida Administrative Code (FDEP, 2006)
e. Chapter 64E-9.013, FAC (FDOH, 2004)
f. 2008 Draft Rule Revision Chapter 64E-9, FAC (FDOH, 2008)

The above criteria and the designated uses for surface waters are currently being reviewed by FDEP. The FDEP 800 CFU/100 mL standard was proposed by FDEP in 1995 as a result of their Triennial Review of FDEP Water Quality Criteria (Williams, 2008). The additional standards, 400 CFU/100 mL in 10% of the samples and a geometric mean of 200 CFU/100 mL in 20 or more samples over 30 days, were adopted from United States Environmental Protection Agency criteria (EPA, 1974; Williams, 2008). The FDEP withdrew the total coliform water quality standard in 2006 due to the lack of specificity of the total coliform group. Strategies for reducing fecal coliform bacteria in recreational waters will in many cases substantially improve total coliform concentrations, since fecal coliform bacteria are a subset of total coliforms.

Through its ambient surface water monitoring program, Alachua County Environmental Protection Department (ACEPD) identified eight locations where the fecal coliform state water quality standard was routinely exceeded. These areas of concern include Hogtown Creek in the vicinity of NW 23rd Avenue, Rattlesnake Branch, Sweetwater Branch at NE 10th Avenue, Rosewood Branch, upper Elizabeth Creek, Lake Forest Creek at NE 25th Street, Tumblin Creek at SW 5th Avenue, and Little Hatchet Creek at NE Waldo Road (Figure 1-2). Although the fecal coliform data clearly demonstrated high bacterial populations at the identified "Hot Spots," the limitations of fecal coliforms as an indicator of fecal pollution became apparent.

The limitations of conventional testing methods for coliform bacteria are well documented (Scott, 2002; Bitton, 2005; Harwood, 2006). One limitation that is specifically problematic is that fecal coliform counts do not distinguish between the many possible sources of fecal coliform, which include:

- Exfiltration and releases from public and private centralized wastewater collection and treatment systems
- Malfunctioning on-site wastewater treatment facilities such as septic tank systems
- Direct use of creeks for human sanitation
- Runoff from urban campsites near creeks
- Domestic animal waste
- Wildlife populations that use riparian corridors and adjacent areas

A secondary source once bacteria enter a water body is the persistence and potential growth of fecal coliform bacteria in favorable environments (Desmarais et al., 2000; Griffin et al., 2001). Laboratory studies to evaluate persistence and re-growth of coliform bacteria in sediments have produced varying results (Anderson et al., 2005). Persistence in sediments has been confirmed in laboratory mesocosm experiments, but verification of re-growth was not confirmed under laboratory conditions (Anderson et al., 2005).

In an effort to distinguish the sources of fecal contamination at the identified "Hot Spots" and to refine sampling methods, ACEPD conducted several studies. Studies include the use of alternative indicators including FWAs, *Escherichia coli*, *Clostridium perfringens*, *Enterococcus* spp., and MST, using more sophisticated techniques that detect human specific DNA markers of microorganisms (*Enterococcus faecium*, *Bacteroides* spp., and human polyomaviruses). High frequency short duration sampling regimes for fecal

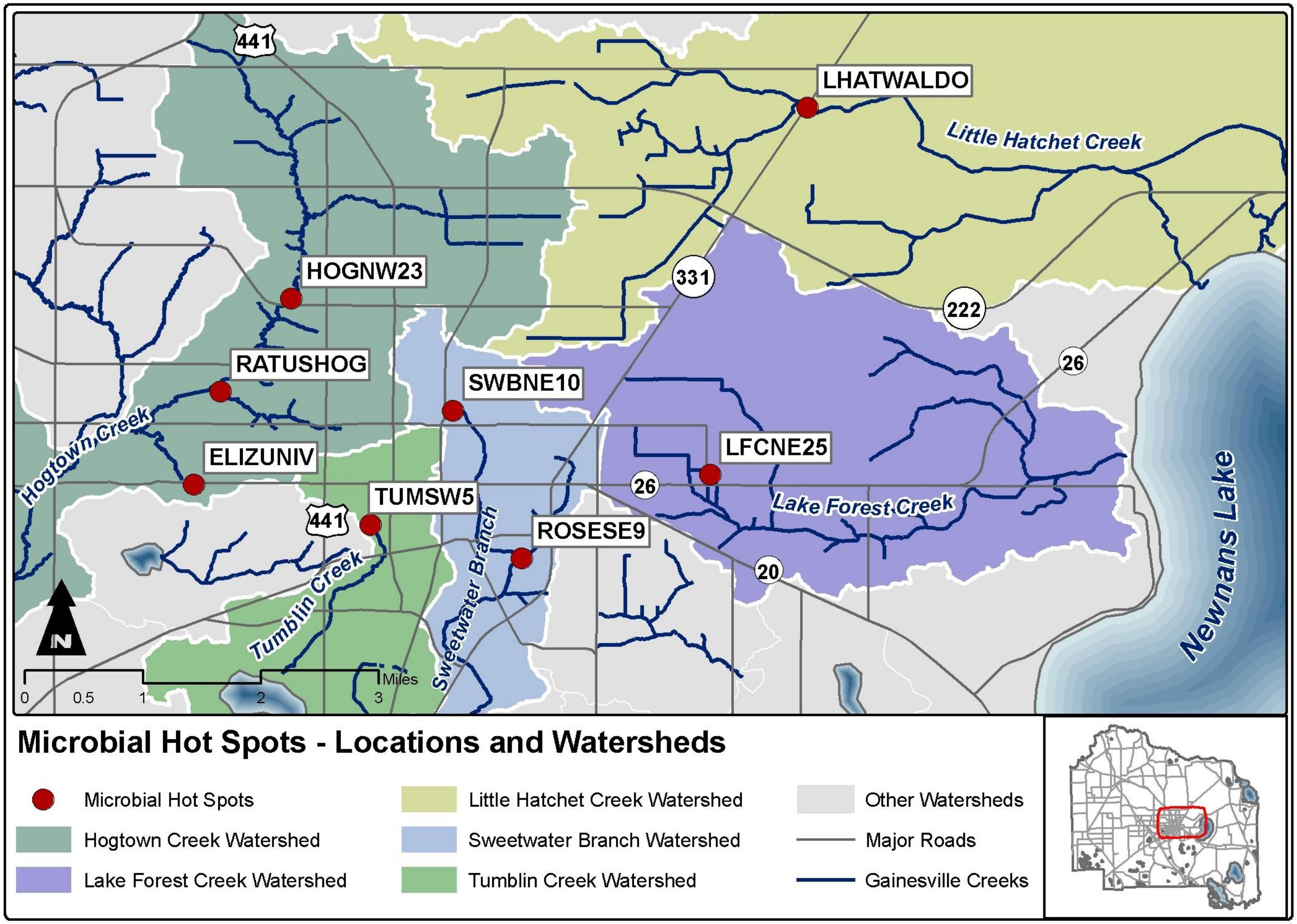


Figure 1-2. Locations of the Eight Microbial "Hot Spots" within the Gainesville Urban Area

coliform were also conducted. One of the most difficult aspects of the monitoring, whether using traditional or MST techniques, is the short "snap shot" of time for which the data are valid given the transient nature of flowing water. The results of these studies are described in the following sections of this report and comprehensive data tables are provided in the appendices.

1.3 Watershed Descriptions

Rainfall recharges surface water directly and indirectly in the Gainesville area. Groundwater from the surficial and intermediate aquifers or aquifer systems provides the primary source of baseflow to these streams. There are three major basins or watersheds within the Gainesville area: Hogtown Creek, Paynes Prairie, and Newnans Lake (Figure 1-1). The data discussed in this report are confined to these three watersheds.

The Hogtown Creek Watershed is a closed basin discharging to the Floridan aquifer system via Haile Sink on Hogtown Prairie west of the Cody Scarp. There are many tributaries to Hogtown Creek, the largest being Possum Creek. Hogtown and Possum creeks and their associated tributaries drain most of northwestern Gainesville. The direction of flow is generally south and west, although Possum Creek flows south and east to its confluence with Hogtown Creek. Hogtown Creek flows through a series of wetlands between SW 2nd Avenue in Gainesville and its terminus at Haile Sink. Two major tributaries of interest in this watershed are Rattlesnake Branch and Elizabeth Creek.

The Paynes Prairie Watershed includes two major streams: Sweetwater Branch and Tumblin Creek. Both of the creeks flow through the Gainesville urban area to Paynes Prairie State Preserve. Tumblin Creek flows south to Bivens Arm Lake; the lake outfall continues onto Paynes Prairie. Sweetwater Branch flows directly onto Paynes Prairie. Sweetwater Branch has one major tributary, Rosewood Branch. On the prairie itself there are two major water bodies: Alachua Sink and Alachua Lake, a large wetland system. The water on Paynes Prairie discharges to the Floridan aquifer system via the Primary Sink Feature at Alachua Sink.

Newnans Lake receives water from three primary sources: Hatchet, Little Hatchet, and Lake Forest creeks. Hatchet Creek, flowing east and south, contributes the greatest volume of water to Newnans Lake and has several large tributaries including Bee Tree Creek, which converges with Hatchet Creek north of SR 26 above the north end of Newnans Lake. Little Hatchet and Lake Forest creeks enter Newnans Lake from the northwest and west, respectively. All three creeks are intermittently dry during drought periods. Newnans Lake discharges water to Prairie Creek on the southern side of the lake at SR 20.

2.0 Fecal Coliform Monitoring

Alachua County Environmental Protection Department (ACEPD) has monitored fecal coliform bacteria levels throughout Alachua County since the 1970s. Fecal coliform levels vary widely in Alachua County's waters (Table 2-1). Median levels of fecal coliform concentrations in Hogtown Creek, Sweetwater Branch, and Tumblin Creek from 2001 through 2005 ranged from less than 290 to 2,400 colony forming units per 100 milliliters (CFU/100 mL). Fecal coliform levels often greatly exceed the one-time allowable maximum of 800 CFU/100 mL Class III Recreational Waters Standard (FDEP, 2006).

Table 2-1. Summary of Fecal Coliform Data for Hogtown Creek, Sweetwater Branch, Tumblin Creek, Little Hatchet Creek, and Lake Forest Creek, 2001 through June 2005

Year	Watershed	Fecal Coliform[a] (CFU/100 mL)			Number (n)	Annual Rainfall[b] (in.)
		Arithmetic Mean	Median	Geometric Mean		
2001	**Hogtown Creek**	1,152	290	462	42	44.55
	Sweetwater Branch	5,019	1,700	1,556	47	
	Tumblin Creek	2,294	1,300	892	27	
	Little Hatchet Creek	278	110	138	23	
	Lake Forest Creek	290	290	216	14	
2002	**Hogtown Creek**	1,746	700	614	81	47.10
	Sweetwater Branch	3,552	1,700	1,310	54	
	Tumblin Creek	5,167	2,400	1,828	47	
	Little Hatchet Creek	504	170	202	51	
	Lake Forest Creek	618	300	307	26	
2003	**Hogtown Creek**	2,291	1,300	1,022	52	43.04
	Sweetwater Branch	1,514	1,110	781	30	
	Tumblin Creek	3,209	2,000	2,152	18	
	Little Hatchet Creek	615	170	158	27	
	Lake Forest Creek	321	130	167	9	
2004	**Hogtown Creek**	1,797	540	558	160	55.73
	Sweetwater Branch	2,751	565	582	102	
	Tumblin Creek	3,526	920	1,070	33	
	Little Hatchet Creek	1377	350	256	51	
	Lake Forest Creek	642	230	287	35	
2005	**Hogtown Creek**	1,005	685	544	46	25.94
	Sweetwater Branch	1,236	720	617	29	
	Tumblin Creek	1,187	800	895	13	
	Little Hatchet Creek	532	560	266	6	
	Lake Forest Creek	646	210	259	26	

a. Fecal coliform values are for all stations monitored on the creeks
Samples were analyzed using either most probable number or membrane filtration analyses
b. Rainfall data was collected at Gainesville Regional Utilities (GRU) John R. Kelly Generating Station

2.1 Background

ACEPD monitoring and assessment activities focus on fecal coliform, since it is a more specific fecal indicator than total coliform and is currently the only indicator of microbial quality for recreational fresh waters recognized by the Florida Department of

Environmental Protection (FDEP). This analysis is relatively inexpensive and there are state standards for reference. ACEPD has historically monitored fecal coliform bacteria in the Gainesville urban creeks and Newnans Lake tributaries within the Orange Creek Basin (OCB). Sampling locations and descriptions are included in Appendix I. For the past three years analyses have been conducted by membrane filtration (MF) techniques, which is an accepted standard method for enumerating indicator organisms in surface waters.

Fecal coliform bacteria concentrations are determined by laboratory analyses of field collected samples. The two traditional and accepted methods for fecal coliform analyses are: multiple tube fermentation, where replicate tubes and dilutions are reported in terms of the most probable number (MPN) and the direct plating technique of membrane filtration (MF) (APHA, 1998). In either case, results are reported relative to a 100 mL sample. Results of multiple tube fermentation tests are reported in terms of the MPN which is determined from probability formulas that estimate the average density of coliform bacteria in a sample based on the results of a number of dilutions from a single sample (APHA, 1998). MF results are based on colony counts, typically as colony forming units (CFU), and are reported as a concentration in a 100 mL sample.

Results from MPN analyses may differ considerably from results derived from MF methodologies for a number of reasons. The two most commonly reported reasons are: (1) the multiple tube fermentation media and incubation temperature allows non-target bacteria from other groups to grow and (2) the media used in multiple tube fermentation analyses revives stressed or injured organisms that would not be enumerated by MF techniques (Griffin, 2004). Although MF methodologies may result in a lower reported bacterial concentration, the results are highly reproducible. Constraints with MF techniques include poor results with turbid water samples and the growth of non-target bacteria on the medium, which results in samples where the colonies cannot be accurately counted. The presence of large quantities of algal cells can interfere with MF analyses, making MPN methodologies more representative for some natural waters (APHA, 1998).

2.2 Purpose

There are three purposes for fecal coliform monitoring of Gainesville's creeks: (1) to monitor ambient levels to determine water quality, (2) to detect illicit discharges to the creeks and (3) to determine if established total maximum daily loads (TMDLs) and associated reduction goals for fecal coliform bacteria in Hogtown Creek, Sweetwater Branch, and Tumblin Creek are being achieved. Two sampling regimens for monitoring fecal coliform bacteria levels have been implemented. The first sampling regimen was quarterly monitoring for fecal coliform when water samples are also collected for routine analyses of nutrients and general chemical characteristics. The second sampling regimen was high frequency short duration sampling in the winter and summer. This second regimen was recommended by FDEP and was implemented for the first time in 2006/2007. These sampling regimens are being used in an attempt to establish trends in fecal coliform levels of the Gainesville Creeks. Sampling related to complaint investigations and illicit discharges, such as sewage releases, are not included in the data discussed in this section or presented in Appendix IIa.

2.3 Materials and Methods

Quarterly fecal coliform samples are obtained during ambient (routine scheduled) surface water quality monitoring that is conducted four to five time per year. Site descriptions, latitude, longitude, and travel directions for all sample locations are in Appendix I. Samples are collected from flowing water in mid-channel at mid-depth in the stream. During quarterly monitoring, field constituents are measured and include dissolved oxygen, pH, specific conductance, water temperature, and turbidity. Samples are also obtained for laboratory analyses of nutrients and general chemical constituents. Water velocity is measured or estimated so that stream flow can be calculated. The sample containers used are Whirl-Pak bags with thiosulfate tablets or sterile hard plastic containers supplied by the laboratory analyzing the samples. Historically, most of the samples have been analyzed by ABC Research, Inc. (ABC) Gainesville, FL. Beginning in the fourth quarter of 2006, Advanced Environmental Laboratories, Inc. (AEL) Gainesville, FL has been conducting the analyses. Both analytical laboratories use the membrane filtration method SM 9222D (APHA, 1999) and are certified for such by the Florida Department of Health. Extended dilutions were run to allow fecal coliforms to be quantified when concentrations were elevated. All samples were submitted to the laboratory within six hours of collection. External quality control samples included replicate sample collection (field duplicates) and occasional blanks prepared from tap water. Beginning in early 2007 high frequency short duration sampling was conducted in an effort to better define fecal coliform levels in the creeks and to provide data for trend analyses. The initial sampling was conducted in December 2006 (Section 6.0) and January 2007 (Section 7.0) and was repeated in July 2007 (Section 8.0).

2.4 Results and Discussion

In the following two sections the results of ambient fecal coliform monitoring and one sampling event specifically designed to evaluate stormflow concentrations of fecal coliform bacteria are discussed. Under stormflow conditions, fecal coliform levels can be orders of magnitude higher than those found under baseflow conditions. Ambient samples are typically not collected during rain events, but are often collected a day or two after storm events. Rainfall data collected at Gainesville Regional Utilities (GRU) John R. Kelly Generating Station are included in Appendix III. Some of the ambient samples have fecal coliform levels typical of stormflow conditions. The TMDL program does not differentiate between baseflow and stormflow samples; instead all results are combined to determine water quality conditions.

To evaluate the fecal coliform results simple statistical methods were used to evaluate the central tendency of the data: arithmetic mean, geometric mean, and median. The geometric mean is frequently used when comparing or analyzing data that varies over several orders of magnitude and can be thought of as a log-transformation of the data. This makes the geometric mean a better measure of central tendency because, unlike the arithmetic mean, the effects of very high or low values are reduced. The median is another measure of central tendency that is not as greatly influenced by extreme values and in the case of the data presented here, compares well (in most instances) to the geometric mean.

2.4.1 Ambient Monitoring

Several monitoring stations were added to the existing monitoring network in the eight areas of concern identified above as “Hot Spots” with consistently high levels of fecal coliform bacteria (Figure 1-2). These areas include Hogtown Creek in the vicinity of NW 23rd Avenue, Rattlesnake Branch, Sweetwater Branch at NE 10th Avenue, Rosewood Branch, upper Elizabeth Creek, Lake Forest Creek at NE 25th Street, Tumblin Creek at SW 5th Avenue, and Little Hatchet Creek at NE Waldo Road. The highest fecal coliform concentrations were found in Sweetwater Branch and Tumblin Creek watersheds (Table 2-2), which are the most urbanized creeks in this study.

Table 2-2. Summary of Fecal Coliform Data by Watershed and “Hot Spot”; Analyzed by Membrane Filter Technique, June 2004 through June 2007

Watershed	Arithmetic Mean (CFU/100 mL)	Median (CFU/100 mL)	Geometric Mean (CFU/100 mL)	Minimum (CFU/100 mL)	Maximum (CFU/100 mL)	Number (n)
Hatchet Creek	331	150	129	<1	4,800	62
Little Hatchet Creek	453	230	162	<1	4,600	62
Lake Forest Creek	4,069	220	342	20	128,000	69
Possum Creek	668	400	370	<1	6,000	101
Hogtown Creek	1,416	530	513	<1	16,000	278
Sweetwater Branch	12,720	715	800	<1	1,300,000	154
Tumblin Creek	1,178	590	577	20	42,000	134
Bivens Arm Lake	146	64	62	<2	500	13
Paynes Prairie[a]	475	130	141	10	9,200	69
"Hot Spot"						
LHATWALDO	899	650	566	100	4,600	24
LFCNE25	9,008	520	694	110	128,000	16
HOGNW23	1,973	700	893	120	11,840	27
SWBNE10	54,063	460	739	<1	1,300,000	31
TUMSW5	2,286	810	949	130	42,000	43
Rattlesnake Branch	1,800	880	874	56	7,600	23
Rosewood Branch	8,890	1,100	1,523	56	132,000	37
Elizabeth Creek	3,617	2,730	2,896	790	10,000	22

a. Paynes Prairie Watershed includes sampling locations on Sweetwater Branch at SR 331 (Williston Road) and downstream as well as locations within Paynes Prairie

The highest levels of fecal coliform bacteria in the Tumblin Creek Watershed occurred where the creek becomes “day lighted”, which is where the creek opens to its above ground section just upstream of SW 5th Avenue. Much of the headwaters and upper reaches of the watershed are underground and flow through culverts. The median concentration throughout the watershed was 590 CFU/100 mL (Table 2-2) with a median value at SW 5th Avenue (TUMSW5) of 810 CFU/100 mL. This is a very urban portion of the watershed with narrow riparian buffers, public and private wastewater collection systems, septic tank systems, and a transient homeless population.

Similar to Tumblin Creek, the highest levels of fecal coliform in Sweetwater Branch occurred downstream of the headwaters, which are primarily underground and flow through culverts. Sweetwater Branch has a small intermittent section in the upper reaches of the watershed that is above ground north of NW 14th Avenue; it then disappears to the subsurface stormwater system before reappearing at NE 10th Avenue. It is in this area at SWBNE10 where high fecal coliform concentrations are often observed with a median value of 460 and an arithmetic mean value of 54,063 CFU/100 mL (Table 2-2). Flow is intermittent at SWBNE10 and there is little water to dilute fecal coliform concentrations. The hydrodynamic separator (Vortex Unit), a trap for sediments and leaves, in the stormwater collection system directly upstream of this site may be a possible source since many bacteria can persist in moist sediments not exposed to sunlight. The wastewater collection system in the urban watersheds is old; much of it is vitreous clay pipe that was installed before other materials were in common usage. Many of the private connections to the system are also old, and some are constructed of clay pipe or less durable materials. This infrastructure may also contribute fecal coliform to surface waters.

Rosewood Branch, the largest tributary to Sweetwater Branch, was also found to have high levels of fecal coliform bacteria, with a median value of 1,100 CFU/100 mL. Rosewood Branch is a highly urbanized watershed with narrow or non-existent riparian buffers, public and private wastewater collection systems, septic tank systems, and a transient homeless population in portions of the watershed.

The main channel of Hogtown Creek was found to have the highest concentrations of fecal coliform bacteria in the vicinity of NW 23rd Avenue (HOGNW23). This area is in the upper middle portion of the watershed. Concentrations of fecal coliform bacteria in this area had a median value of 700 CFU/100 mL (Table 2-2). This is an area with public and private wastewater collection systems, septic tank systems, and a transient homeless population. Two tributaries, Rattlesnake Branch and Elizabeth Creek were also found to have high levels of fecal coliform bacteria. Rattlesnake Branch had a median value of 880 CFU/100 mL; while Elizabeth Creek had a median value of 2,730 CFU/100 mL.

The major tributaries of Newnans Lake (Lake Forest, Little Hatchet, and Hatchet creeks) and samples collected from Bivens Arm Lake were found to have the lowest levels of fecal coliform bacteria. Although Lake Forest Creek and Little Hatchet Creek both had relatively low levels of fecal coliform bacteria, there is one location in each of these watersheds where elevated levels of fecal coliform bacteria have been recorded. In the Lake Forest Creek Watershed, the sampling site at NE 25th Street and north of University Avenue (LFCNE25) was found to have median concentrations of 520 CFU/100 mL, in a watershed where the overall median is 220 CFU/100 mL (Table 2-2). Little Hatchet Creek downstream of Waldo Road has also been found to have elevated levels of fecal coliform bacteria. Results for samples collected at Waldo Road (LHATWALDO) had a median value of 650 CFU/100 mL as compared to the watershed median value of 230 CFU/100 mL. The fecal coliform levels found in samples from Hatchet Creek were the lowest of any of the creeks in the Gainesville urban area, with a median value of 150

CFU/100 mL. Hatchet Creek is the most rural in character of the major tributaries to Newnans Lake.

Fecal coliform bacteria are an indicator of fecal pollution; however it does not discriminate between sources. Multiple sources of fecal coliform bacteria are almost certainly present in all of the urban watersheds. In the very urbanized watersheds the riparian areas surrounding the creeks are often the only refuges for wildlife. Most of the subsurface public wastewater collection system infrastructure in these areas includes gravity systems which are located in topographic lows that coincide with or cross stream channels. The urban creeks are also part of the municipal separate storm sewer system (MS4) and they receive large volumes of untreated stormwater, especially in areas that were developed before stormwater regulations required on-site stormwater treatment. Determining the source(s) of the bacteria is extremely important, as the presence of fecal coliform bacteria from human sources presents a higher risk of water being contaminated by human pathogens that could be transferred to individuals in contact with water in the creeks.

2.4.2 Stormflow Monitoring Event

When reporting fecal coliform data it is important to note if the samples were collected under baseflow or stormflow conditions. Fecal coliform concentrations in surface waters generally experience a transient increase during storm events, as rainwater washes pollutants off of the landscape and into water bodies. Sources of fecal contamination during storm events may be different than the sources that most influence fecal coliform concentrations under baseflow conditions. Stormwater runoff likely transports the feces of domestic and wild animals from riparian areas to creeks. Sanitary sewer overflows may also be more likely during rain events since infiltration into the collection system increases. The intensity, duration, and frequency of storm events affect the quality of the runoff entering water bodies. For example, a rain event following a three week dry spell will wash three weeks worth of accumulated pollutants into a creek, whereas the routine afternoon storms in the summer contribute roughly a day’s worth of pollutants into a creek. In contrast, sustained rains over a period of days tend to bring about a reduction in bacterial loading, as the pollutants are eventually diluted by continuing rainfall.

To characterize the affects of a storm event on fecal coliform levels, ACEPD monitored a routine sample site, Tumblin Creek at US 441 (TUM441), during a storm event. Tropical Storm Ernesto was forecasted to hit Gainesville on 8/30/2006. Baseflow fecal coliform samples and field parameters (temperature, pH, specific conductance, dissolved oxygen, and turbidity) were collected at the site on 8/29/2006 and are presented in Appendix IIb with the stormflow fecal coliform data from this event. As is typical in August, near-daily storm events led up to the study. However, the stage and water quality parameters were under baseflow conditions when the site was sampled on 8/29/2006. Baseflow conditions were determined based on historical data.

On 8/30/2006 baseflow conditions were monitored beginning at 9:55 (Figure 2-1). Fecal coliform levels, stream flow, and field parameters were monitored throughout the mild storm event which began at approximately 13:30. Turbidity spiked from 1.97 to 44.7

NTU at 14:00 when runoff began to make it to Tumblin Creek. As turbidity increased, fecal coliform concentrations increased also. These increases represent the beginning of the first flush, which brings the highest concentrations of pollutants into the creek. Fecal coliform levels started at 2,400 and reached a maximum of approximately 1,000,000 CFU/100 mL at 14:15 which coincided with the highest turbidity value, 70.2 NTU. These values represent the peak of the first flush. The fecal coliform concentration was estimated because insufficient dilutions of the sample were made to accurately enumerate the colonies at such high concentrations. Fecal coliform concentrations and turbidity began to decrease at 14:45, signifying the end of the first flush. An additional fecal coliform sample was collected on 8/31/2006 and the count had returned to typical baseflow levels, 2,400 CFU/100 mL.

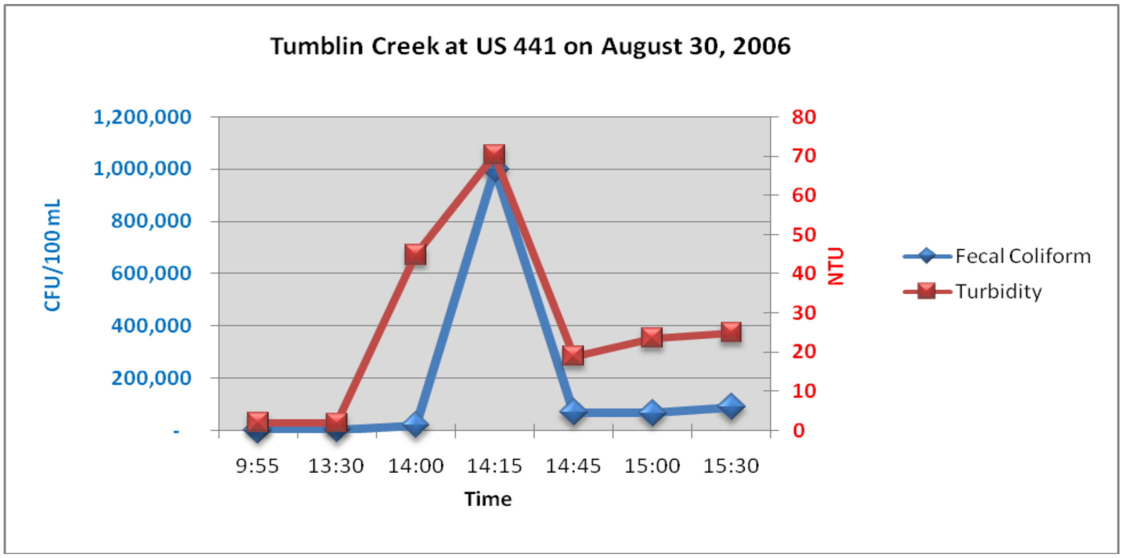


Figure 2-1. Relationship of Fecal Coliform and Turbidity during Storm Event Monitoring on Tumblin Creek, August 30, 2006

The tropical storm did not bring as much rain as expected; only 0.1 inches of rain was collected in the rain gage at the sampling station. This was enough rainfall to increase stage 0.03 feet during the peak of the event. The typical increases in turbidity and fecal coliform concentrations were observed with the low intensity storm event, as demonstrated in Figure 2-1.

2.5 Summary and Conclusions

While evaluating fecal coliform data for the Gainesville urban creeks, it has become clear that increased fecal coliform levels occur during and sometimes after storm events. Even with the use of turbidity as an indicator of stormflow conditions, it remains difficult (if not impossible) to accurately designate a water sample as representative of stormflow or baseflow conditions. The "first flush" during a storm event can be distinguished by elevated turbidity, rising water levels and increased fecal coliform concentrations, as was observed in Tumblin Creek. Under storm event conditions, this increase in fecal coliform levels can be several orders of magnitude. Storm events can affect fecal coliform levels differently, depending upon the duration and intensity of the storm event, the season of the year and the length of the antecedent dry period (ACEPD, 2007).

Watershed configuration and intensity of use also impact levels of fecal coliform bacteria observed during a storm event. Watersheds with substantial impervious area, such as Tumblin Creek, are flashy, and the response to a storm is immediate. Watersheds with less impervious area, such as Hatchet or Little Hatchet creeks, tend to respond more slowly to rainfall. Some of the Newnans Lake tributaries take hours or days to show a response to storm events (ACEPD, 2007). In urbanized areas where there is much impervious surface, pet waste can be a major contributor to fecal coliform levels during storm events. Other variables, such as wastewater collection system infiltration and subsequent exfiltration, can also change water quality during and sometimes hours or days after storm events.

The sources of fecal coliform bacteria may be different under stormflow and baseflow conditions. For example: pet wastes may become a larger contributor when a rain event washes wastes down the stormdrains. Illicit discharges, failing wastewater infrastructure (such as public and private collections system or failing septic tank systems), and wildlife may be the primary sources of high levels of fecal coliform under baseflow conditions. It is important to note these differences, since improving water quality must take these differing sources into account when developing remedial strategies. However, for continued ambient fecal coliform monitoring and data analysis ACEPD will sample and analyze data in total without regard to stormwater inputs. This approach is consistent with the TMDLs developed for the urban creeks, as they do not differentiate between baseflow and stormflow conditions.

Using fecal coliform data alone, one cannot determine the source(s) of fecal coliform bacteria in Gainesville urban creeks. ACEPD contracted with the University of South Florida and Biological Consulting Services, Inc. to conduct antibiotic resistance analyses and ribotyping, two types of microbial source tracking (MST), in hopes of determining the sources of fecal coliform bacteria (Harwood, 2003a; Harwood, 2003b). Although the results were not as easy to interpret as one might like, a significant human source of bacterial contamination was indicated by both ribotyping and antibiotic resistance analysis in studies conducted in Sweetwater Branch and Tumblin Creek. ACEPD conducted additional studies to evaluate techniques for determining the sources of fecal coliform contamination (Sections 4.0 and 5.0). GRU also conducted work in the urban creeks including synoptic sampling to determine “Hot Spots” and detailed MST using a variety of techniques. GRU recently completed a report on the project (CH2MHILL, 2007), which is evaluated in Appendix IV.

Without knowing the sources of the bacteria, targeted measures to improve water quality cannot be developed or implemented. Additional work will be required on a site or creek specific basis to develop a methodology for determining the sources of fecal coliform bacteria and evaluating the potential sources. This work will require the combined efforts of all the agency stakeholders in these watersheds, including ACEPD, GRU, the Alachua County Health Department, the City of Gainesville Public Works Department, the Florida Department of Transportation, and FDEP.

3.0 Fluorescent Whitening Agents in Gainesville Creeks, May 2005

3.1 Introduction

Fluorescent whitening agents (FWAs), also called optical brighteners, are dyes that are found in most laundry detergents. FWAs make "whites whiter and brights brighter" by adsorbing ultraviolet and fluorescent blue light in the visible spectrum (Aley, 1985; Boving et al., 2004). FWAs are highly substituted large aromatic organic compounds, which commonly contain double bonds that are activated by UV light (Hagedorn et al., 2005). The most common FWAs used in laundry detergents are reportedly carbocycles (primarily distyrylbiphenyls) and the triazinylaminostilbenes (Hagedorn et al., 2005). FWAs do not naturally occur in nature, and their detection in aquatic ecosystems indicates the presence of laundry wash water (which can come from faulty septic systems, failing wastewater collection infrastructure, or illicit discharges). Their reported ease of use and affordability makes FWAs an attractive candidate as an indicator of illicit discharges and potential fecal pollution. However, naturally occurring fluorescent compounds in water such as humic acid, tannic acid, and other dissolved organic compounds can interfere with the detection of FWAs (Boving et al., 2004) and FWAs do photodegrade over time. Stoll and Giger (1997) found FWAs in a wastewater treatment plant to be resistant to biodegradation over a 28 day period; however sorption and photodegradation did reduce concentrations during their studies.

There are several techniques used to analyze FWAs, each with its own advantages and disadvantages. As a screening or reconnaissance tool, FWAs in natural waters can be concentrated and evaluated by use of cotton swatch samplers (Aley, 1985). In this simple technique a cotton cloth can be fixed in place and left in a stream for several days to adsorb FWAs present in the water. Analyses of the cotton cloth are then conducted with a spectrofluorophotometer in a laboratory. This technique has the advantage of collecting data over an extended period of time, which is important when discharges are periodic in nature. One disadvantage of this technique is that it only provides qualitative results. One method to obtain quantifiable results is high-performance liquid chromatography (Poiger et al., 1996), which is more costly and evaluates the presence of FWAs in the water at the time the sample was collected, giving only a brief "snap shot." Alachua County Environmental Protection Department (ACEPD) chose to conduct qualitative screening for the presence of FWAs in Gainesville's urban creeks using cotton fabric swatches and spectofluorophotometric analyses to evaluate FWAs. Analyses were conducted by Ozark Underground Laboratory, Inc. in Protem, Missouri. Results from these analyses are included in Appendix V.

3.2 Materials and Methods

Forty sampling locations were selected within the upper Orange Creek Basin (OCB) in proximity to Gainesville (Figure 3-1). Most of the sites were established sampling sites within ACEPD's ambient surface water monitoring network in the Hogtown Creek (includes Possum and Elizabeth creeks and Rattlesnake Branch), Newnans Lake (includes Lake Forest, Hatchet, and Little Hatchet creeks), and Paynes Prairie (includes Sweetwater and Rosewood branches and Tumblin Creek) watersheds. Several small unnamed tributaries of Hogtown Creek were also sampled.

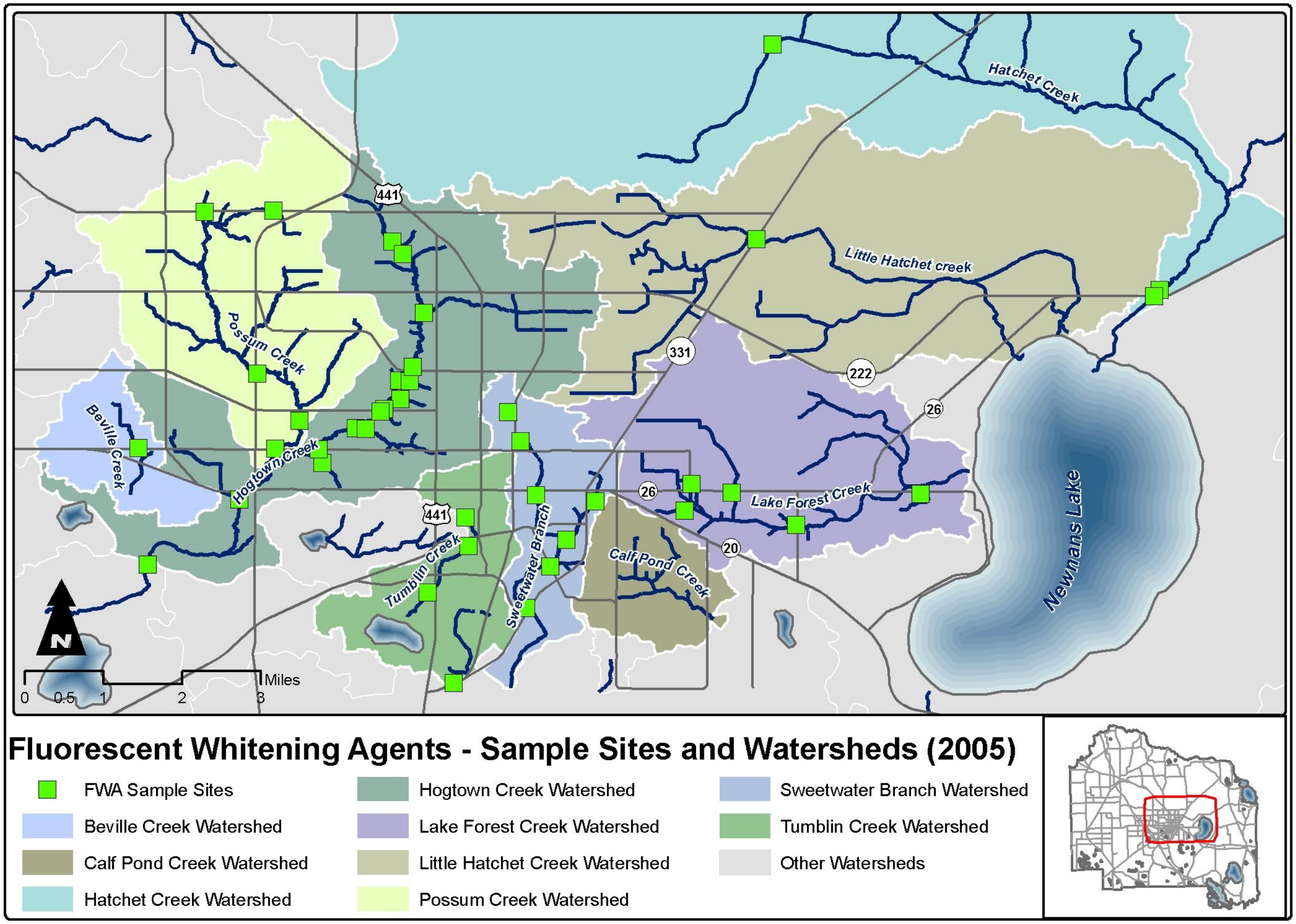
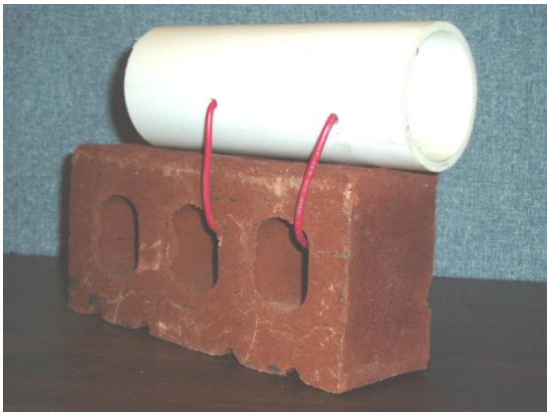


Figure 3-1. Locations of Gainesville Urban Creek Fluorescent Whitening Agents Sample Sites in the Gainesville Urban Area, May and June 2005

FWAs sampling devices were made by using wire to attach a 4-inch piece of 2-inch diameter PVC pipe to a brick. Square untreated cotton cloth swatches approximately 2.5-inches on a side were obtained from the Ozark Underground Laboratory, Inc. and were attached to one end of the PVC pipe using a rubber band. In theory, FWAs present in the water column will adsorb to the fabric. Gloves were worn when handling the sampling cloths and samplers were instructed to refrain from doing laundry the day of and before sampling events to reduce the possibility of contaminating the fabric swatches with FWAs.



Two samplers were placed in flowing water at each sampling location between 5/8/2005 and 6/8/2005. When possible, the samplers were placed in shaded areas to avoid photodegradation of any FWAs that adsorbed onto the cloth swatch. Fecal coliform samples were also collected at most sites. Each sampler was left in a stream segment for seven days, the recommended deployment period by Ozark Underground Laboratory, Inc. The condition (buried in sand, covered with algae, etc.) of each sampler was recorded during the collection process. After the seven day deployment period, the cloth swatches were removed from the samplers, lightly squeezed of excess water, and stored in labeled sterile “Whirl-Pak” plastic bags. The cloth swatches were placed in a dark freezer until they were sent to the Ozark Underground Laboratory, Inc. for analysis.

Laboratory staff rinsed off the swatches, analyzed the adsorbed compounds with a Shimadzu RF-540 Spectroflurophotometer, and interpreted output charts. Each side of the cloth swatch was analyzed by the laboratory, since they could not determine which side was facing the flowing water. Results for both sides of the cloth were reported for each sampler deployed (Appendix V).

For Quality Assurance purposes field blank samples were collected. During each day in which samplers were deployed, a sampler was placed in a plastic container filled with deionized water in the ACEPD field support building. Each sampler remained in the container for seven days and was then processed using the same methods as all of the other cloth swatches. Two sites directly downstream of water reclamation facilities (domestic wastewater treatment plants) on Little Hatchet Creek and Sweetwater Branch served as positive controls, since FWAs can pass through the wastewater treatment process.

Fecal coliform samples were collected at all of the sites using Whirl-Paks with a thiosulfate preservative. Samples were collected from approximately mid-depth of the water column in flowing water near the FWAs samplers. Samples were stored on ice and then analyzed by ABC Research, Inc. in Gainesville, FL within six hours of collection. Samples were analyzed using the membrane filtration SM 9222D method (APHA, 1998). Extended dilutions were run to allow fecal coliform concentrations to be quantified (Appendix V).

Field parameters were analyzed using an YSI556 Multi-Probe System field multi-parameter meter for pH, specific conductivity, temperature, and dissolved oxygen. The probe was placed facing the flow of water and parameters were allowed to stabilize before they were recorded. Turbidity was measured using a Hach2100P turbidimeter and water velocity was measured with a Marsh McBirney FlowMate 2000 for flow calculations. Sample location characteristics were recorded for each site (Appendix V).

3.3 Results and Discussion

The samplers were deployed for seven days; therefore the results are composites reflecting the in-stream site conditions during those seven days. Composite samples are more representative of site conditions compared to grab samples, which reflect site conditions during the one moment in time when the samples are collected. Detection of FWAs indicates that laundry wastewater or water to which laundry detergent had been added was introduced to this site at some point during the seven days. Unfortunately, the converse is not necessarily true; a “not detected” result does not necessarily mean that FWAs are absent from that site. The levels could be too low to detect or FWAs could be absent during the seven day window but present outside of that window.

FWAs were not detected at any of the sampling sites except SWB331, LHATWALDO, and HOGNW8 (Appendix V). SWB331 is approximately 2,000 feet downstream of the permitted discharge of the treated wastewater effluent from Gainesville Regional Utilities (GRU) Main Street Water Reclamation Facility and LHATWALDO is approximately 650 feet downstream of the permitted discharge of treated wastewater effluent from Brittany Estates Mobile Home Park’s Water Reclamation Facility. FWAs are known to pass through the treatment processes at wastewater treatment plants (Stoll and Giger, 1997); therefore we would expect their detection downstream of these two water reclamation facilities. These sites served as positive controls, demonstrating that FWAs were detected in samples with known wastewater contributions. No FWAs were detected in the blank sample, which indicates that sampling protocols were sufficient and false positive results are unlikely.

Both sides of the sampler cloth yielded non-detectable results for one of the samplers from HOGNW8, whereas a weak detection of FWAs was reported for one side of the other sampler from this site. HOGNW8 was re-sampled to determine if FWAs were present or absent at this site. Unfortunately both of the samplers were lost in a storm event. The results for this site are inconclusive since one of the samples yielded a not detected result while the other yielded a weak positive. This site was re-sampled in April 2006 (Section 4.0) and FWAs were not detected.

Large storm events may have influenced the results of this study. This sampling was conducted in May, which is normally a dry month. Rainfall events that occurred during the deployment did not allow evaluation of the streams under baseflow conditions, but a mixture of baseflow and stormflow conditions. Several of the samplers became completely buried in sediment during the seven day deployment period. It is unlikely that adsorbed FWAs would come off during these conditions. However, if the cloths were completely covered with sediment, additional FWAs in the water column would not come in contact with or become adsorbed by the cloth. An intense storm surge is characteristic for several of these urban creeks and several samplers could not be located after the seven day deployment period. It is likely that they were transported downstream, completely buried in the sediments, or removed by curious people.

Another factor possibly affecting the results was a growth that was observed coating several of the samplers. The growth was most likely a combination of bacteria, fungal mycellium, diatoms, and algae. The coating on the surface of the cloth swatches could inhibit the adsorption of FWAs from the water column. Using a shorter deployment period and/or deploying samplers in the colder months may decrease the confluent microbial and algal growth.

Some of the sample sites lacked a shade creating canopy. At these sites the samplers were exposed to sunlight for seven days. FWAs have been shown to photodegrade over time, so it is possible that photodegradation contributed to the large number of samples where FWAs were not detected.

Interference by dissolved organic compounds is a known limitation of FWAs as an indicator. Little Hatchet, Hatchet, and segments of Lake Forest creeks are characterized by “tannic” water. The tannic and fulvic acids naturally occurring in these creeks could interfere with the FWAs in these watersheds. However, these organic compounds would have been more likely to lead to false positive results over false negative results. The weakly positive FWA result downstream of the Brittany Estates Mobile Home Park reclaimed water discharge may have been a result of interference from dissolved organic acids or the result of dilution of the reclaimed water with natural surface water in the stream.

The FWAs swatch method has limitations and is not the perfect “one stop-shopping” indicator for which researchers are searching for. Sediment smothering, microbial and algal coatings on the cloth swatches, photodegradation, and organic interferences are some of the factors affecting FWAs value as an indicator. However, by combining indicators you can often more accurately characterize conditions at a site.

Fecal coliform concentrations were measured when the FWAs samplers were deployed and again during sampler collection at selected sites (Appendix V). Fecal coliform data can be difficult to interpret due to the large variance between sites, sample dates, and individual grab samples. Fecal coliform concentrations during this study varied from 21 to 8,800 CFU/100 mL. Upon deployment of the FWAs samplers, 17 of the 41 (41%) fecal coliform samples collected exceeded the 800 CFU/100 mL one time maximum state water quality standard. Most of the fecal coliform concentrations were less than 2,000 CFU/100 mL, with HOGGHSN standing out with concentrations in the 8,000 CFU/100 mL range. This sample site is located directly downstream of a GRU lift station in a small tributary of Hogtown Creek in the vicinity of Gainesville High School. Fecal coliform counts in this range likely can be attributed to anthropogenic sources or wildlife. FWAs were not detected and interference was unlikely since the samplers were placed in a shaded area, the water was clear (free of tannic acids), and only minor silt smothering was noted on the cloths' surfaces. One of the samplers could not be located upon collection, so there are only two FWAs results for this site instead of four. The flow was too low to measure during the 2005 sampling event, but ranged from 0.0001 to 0.0043 cubic meters per second during subsequent studies. At such low flows there is not a lot of water to dilute sources of fecal coliforms, regardless of the source. The source of fecal coliform bacteria at HOGGHSN may be the wild animals frequenting this site as noted by numerous tracks and reports of feral cat populations in the area (Bird, 2007); however this hypothesis cannot be proven with fecal coliform and FWAs results alone. Harwood (2008) disagrees that feces from non-concentrated animals could result in fecal coliform levels this elevated and considers the wastewater infrastructure in the area as a more likely source of the elevated fecal coliform levels.

3.4 Summary and Conclusions

The use of FWAs as a qualitative screening tool to detect illicit discharges was chosen for its ease of use and the cost effective nature of the analyses. FWAs were detected at three of 40 sample sites. Two of these sites were downstream of permitted reclaimed water (treated domestic wastewater) discharges; only one was in an area where a possible illicit discharge was likely to occur. Subsequent sampling yielded negative results for this site (Section 4.0). The low sensitivity of the analytical screening method and variable field conditions likely limited the usefulness of this technique to identify illicit discharges of domestic wastewater.

FWAs were detected at sites with known wastewater effluent contributions, so they are a potential indicator of wastewater releases. FWAs were not detected in areas with consistently high fecal coliform counts. However, the use of FWAs using the techniques employed in this assessment is limited due to the sensitivity of the laboratory analysis, the potential for interference by dissolved organics, sedimentation, surface growth on the cotton fabric swatches, and photodegradation. The samplers may have been deployed for too long for the warm water conditions present throughout this study. Additional studies, described in Sections 4.0 and 5.0, utilized FWAs and Microbial Source Tracking techniques to investigate sites with consistently high fecal coliform concentrations.

4.0 Fluorescent Whitening Agents, Alternative Microbial Indicators, and Microbial Source Tracking at "Hot Spots," April 2006

4.1 Introduction

Alachua County Environmental Protection Department (ACEPD) routinely monitors creeks for the presence of fecal coliform bacteria. The sampling program has identified several areas with consistently high concentrations of fecal coliform. These concentrations may indicate the presence of fecal contamination, but the source of the contamination is unknown and cannot be determined by fecal coliform sampling alone.

Past efforts (described above in Section 3.0) have included the use of fluorescent whitening agents (FWAs) to determine if leaking wastewater collection infrastructure, failing septic tanks, and/or illicit discharges serve as sources of fecal contamination. The only conclusive results were that FWAs were present directly downstream of permitted water reclamation facility discharge points. The following sections describe monitoring efforts that include the use of alternative microbial indicators, FWAs, and microbial source tracking (MST) techniques in an attempt to identify the sources of elevated fecal coliform levels in Gainesville's urban creeks. Results from these analyses are provided in Appendix VI with corresponding fecal coliform data and field parameters.

4.1.1 Alternative Microbial Indicators

In an effort to further characterize the stream segments with high fecal coliform counts, expanded microbial indicators were used: *Escherichia coli*, *Clostridium perfringens,* and *Enterococcus* spp. (Table 4-1). Each of these bacteria has unique traits that were reported to be potentially useful in determining the source(s) of fecal coliform bacteria (Griffin, 2004). Fecal coliform bacteria alone often cannot predict the occurrence of pathogenic bacteria, protozoans (*Crytosporidium* and *Giardia*), and enteric viruses in environmental waters (Fong et al., 2005). While the search for the ideal indicator organism continues, combining the analyses of several different bacteria can provide more information on potential fecal contamination sources.

E. coli is one of many species of bacteria living in the lower intestines of warm blooded animals. *E. coli* is a gram-negative, non-sporulating bacterium in the family *Enterobacteriaceae* (Phylum Proteobacteria). It is thought to be a better indicator of fecal pollution than the broader group of fecal coliform bacteria because *E. coli* concentrations in fresh water have been shown to correlate with increased risk of gastroenteritis in recreational water users, while the same has not been found for fecal coliforms. The United States Environmental Protection Agency (EPA) has recommended that *E. coli* (a subset of fecal coliform bacteria group) replace fecal coliform bacteria in state water quality standards. The EPA criteria for bathing in recreational freshwaters states that a single sample shall not exceed 235 CFU/100 mL. This standard is not recognized in Florida, but will be used for comparative purposes in this report. Some states have adopted *E. coli* standards to replace fecal coliform, but Florida continues to use fecal coliform as a standard for fecal pollution of recreational fresh waters (FDEP, 2006).

Table 4-1. Alternative Bacterial Indicators and Florida Water Quality Criteria

Indicator	**Description**	**Florida Recreational Use Standards**[a]	**Advantages of Use as an Indicator**	**Disadvantages of Use as an Indicator**
Fecal Coliform	Members of the family *Enterobacteriaceae* Make up 30% of intestinal organisms in humans and warm blooded animals (Bitton, 2005) Facultative anaerobic, gram-negative, non-spore forming, rod-shaped bacteria (Bitton, 2005)	Fresh or Marine Waters (FDEP, 2006): 800 CFU/100 mL (SSM); 400 CFU/100 mL in 10% of the samples; 200 CFU/100 mL (GM) in a monthly average based on a minimum of 20 samples taken over a 30 day period Marine Waters (EPA, 2004) Florida Healthy Beaches Initiative: 400 CFU/100 mL (SSM)	Used as an indicator of bacteriological water quality, inexpensive, and relatively easy to analyze Lose viability in fresh water at a slower rate than most other intestinal bacterial pathogens (Rose *et al*, 2007) Will usually be detected in waters impacted by animal feces or sewage	Fecal coliform represents a relatively large class of bacteria. Bacteria can persist or re-grow in sediments Not human specific
Escherichia coli	A type of fecal coliform bacteria that occur naturally in the intestinal tract of warm blooded animals	No Florida Criteria	Reported to have a high correlation with gastroenteritis associated with bathing in fresh water (Dufour, 1984) Concentrations may correlate well with fecal coliform	May persist or re-grow in the sediments, especially in warm waters Reported to be poorly correlated with gastroenteritis in marine waters (Cabelli, 1983) Presence does not correlate well with the presence of enteric viruses and parasites (Rose *et al*, 2007) Not human specific

Indicator	**Description**	**Florida Recreational Use Standards**[a]	**Advantages of Use as an Indicator**	**Disadvantages of Use as an Indicator**
Enterococcus spp.	A gram-positive non-spore forming member of the fecal Streptococci bacteria group (Bitton, 2005) Many strains are not pathogens, but may indicate the presence of enteric pathogens (Rose *et al*, 2007)	Marine Waters (EPA, 2004) Florida Healthy Beaches Initiative: 35 CFU/100 mL (GM); 104 CFU/100 mL (SSM) Fresh Waters (EPA, 2004) Florida Healthy Beaches Initiative: 33 CFU/100 mL (GM); 61 CFU/100 mL (SSM)	Used as an indicator of bacteriological quality of recreational waters *Enterococci* may die at a slower rate that fecal coliforms in water and sediments, possibly a indication of recent contamination (Rose *et al*, 2007) Reported to be poorly correlated with gastroenteritis in marine waters (Cabelli, 1983)	Can persist or re-grow in the environment In tropical or other warm waters elevated concentrations may be common and not indicative of recent contamination (Hawaii State Dept. of Health, 2006) Not human specific
Clostridium perfringens	Obligate anaerobic gram-positive bacteria that forms endospores Found in sewage An opportunistic pathogen that produces enterotoxins (Rose *et al*, 2007)	No Florida Criteria	Potential indicator for the presence of persistent intestinal pathogens, such as viruses (Rose *et al*, 2007) May be useful in combination with other bacterial indicators (Griffin, 2004)	Found in low concentrations in the environment Not human specific

a. SSM is the Single Sample Maximum and GM is the Geometric Mean

C. perfringens is an anaerobic gram-positive, spore forming sulfite-reducing bacterium found in human and animal feces (Bitton, 2005). In the human colon it reportedly represents 0.5% of the fecal microflora (Bitton, 2005). It has been used as a fecal pollution indicator in many areas and is a widely used fecal pollution indicator in Europe (Bitton, 2005). *C. perfringens* can be found in soils, the intestines of humans and animals and wastewater. Fujioka and Shizumura (1985) found the bacterium to be consistently present in wastewater at concentrations of 10^3 to 10^4 CFU/100 mL. Although *C. perfringens* is an anaerobe, its ability to form spores may allow it to persist in the environment, reducing its predictive value for recent fecal contamination (Scott et al., 2002). Although concentrations are usually lower than fecal coliform, *C. perfringens* is used in Hawaii as a secondary indicator of fecal pollution in marine waters in conjunction with *Enterococcus* when *Enterococcus* levels are high (Hawaii State Department of Health, 2007). For inland waters Fujioka and Shizumura (1985) suggest a *C. perfringens* standard of 50 CFU/100 mL as an indicator of microbial water quality. This standard is not recognized in Florida, but will be used for comparative purposes in this report.

Enterococcus spp. are a common inhabitant of the intestinal tract of humans and animals. Fecal streptococci, along with fecal coliform, have been used historically as indicators for fecal pollution in recreational waters. *Enterococcus* spp. is a subgroup of the fecal streptococci which includes *E. faecalis, E. faecium, E. durans, E. gallinarum and E. avium* (Scott et al, 2002; Bitton, 2005). Bitton (2005) reports that *Enterococcus* spp. do not reproduce in the aquatic environment, making them a useful indicator. Florida has adopted a recreational water standard of 61 CFU/100 mL (confirmed with a second sample) for *Enterococcus* spp. in fresh waters as part of the Florida Healthy Beaches Program implemented by the Florida Department of Health.

4.1.2 Microbial Source Tracking (MST)

The non-specific nature of traditional microbial indicators, i.e. the fact that they can originate from a myriad of possible sources, has led to the emerging technologies of MST. This term refers collectively to methodologies used to detect and differentiate among sources of fecal pollution in waters (Scott et al., 2002; Scott et al., 2005; McQuaig et al., 2006). MST methods can be divided into broad categories: chemical methods, microbiological methods, phenotypic library-dependent methods, genotypic library dependent methods, and library independent methods (Scott et al., 2002; Scott et al., 2005; McQuaig et al., 2006).

This study utilized library independent methods which use host specific molecular markers to identify sources of fecal pollution. Select samples were analyzed for three human specific DNA markers: *Enterococcus faecium* (surface protein *esp* gene), *Bacteroides* spp. (16S rRNA), and human polyomaviruses (JC virus and BK virus) (Table 4-2). Each of these markers are detected by a polymerase chain reaction (PCR) based assay. The use of these markers yields results of presence or absence, which are highly sensitive and microbe specific and can be used to provide a framework for evaluating the results of water quality monitoring (McQuaig et al., 2006). These various MST techniques, or “tools”, can be combined to better define the sources of fecal pollution in impacted surface waters.

Table 4-2. Microbial Source Tracking Indicator Comparison for Microbial Source Tracking Using a Polymerase Chain Reaction (PCR) Based Assay Method

Indicator	**Description**	**Advantages of Use as an Indicator**	**Disadvantages of Use as an Indicator**
Enterococcus faecium (surface protein *esp* gene)	Human specific DNA marker	Human specific May eliminate septic systems as a possible source of contamination	Can persist or re-grow in sediments, No criteria or standards, Analyses are costly, and Repeated analyses must be conducted to allow statistical analyses of the data to validate occurrence
Bacteroides spp. (16S rRNA)	Human specific DNA marker	Human specific Lack of persistence may indicate recent sources	No criteria or standards, Analyses are costly, and Repeated analyses must be conducted to allow statistical analyses of the data to validate occurrence
Human Polyomaviruses (JC virus and BK virus)	Human specific DNA marker	Human specific	No criteria or standards, Analyses are costly, Repeated analyses must be conducted to allow statistical analyses of the data to validate occurrence, and A high volume of sample is required to reduce the chance of false negative results
Bacteroides spp.	General marker for sediments	Presence in sediments indicates sediments as a source	No criteria or standards, Analyses are costly, Some professionals do not recommend analyzing sediments for human specific markers, and Repeated analyses must be conducted to allow statistical analyses of the data to validate occurrence

The first MST technique used in this study involved detection of the *E. faecium* surface protein *esp* gene. The *esp* gene can be detected in these bacterium and may be used in a data-library independent determination of the presence of human waste (Jenkins et al., 2005). Analyses are conducted using standard *Enterococcus* culture procedures and PCR, a type of DNA replication technology, which pinpoints the specific bacteria that carry the *esp* gene, which is a human specific DNA marker. Scott et al. (2005) reported that use of PCR primers specific for the *esp* gene in *E. faecium* is another useful technique in evaluating human fecal pollution in environmental waters.

A second MST technique used to better assess the extent of human fecal pollution of Gainesville's urban creeks was the *Bacteroides* spp. 16S rRNA marker. The genus *Bacteroides* is a dominant constituent of human feces, contributing to approximately 30% of the intestinal tract flora in humans (Bitton, 2005). Bernhard and Field (2000) identified a *Bacteroides* spp. 16S rRNA marker by screening DNA from human feces and developed a conventional host-specific PCR assay to detect the human specific DNA marker in environmental samples. Previous work in the Gainesville urban area found the *Bacteroides* 16S rRNA marker in 35% of the water samples from the urban creeks (McQuaig et al., 2006; CH2MHill, 2007).

The third MST technique utilized was the human polyomaviruses (JC virus and BK virus). Human polyomaviruses are only found in humans and are highly prevalent in the general population. High concentrations of these viruses are reportedly found in wastewater (McQuaig et al., 2006). These viruses are usually asymptomatic within the host and high concentrations are frequently excreted in human urine (McQuaig et al., 2006), thus these viruses are indicators of sewage. A method was developed to concentrate the polyomaviruses and extract the DNA in environmental waters (McQuaig et al., 2006). The results of human specific PCR tests for the *esp* gene, *Bacteroides* spp. and polyomaviruses in surface water samples from Florida were highly correlated (McQuaig et al., 2006), providing confidence in the ability of these markers to indicate human sewage in environmental waters.

It has been well documented that conventional parameters, such as fecal coliform, do not adequately or accurately reflect the human health risk posed by bacterial contamination or fecal pollution (McDonald et al., 2006). The MST techniques outlined above are some of the "tools" in the "toolbox" that can be used to better define fecal pollutants in surface waters. Detailed summary and discussion of these methodologies and recent results are presented in Appendix VII, a white paper by T.M. Scott and G. Lukasik, *Identification and Quantification of Human Fecal Sources in Alachua County Urban Creeks*.

4.2 Materials and Methods

Sample sites upstream and downstream of the "Hot Spots" (Figure 1-2) were selected using historic fecal coliform data and maps of wastewater collection systems, stormwater systems, and septic systems locations. The goal of site selection was to spatially isolate the source of the fecal contamination. Sites needed to be accessible and have flowing water under baseflow conditions.

Water column samples were collected from approximately mid-depth in flowing water at each site once a week for three consecutive weeks starting April 25, 2006. Samples were analyzed for fecal coliform using standard method SM 9222D (APHA, 1998), *E. coli* EPA Method 1603 (EPA, 2002a), the *C. perfringens* method of Bisson and Cabelli (1979), and *Enterococcus* spp. (EPA, 2002b) by Biological Consulting Services, Inc. (BCS) of Gainesville, FL. Water column samples were archived (DNA was extracted and then frozen) to allow for future MST analysis on select samples with high indicator counts. MST analyses were not run on every sample due to the expense of these analyses.

FWAs samplers were placed at all of the sites during the week two sampling event and were collected during week three. Each sampler was exposed to site conditions for seven days. The FWAs sampling and analyses were conducted as described in Section 3.2.

Field parameters including pH, specific conductivity, temperature, and dissolved oxygen were analyzed using an YSI556 Multi-Probe System field multi-parameter meter. The probe was placed facing the flow of water and parameters were allowed to stabilize before they were recorded. Turbidity was measured using a Hach 2100P turbidimeter. Water velocity was measured with a Marsh McBirney FlowMate 2000 for flow calculations and sample location characteristics were recorded for each site.

4.3 Results and Discussion

Data for this study are located in Appendix VI. Conditions during week one were characterized by varied stormflow. There were scattered rain events during the week and it was raining while the Tumblin Creek sites were sampled. Fecal coliform concentrations at several of the sites were typical of stormflow conditions, with concentrations an order of magnitude greater than historic baseflow levels. Week two was characterized by baseflow conditions with each site experiencing at least four days without rainfall. It rained throughout week three and all sites were considered to be under stormflow conditions.

Microbial data can have considerable variability, making it difficult to interpret. To reduce the effects of this variability, it is common to log transform the data during the data analysis process. Log transformation allows for an easier assessment of the presence or absence of an overall trend for data that are non-normal in their distribution (Tyler, 2006). Fecal coliform concentrations tended to be the highest, as one would expect since they are the broadest group of bacteria analyzed as part of this study. The concentrations of *Enterococcus* spp. tended to exceed *E. coli* counts. *C. perfringens* concentrations were low (compared to the other indicators) because it is found in lower concentrations in human wastes as discussed by Bitton (2005) and evident in the concentrations detected in wastewater effluent (Table 4-3). Several anomalies to the general trends were noted. During the week two baseflow sampling, median values of *Enterococcus* spp. were higher (13,000 CFU/100 mL) than the other indicator bacteria (fecal coliform = 5,200 CFU/100 mL, *E. coli* = 3,300 CFU/100 mL, and *C. perfringens* = 147 CFU/100 mL) at ELIZNW7DS. A similar trend of higher *Enterococcus* spp. counts was seen at ELIZ309NW23 (Appendix VI).

Although the data confirmed that there are sites with higher bacteriological counts, no meaningful patterns emerged. The values for each “Hot Spot” watershed were combined to calculate a watershed median over the three week study (Figure 4-1). Median fecal coliform values were highest in Tumblin and Hogtown creeks and lowest in the Sweetwater Branch Watershed (Table 4-3). *Enterococcus* spp. median values were the highest in Tumblin and Elizabeth creeks. The median value of *E. coli* was reportedly highest in samples from Lake Forest Creek. *C. perfringens* median values exceeded the 50 CFU/100 mL standard in all of the creeks (except for Hogtown Creek) and was the highest in Lake Forest Creek.

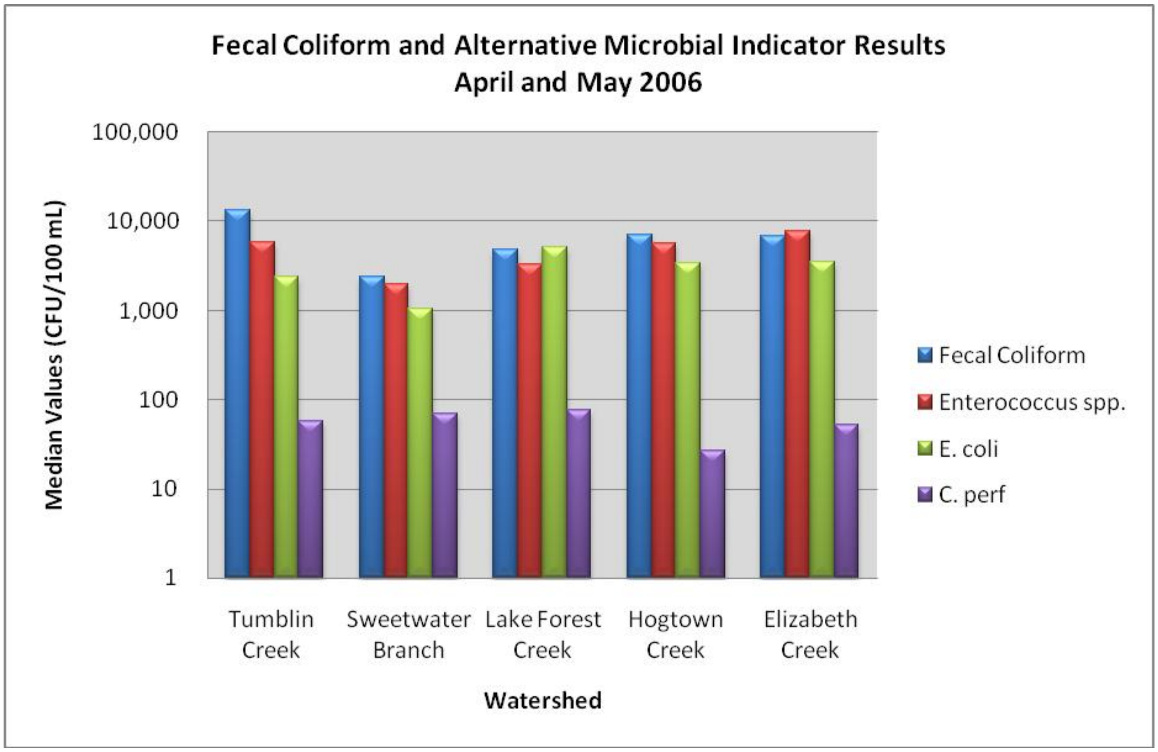


Figure 4-1. Median Concentrations of Fecal Coliform, *Enterococcus* spp., *E. coli,* and *C. perfringens* during April and May 2006

Table 4-3. Median and Range (Minimum and Maximum) of Concentrations of Fecal Coliform, *Enterococcus* spp., *E. coli,* and *C. perfringens* during April and May 2006

Watershed	Fecal Coliform CFU/100 mL	*Enterococcus* spp. CFU/100 mL	*E. coli* CFU/100 mL	*C. perfringens* CFU/100 mL	Number (n)
Tumblin Creek	13,550 (280-180,000)	5,850 (490-26,000)	2,400 (52-11,000)	58 (1-208)	12
Sweetwater Branch	2,400 (800-76,000)	2,000 (460-23,000)	1,035 (290-36,000)	69 (5-221)	12
Lake Forest Creek	4,800 (2,100-62,000)	3,300 (900-24,000)	5,100 (1,200-6,800)	76 (8-200)	9
Hogtown Creek	7,050 (1,100-97,000)	5,650 (1,600-24,000)	3,450 (260-33,000)	29 (0-220)	18
Elizabeth Creek	6,800 (1,900-76,000)	7,900 (1,400-15,000)	3,500 (290-8,600)	52 (0-150)	15
Untreated Wastewater[a]	4,100,000 (3.5E6-1.0E7)	1,200,000 (4.5E5-2.5E6)	2,700,000 (2.1E6-3.6E6)	60,000 (16,000-76,000)	3

a. Sample collected from GRU Main Street Water Reclamation Facility

Sampling event median fecal coliform levels for all samples collected in April and May 2006 greatly exceeded the water quality criteria of 400 CFU/100 mL in 10% of the samples in each area (Table 4-4). Median *C. perfringens* levels ranged from 5 to 201 CFU/100 mL, with the highest values occurring during the stormflow events of week three.

Table 4-4. Median Values for Fecal Coliform, *Enterococcus* spp., *E. coli*, and *C. perfringens*, Summarized by Sampling Event and Watershed

Date	**Watershed**	**Fecal Coliform** CFU/100 mL	***Enterococcus* spp.** CFU/100 mL	***E. coli*** CFU/100 mL	***C. perfringens*** CFU/100 mL	**Number (n)**
Week One 4/25/2006 – 4/27/2006	Tumblin Creek	25,000	5,850	2,400	6	4
	Sweetwater Branch	1,700	2,000	585	14	4
	Lake Forest Creek	4,800	3,300	5,100	31	3
	Hogtown Creek	21,500	4,250	5,400	5	6
	Elizabeth Creek	6,800	7,300	2,500	23	5
Week Two 5/2/2006 – 5/4/2006	Tumblin Creek	875	620	170	31	4
	Sweetwater Branch	1,150	1,250	1,035	69	4
	Lake Forest Creek	2,600	1,300	1,800	76	3
	Hogtown Creek	2,150	5,200	2,200	42	6
	Elizabeth Creek	5,200	7,800	4,200	65	5
Week Three 5/9/2006 – 5/11/2006	Tumblin Creek	44,000	14,500	4,500	162	4
	Sweetwater Branch	44,000	18,500	19,000	201	4
	Lake Forest Creek	43,000	22,000	5,900	176	3
	Hogtown Creek	7,050	9,250	3,450	41	6
	Elizabeth Creek	23,000	8,300	4,000	40	5

In order to evaluate the relationship between fecal coliform and *E. coli* a simple linear regression analysis was performed, and the R^2 value, the square of the correlation coefficient, was determined (Table 4-5). The closer R^2 is to 1 the greater the correlation between the variables fecal coliform and E. coli. Sweetwater Branch had the highest R^2 value, 0.98. Tumblin Creek had an R^2 of 0.73; while the remaining creeks had R^2 values of <0.3. A strong correlation between fecal coliform and *E. coli* indicates the source is likely a recent source (Harwood 2008).

Table 4-5. Results from Linear Regressions Based on All Samples Collected for Fecal Coliform

	E. coli		**Turbidity**	
Watershed	**R^2**	**Number (n)**	**R^2**	**Number (n)**
Tumblin Creek	0.73	12	0.93	11
Sweetwater Branch	0.98	12	0.90	12
Lake Forest Creek	0.29	9	0.83	9
Hogtown Creek	0.22	18	0.00	17
Elizabeth Creek	0.12	15	0.01	15
All Samples	0.26	66	0.51	64

There was also a strong correlation between turbidity and fecal coliform; samples from Tumblin Creek had an R^2 of 0.93, Sweetwater Branch samples had an R^2 of 0.90 and samples from Lake Forest Creek had an R^2 of 0.83. This analysis suggests that fecal coliform and turbidity (indicative of stormflow conditions) are strongly correlated. Recall that two of the three sampling events were conducted under stormflow conditions. There was no correlation between fecal coliform and turbidity for Hogtown Creek and Elizabeth Creek, indicating that the source of fecal coliform is likely not related to stormwater inputs.

Using *E. coli*, *C. perfringens*, and *Enterococcus* spp. did not provide much additional information as to the source or location of fecal contamination as compared to using fecal coliform data alone. Due to their lack of specificity, expense, and the fact that there are no FDEP standards for these alternative indicators ACEPD does not plan to use this suite of indicators on a continuing basis. That being said, combining the microbial results with the FWAs and MST analyses does contribute to our understanding of these fecal coliform "Hot Spots" as demonstrated in the following sections.

4.3.1 Tumblin Creek

Four sites in the upper reaches of the Tumblin Creek Watershed were sampled to evaluate high fecal coliform levels observed upstream of SW 5th Avenue (Figure 4-2). Two sites, TUMSW1 and MHSW2NDAVE, are upstream of SW 5th Avenue. The most upstream sample site in the Tumblin Creek watershed is TUMSW1. Samples at this site were collected from the stormwater collection system just south of SW 1st Avenue and west of SW 8th Street. Based on the City of Gainesville's stormwater collection system GIS layer, this site is part of the stormwater collection system servicing the highly developed area of Gainesville along University Avenue between NW 11th Street and possibly extending to NW 7th Street. The FWAs results at TUMSW1 were a weak positive and a moderate positive (Table 4-6). During baseflow conditions (week two) the microbial indicators were relatively low at this site, but still exceeded water quality standards for fecal coliform and *Enterococcus* spp. The archived baseflow and stormflow (weeks two and three) samples from TUMSW1 were analyzed for the three human specific DNA markers; however they were not detected in these samples. Combining the positive FWAs results with the negative MST results leads one to suspect an illicit discharge of grey water (sink and shower, but not domestic wastewater) or other illicit discharge to the stormwater collection system as a source of contamination.

A weak positive FWAs result was also reported downstream at a manhole in front of Shands at Alachua General Hospital (AGH), MHSW2NDAVE, and at TUMSW5. During baseflow sampling (week two) the microbial indicators were relatively low at MHSW2NDAVE and an order of magnitude higher at TUMSW5. Fecal coliform counts have historically been high at TUMSW5, hence identifying this area as a "Hot Spot". The FWAs detected may be from the same source detected upstream at TUMSW1. The three human specific DNA markers were not detected in the archived (frozen DNA extracts) baseflow and stormflow (weeks two and three) samples from TUMSW5. High bacteria populations at TUMSW5 may be due to persistence in the sediments or the presence of wildlife. Cats and raccoons are commonly seen at this site. It is also possible that the three human markers were missed because the site was not sampled very often.

TUMPORTERS was the site located the furthest downstream in the watershed. Samples were collected from the stormwater culvert south of Depot Avenue and west of Main Street. The culvert comes from the Porters neighborhood and discharges to Tumblin

Creek directly downstream of the sample site. Fecal coliform values were very high during the week three sampling event (180,000 CFU/100 mL), which was clearly under stormwater conditions. The microbial indicators were relatively low during the week one and two sampling events. Additionally, no FWAs were detected at this site and, therefore MST was not run on the archived samples.

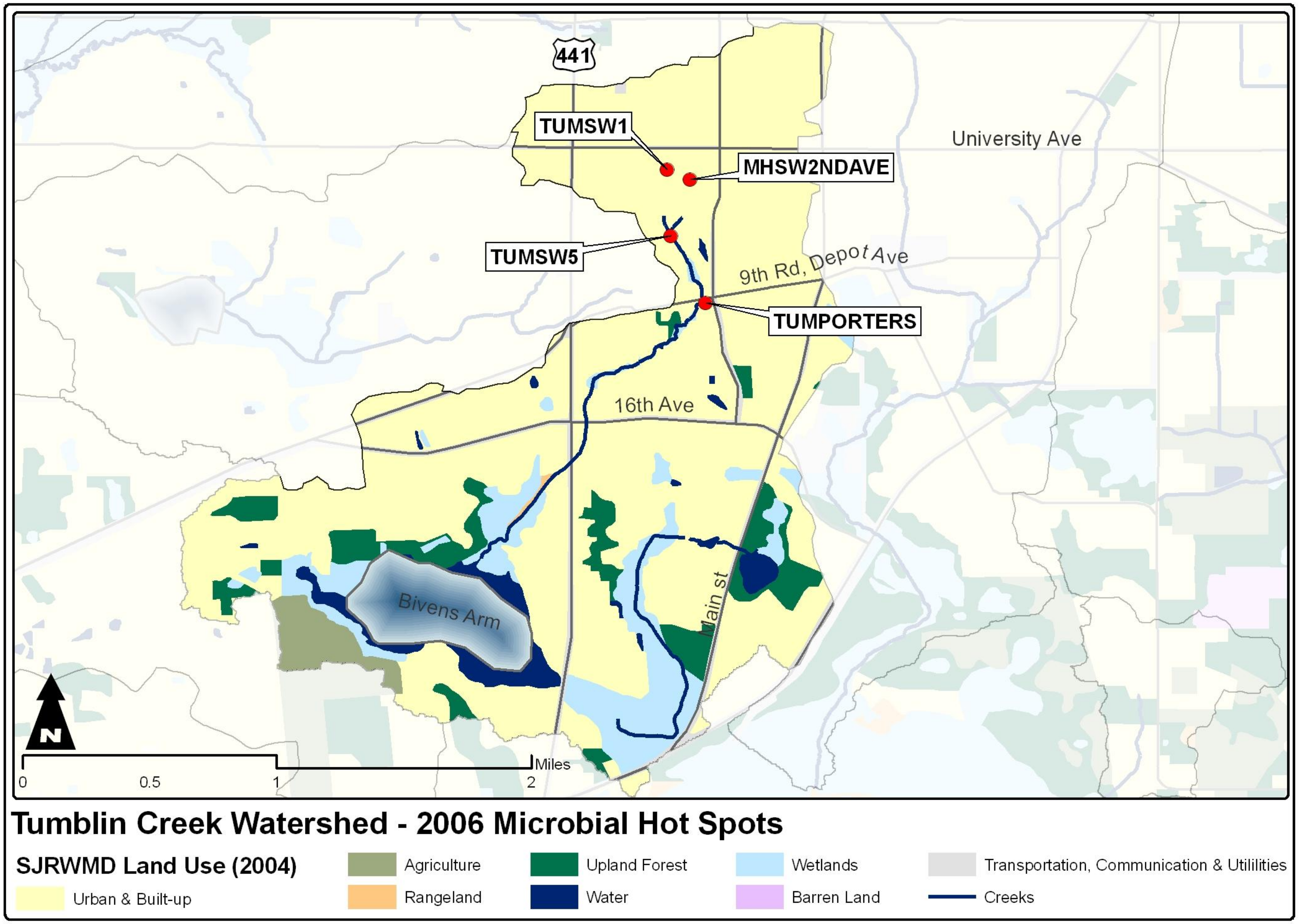


Figure 4-2. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, Alternative Indicators, and Microbial Source Tracking Sample Sites in Tumblin Creek, April and May 2006

Table 4-6. Summary of Fecal Coliform, Alternative Microbial Indicators, Fluorescent Whitening Agents, and Microbial Source Tracking Data for the Tumblin Creek Watershed, April through May 2006

Date	**Sample Site**	**Days with no rain prior to sampling**	**Rainfall for last 5 days (inches)**	**Fecal Coliform[a] (CFU/100 mL)**	***Enterococcus*[b] spp. (CFU/100 mL)**	***E. coli*[c] (CFU/100 mL)**	***C. perfringens*[d] (CFU/100 mL)**	**FWA[e]**	**MST[e,f]**
4/27/2006	TUMSW1	0	1	**21,000**	**9,100**	**1,600**	1	NM	NM
	MHSW2NDAVE	0	1	**35,000**	**1,500**	**6,700**	**79**	NM	NM
	TUMSW5	0	1	**29,000**	**21,000**	**3,200**	4	NM	NM
	TUMPORTERS	0	1	**1,700**	**2,600**	**770**	8	NM	NM
5/4/2006	TUMSW1	6	0	**900**	**580**	190	18	Weak Pos/ Moderate Pos	Negative
	MHSW2NDAVE	6	0	**850**	**490**	150	26	Weak Pos/ ND	NM
	TUMSW5	6	0	**6,100**	**12,000**	**5,500**	**115**	Weak Pos/ ND	Negative
	TUMPORTERS	6	0	280	**660**	51	36	ND	NM
5/11/2006	TUMSW1	0	0.27	**4,200**	**1,000**	**800**	**136**	NM	Negative
	MHSW2NDAVE	0	0.27	**45,000**	**18,000**	**4,900**	**144**	NM	NM
	TUMSW5	0	0.27	**43,000**	**11,000**	**4,100**	**180**	NM	Negative
	TUMPORTERS	0	0.27	**180,000**	**26,000**	**11,000**	**208**	NM	NM

a. Bold values exceed the FDEP one time maximum standard of 800 CFU/100 mL for fecal coliform (FDEP 2006)
b. Bold values exceed the FDOH recreational water standard for fresh waters of 61 CFU/100 mL for *Enterococcus* spp. (FDEP 2006)
c. Bold values exceed the USEPA criteria for bathing in recreational freshwaters of 235 CFU/100 mL for *E. coli* (USEPA 1986)
d. Bold values exceed the standard recommended by Fujioka and Shizumura of 50 CFU/100 mL for C. perfringens (1985)
e. NM = Not Measured; ND = Not Detected; Pos = Positive
f. Negative result indicates that three human specific DNA markers (*esp* gene, *Bacteroides* spp, and polyomaviruses) were not detected

4.3.2 Sweetwater Branch and Rosewood Branch

In the Sweetwater Branch Watershed, two fecal coliform “Hot Spots” were identified: one in Sweetwater Branch and a second on Rosewood Branch, the largest tributary in the watershed (Figure 1-2). For this study one sample site was located on the main branch of Sweetwater Branch and three sites were located on Rosewood Branch (Figure 4-3). Water quality standards for fecal coliform, *Enterococcus* spp. and *E. coli* were exceeded at all four sites during each sampling event, except for week one when SWBNE10 had a fecal coliform value of 800 CFU/mL (Table 4-7). Seven out of the 12 samples (58%) exceeded the *C. perfringens* standard of 50 CFU/100 mL, with no samples exceeding the standard during week one.

Consistent with historic data, baseflow (week two) fecal coliform counts were high at SWBNE10 (5,600 CFU/100 mL). As would be expected the microbial indicator counts increased during the stormflow sampling event during week three. Samples from all four sites showed an order of magnitude increase in fecal coliform, *Enterococcus* spp. and *E. coli* counts and increased counts for *C. perfringens* for this third week of sampling. During the first week of sampling, the Sweetwater and Rosewood sites were sampled two days after a rain event, and concentrations in samples from the three Rosewood Branch sites (ROSEUNIV, ROSESE7, and ROSESE9) exceeded those measured at SWBNE10 for all four of the microbial indicators. During this first week, the fecal coliform counts were relatively low (800 CFU/100 mL) at SWBNE10. The lower counts may be indicative of a “flushing out” effect that may follow storm events. However, this trend was not noted in the Rosewood sites. Replicates and numerous sampling events would be needed to test this hypothesis.

The three Rosewood Branch sites behaved similarly to each other throughout the study. However, there was an elevated *C. perfringens* value (210 CFU/100 mL) at ROSESE7 during the baseflow sampling event (week two) and the subsequent stormflow sampling event in week three (221 CFU/100 mL). Since FWAs were not detected at any of the Rosewood sites and funds were limited, the archived samples were not analyzed for the human specific DNA markers. See Section 5.3.3 for a discussion of April 2007 MST results for Rosewood Branch, in which the human specific *esp* gene was detected at ROSESE9.

Samples from Sweetwater Branch (SWBNE10) for fecal coliform were found to be highest following rainfall events (Table 4-7). A weak positive for FWAs was detected at SWBNE10 during the week two sampling event. The archived baseflow sample (5/2/2006) from SWBNE10 tested positive for all three of the human specific DNA markers (*esp* gene, *Bacteroides* spp. and polyomaviruses) while the stormflow sample (5/9/2006) tested positive for the *esp* gene only. These results indicate that the source of fecal contamination at the SWBNE10 “Hot Spot” is likely human in origin. At this point it can not be determined if the human source is wastewater infrastructure, an illicit discharge, failing septic tank systems, or the homeless population upstream of SWBNE10. It is also possible that a contributing source is microbial populations persisting in the sediments, as this area was impacted from a documented sanitary sewer overflow detected a year prior to this study on June 22, 2005. This site may be impacted by a combination of sources, including the sediments in the hydrodynamic separator (Vortex Unit) acting as a continuing source of bacteria directly upstream of SWBNE10.

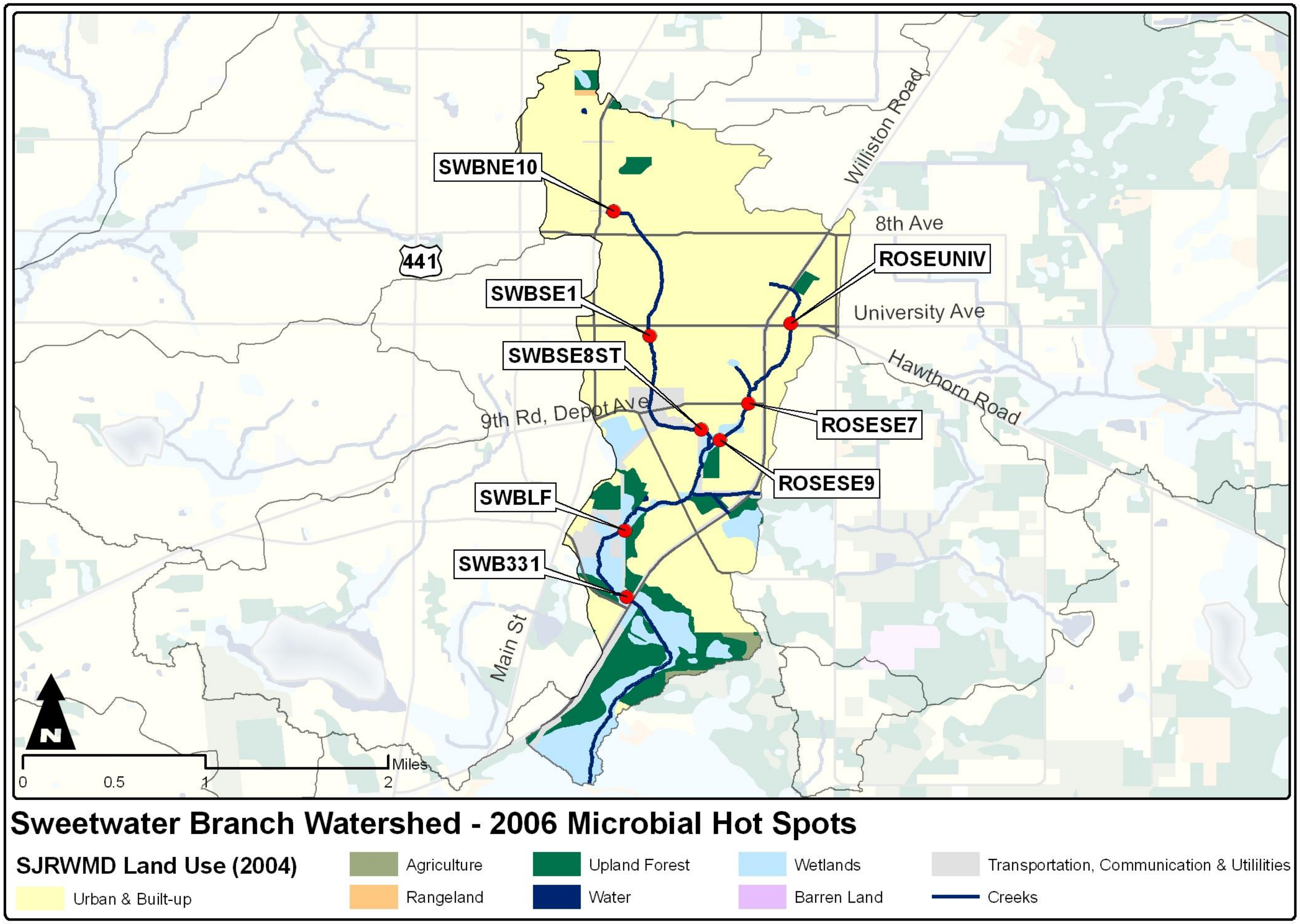


Figure 4-3. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, Alternative Indicators, and Microbial Source Tracking Sample Sites in the Sweetwater Branch Watershed during April and May 2006

Table 4-7. Summary of Fecal Coliform, Alternative Microbial Indicators, Fluorescent Whitening Agents, and Microbial Source Tracking Data for the Sweetwater Branch Watershed, April through May 2006

Date	**Sample Site**	**Days with no rain prior to sampling**	**Rainfall for last 5 days (inches)**	**Fecal Coliform[a] (CFU/100 mL)**	***Enterococcus* spp.[b] (CFU/100 mL)**	***E. coli*[c] (CFU/100 mL)**	***C. perfringens*[d] (CFU/100 mL)**	**FWA[e]**	**MST[e,f]**
4/25/2006	SWBNE10	2	0.7	800	**460**	**290**	10	NM	NM
	ROSEUNIV	2	0.7	**2,300**	**3,600**	**950**	40	NM	NM
	ROSESE7	2	0.7	**2,500**	**2,300**	**600**	5	NM	NM
	ROSESE9	2	0.7	**1,100**	**1,700**	**570**	18	NM	NM
5/2/2006	SWBNE10	4	0.35	**5,600**	**550**	**2,300**	**62**	Weak Positive	Positive (*esp* gene, *Bacteroides* spp, polyomaviruses)
	ROSEUNIV	4	0.35	**1,100**	**1,300**	**590**	**76**	ND	NM
	ROSESE7	4	0.35	**1,100**	**1,500**	**1,100**	**210**	ND	NM
	ROSESE9	4	0.35	**1,200**	**1,200**	**970**	11	ND	NM
5/9/2006	SWBNE10	0	0.06	**76,000**	**11,000**	**36,000**	**186**	NM	Positive (*esp* gene)
	ROSEUNIV	0	0.06	**45,000**	**23,000**	**17,000**	**215**	NM	NM
	ROSESE7	0	0.06	**43,000**	**16,000**	**18,000**	**221**	NM	NM
	ROSESE9	0	0.06	**37,000**	**21,000**	**20,000**	**157**	NM	NM

a. Bold values exceed the FDEP one time maximum standard of 800 CFU/100 mL for fecal coliform (FDEP 2006)
b. Bold values exceed the FDOH recreational water standard for fresh waters of 61 CFU/100 mL for *Enterococcus* spp. (FDEP 2006)
c. Bold values exceed the USEPA criteria for bathing in recreational freshwaters of 235 CFU/100 mL for *E. coli* (USEPA 1986)
d. Bold values exceed the standard recommended by Fujioka and Shizumura of 50 CFU/100 mL for C. perfringens (1985)
e. NM = Not Measured; ND = Not Detected; Pos = Positive
f. Negative result indicates that three human specific DNA markers (*esp* gene, *Bacteroides* spp, and polyomaviruses) were not detected

4.3.3 Hogtown Creek and Rattlesnake Branch

The Hogtown Creek "Hot Spot" is actually a region in the watershed where several tributaries join the main channel of Hogtown Creek. This area extends from NW 23rd Avenue to NW 22nd Street, south of the confluence with Rattlesnake Branch (Figure 4-4). For this study there were three sample sites in the main channel of Hogtown Creek, one site in the Rattlesnake Branch tributary and two sample sites on an un-named tributary near Gainesville High School. Water quality standards for fecal coliform (EPA, 2004), *Enterococcus* spp., and *E. coli* were exceed at all six sites during each sampling event, and the 50 CFU/100 mL *C. perfringens* standard was exceeded in 39% of the samples (Table 4-8). FWAs were not detected at any of the sites. During week one all of the sites were sampled three days after a rain event and the sites appeared to be under baseflow conditions. For this report weeks one and two will be considered to be baseflow conditions, while week three was clearly a stormflow sampling event.

Although weeks one and two were considered baseflow events based on rainfall, fecal coliform levels for week one ranged from 3,000 to 97,000 CFU/100 mL, with a median value of 21,500 CFU/100 mL. The median for weeks two and three were 2,150 and 7,050 CFU/100 mL, respectively. During the first sampling event, the microbial indicators (except *C. perfringens*) were exceptionally high just upstream of the confluence with Rattlesnake Branch (HOGUSRAT).

The two sites on the tributary just north of Gainesville High School (HOGGHSN and HOGGHSNASH) were sampled due to historical high fecal coliform counts in this tributary. HOGGHSNASH is close to the headwaters of the tributary and is located in a densely populated apartment complex. Microbial indicators were high at this site during baseflow, with a week one fecal coliform count of 19,000 CFU/100 mL and week two fecal coliform count of 53,000 CFU/100 mL (Table 4-8). Week three fecal coliform concentrations were 44,000 CFU/100 mL. The three human specific DNA markers were not detected in the archived samples from week two and three from HOGGHSNASH. The negative FWAs and MST results indicate that the fecal contamination may be from a non-human source. Heavy raccoon tracks and an abundance of food related trash, which attracts wildlife and feral cats, were noted at this site. It is also possible that bacteria are persisting in the sediments in this shaded tributary. Sediment samples from HOGGHSN were analyzed in January 2007 and are discussed in Section 7.0 of this report.

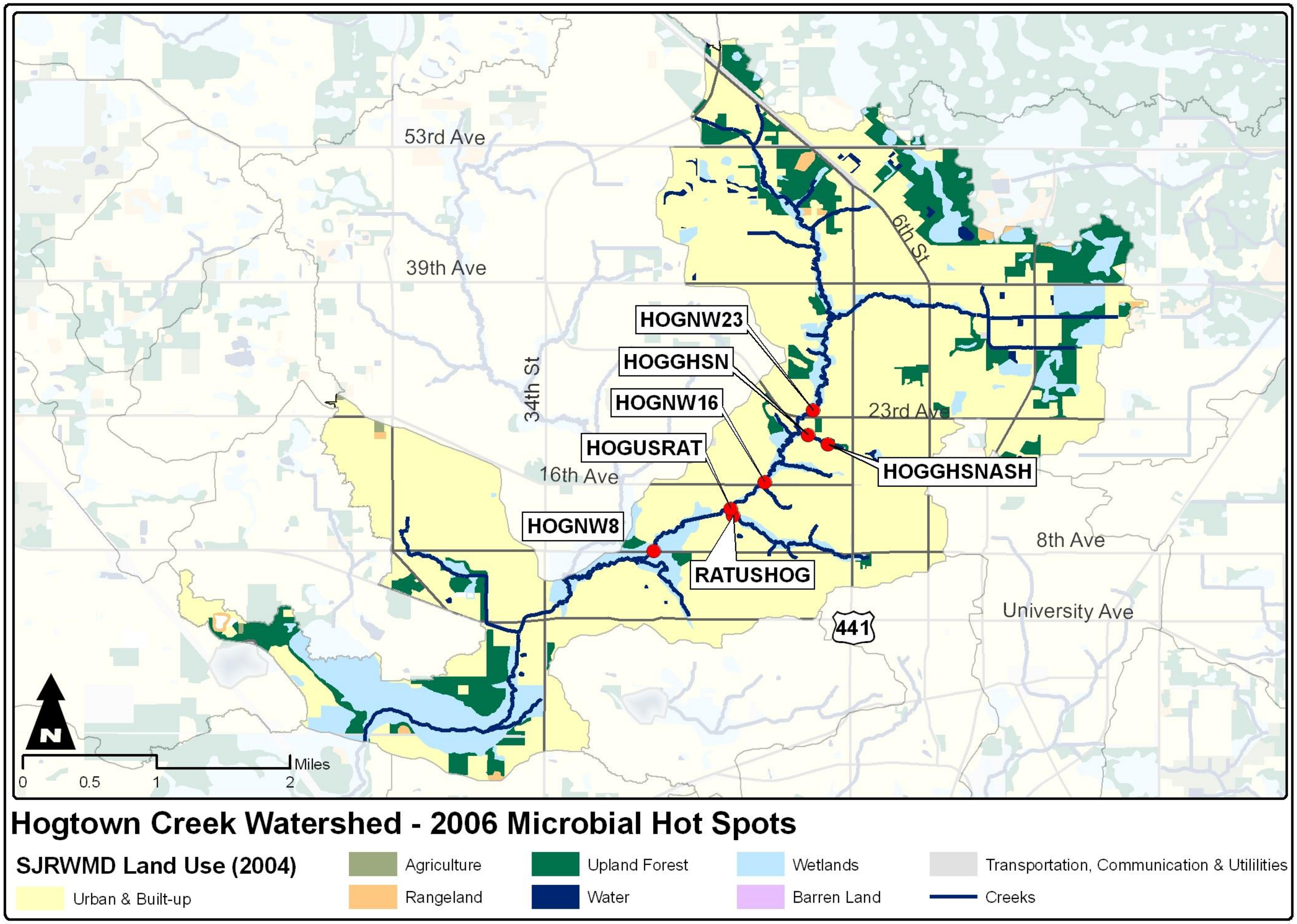


Figure 4-4. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, Alternative Indicators, and Microbial Source Tracking Sample Sites in the Hogtown Creek Watershed during April and May 2006

Table 4-8. Summary of Fecal Coliform, Alternative Microbial Indicators, Fluorescent Whitening Agents, and Microbial Source Tracking Data for the Hogtown Creek Watershed, April through May 2006

Date	**Sample Site**	**Days with no rain prior to sampling**	**Rainfall for last 5 days (inches)**	**Fecal Coliform[a] (CFU/100 mL)**	***Enterococcus* spp.[b] (CFU/100 mL)**	***E. coli*[c] (CFU/100 mL)**	***C. perfringens*[d] (CFU/100 mL)**	**FWA[e]**	**MST[e,f]**
4/26/2006	HOGNW23	3	0.7	**3,000**	**2,900**	**260**	4	NM	NM
	HOGNW16	3	0.7	**24,000**	**3,600**	**6,400**	**123**	NM	NM
	HOGUSRAT	3	0.7	**97,000**	**4,800**	**5,400**	6	NM	NM
	RATUSHOG	3	0.7	**3,400**	**3,700**	**1,400**	29	NM	NM
	HOGGHSNASH	3	0.7	**19,000**	**16,000**	**9,700**	ND	NM	NM
	HOGGHSN	3	0.7	**72,000**	**9,900**	**5,400**	0	NM	NM
5/3/2006	HOGNW23	5	0	**1,100**	**1,600**	**260**	9	ND	NM
	HOGNW16	5	0	**2,700**	**6,700**	**2,400**	**66**	ND	NM
	HOGUSRAT	5	0	**1,600**	**3,700**	**2,000**	25	ND	NM
	RATUSHOG	5	0	**1,400**	**2,300**	**1,700**	5	ND	NM
	HOGGHSNASH	5	0	**53,000**	**10,000**	**33,000**	**220**	ND	Negative
	HOGGHSN	5	0	**10,000**	**9,600**	**7,500**	**59**	ND	NM
5/10/2006	HOGNW23	0	0.16	**5,300**	**3,700**	**2,500**	2	NM	NM
	HOGNW16	0	0.16	**6,800**	**12,000**	**3,100**	**51**	NM	NM
	HOGUSRAT	0	0.16	**3,900**	**6,500**	**2,000**	**96**	NM	NM
	RATUSHOG	0	0.16	**7,300**	**2,000**	**3,800**	3	NM	NM
	HOGGHSNASH	0	0.16	**44,000**	**24,000**	**15,000**	**77**	NM	Negative
	HOGGHSN	0	0.16	**23,000**	**15,000**	**5,700**	30	NM	NM

a. Bold values exceed the FDEP one time maximum standard of 800 CFU/100 mL for fecal coliform (FDEP 2006)
b. Bold values exceed the FDOH recreational water standard for fresh waters of 61 CFU/100 mL for *Enterococcus* spp. (FDEP 2006)
c. Bold values exceed the USEPA criteria for bathing in recreational freshwaters of 235 CFU/100 mL for *E. coli* (USEPA 1986)
d. Bold values exceed the standard recommended by Fujioka and Shizumura of 50 CFU/100 mL for C. perfringens (1985)
e. NM = Not Measured; ND = Not Detected; Pos = Positive
f. Negative result indicates that three human specific DNA markers (*esp* gene, *Bacteroides* spp, and polyomaviruses) were not detected

4.3.4 Elizabeth Creek

The Elizabeth Creek Watershed is a residential area close to the University of Florida main campus (Figure 1-2) and is a tributary of Hogtown Creek. Five sites were sampled in the Elizabeth Creek Watershed (Figure 4-5). The creek has historically had high fecal coliform concentrations, and recreation in its water has been discouraged by warning signs. Water quality standards for fecal coliform, *Enterococcus* spp., and *E. coli* were exceeded at all five sites during each sampling event (Table 4-9). Eight out of 15 (53%) of the samples exceeded the 50 CFU/100 mL *C. perfringens* standard, with weak two having the highest concentrations. During week one all of the sites were sampled three days after a rain event and appeared to be under baseflow conditions. The fecal coliform concentration at ELIZNW23 was more typical of stormflow conditions at 76,000 CFU/100 mL during this sampling event. *Enterococcus* spp. (7,300 CFU/100 mL) and *E. coli* (2,500 CFU/100 mL) counts were also elevated, but *C. perfringens* was not detected.

The most upstream site in the Elizabeth Creek Watershed is ELIZUNIV. Indicator bacteria were relatively low at this site during baseflow conditions and a weak positive FWA result was detected. The archived baseflow (week two) sample was positive for human specific *esp* gene and *Bacteroides* spp. but negative for polyomaviruses. The stormflow sample was positive for *esp* gene and polyomaviruses, but negative for *Bacteroides* spp. This site is clearly impacted by human fecal contamination, but it may be a residual source since the human specific DNA marker for *Bacteroides* spp. was not detected. The presence of *Bacteroides* spp. reportedly often indicates a recent source (CH2MHILL, 2007). There are a number of older septic tank systems in the watershed and a relatively old wastewater collection system in this area. It is unlikely that this area is impacted by the homeless population.

An unnamed tributary of Elizabeth Creek was sampled at ELIZARBOR. There was a weak positive FWA result, but the week two archived (frozen) sample was negative for all three of the human specific DNA markers. This tributary is piped through a neighborhood dominated by rental homes and apartments serving college students. It is possible that there is an illicit connection to the stormwater collection system, discharging laundry water to Elizabeth Creek. FWAs were not detected at the downstream sites (ELIZ309NW23, ELIZNW23, or ELIZNW7US) therefore these sites were not sampled for human specific DNA markers.

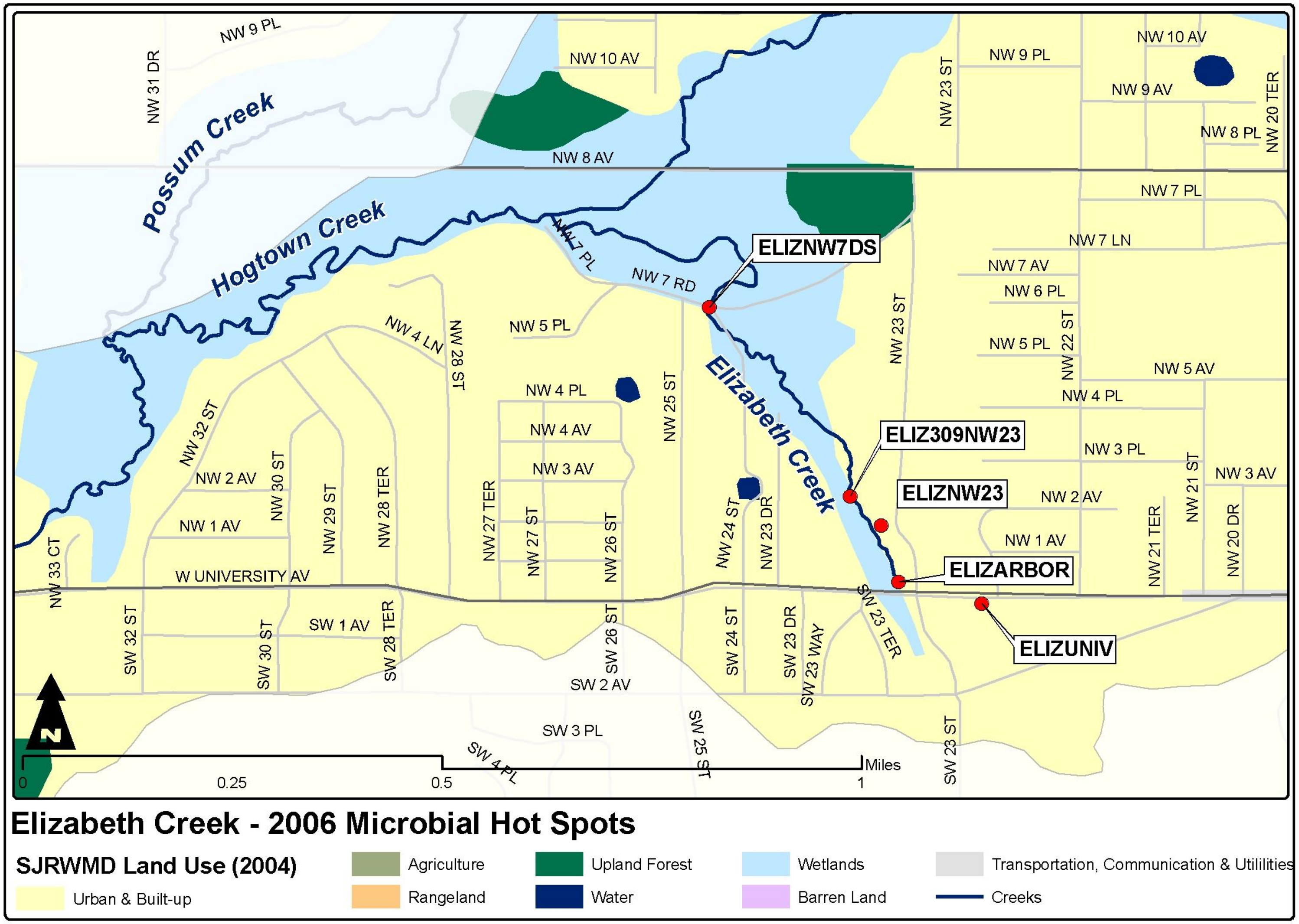


Figure 4-5. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, Alternative Indicators, and Microbial Source Tracking Sample Sites in Elizabeth Creek during April and May 2006

Table 4-9. Summary of Fecal Coliform, Alternative Microbial Indicators, Fluorescent Whitening Agents, and Microbial Source Tracking Data for the Elizabeth Creek Watershed, April through May 2006

Date	**Sample Site**	**Days with no rain prior to sampling**	**Rainfall for last 5 days (inches)**	**Fecal Coliform[a] (CFU/100 mL)**	***Enterococcus* spp.[b] (CFU/100 mL)**	***E. coli*[c] (CFU/100 mL)**	***C. perfringens*[d] (CFU/100 mL)**	**FWA[e]**	**MST[e,f]**
4/26/2006	ELIZUNIV	3	0.7	**3,700**	**1,600**	**420**	20	NM	NM
	ELIZARBOR	3	0.7	**6,200**	**6,800**	**800**	**52**	NM	NM
	ELIZ309NW 23	3	0.7	**6,800**	**9,800**	**3,500**	**133**	NM	NM
	ELIZNW23	3	0.7	**76,000**	**7,300**	**2,500**	ND	NM	NM
	ELIZNW7DS	3	0.7	**12,000**	**10,000**	**4,900**	23	NM	NM
5/3/2006	ELIZUNIV	5	0	**1,900**	**1,400**	**290**	2	Weak Positive	Positive (*esp* gene , *Bacteroids* spp.)
	ELIZARBOR	5	0	**12,000**	**7,900**	**8,600**	**68**	Weak Pos/ND	Negative
	ELIZ309NW 23	5	0	**3,900**	**7,800**	**4,200**	**65**	ND	NM
	ELIZNW23	5	0	**6,000**	**7,000**	**5,800**	**63**	ND	NM
	ELIZNW7DS	5	0	**5,200**	**13,000**	**3,300**	**147**	ND	NM
5/10/2006	ELIZUNIV	0	0.16	**74,000**	**15,000**	**8,400**	1	NM	Positive (*esp* gene, *polyoma-viruses*)
	ELIZARBOR	0	0.16	**19,000**	**8,100**	**4,200**	**92**	NM	NM
	ELIZ309NW 23	0	0.16	**23,000**	**6,200**	**2,600**	40	NM	NM
	ELIZNW23	0	0.16	**25,000**	**8,600**	**4,000**	**150**	NM	NM
	ELIZNW7DS	0	0.16	**6,000**	**8,300**	**1,100**	16	NM	NM

a. Bold values exceed the FDEP one time maximum standard of 800 CFU/100 mL for fecal coliform (FDEP 2006)
b. Bold values exceed the FDOH recreational water standard for fresh waters of 61 CFU/100 mL for *Enterococcus* spp. (FDEP 2006)
c. Bold values exceed the USEPA criteria for bathing in recreational freshwaters of 235 CFU/100 mL for *E. coli* (USEPA 1986)
d. Bold values exceed the standard recommended by Fujioka and Shizumura of 50 CFU/100 mL for C. perfringens (1985)
e. NM = Not Measured; ND = Not Detected; Pos = Positive
f. Negative result indicates that three human specific DNA markers (*esp* gene, *Bacteroides* spp, and polyomaviruses) were not detected

4.3.5 Newnans Lake Watershed

Lake Forest Creek, one of three major tributaries to Newnans Lake, is in a less urbanized area than most of the Gainesville creeks. The Lake Forest Creek Watershed, in the vicinity of LFCNE25, is a system of channelized ditches in a lower income suburban neighborhood in east Gainesville (Figure 4-6). Water quality standards for fecal coliform, *Enterococcus* spp., and *E. coli* were exceeded at all three sites during each sampling event (Table 4-10). Five of 12 (42%) of the samples exceeded the 50 CFU/100 mL *C. perfringens* standard, with weak three having the highest concentrations. FWAs were not detected at any of the sites. During week one all of the sites were sampled two days after a rain event and appeared to be under baseflow conditions. For this report, weeks one and two will be considered under baseflow conditions, while week three was clearly a stormflow sampling event.

During all three sampling events the microbial indicators (except *C. perfringens*) tended to be the highest at LFCNE25, which is the most downstream site sampled in the watershed. Indicators were the second highest at LFCNE3AVE, which is the most upstream site. Concentrations of fecal coliform and *Enterococcus* spp. were the same order of magnitude for all sites sampled during weeks one and two.

During the storm event sampling, fecal coliform and *Enterococcus* spp. concentrations increased by an order of magnitude at all three sites. Baseflow *C. perfringens* counts were higher than the stormflow concentrations at LFCNE3AVE, indicating this bacterium may be persisting in the sediments.

The archived samples from LFCNE3AVE for week two and three were negative for the three human specific DNA markers. The negative FWAs and MST results may indicate that the fecal contamination is from a non-human source; however this area was not sampled sufficiently to make any definitive conclusions. There is a large population of domestic dogs in this densely populated area of Lake Forest Creek, which may be a major source of fecal contamination.

Little Hatchet Creek is another major tributary of Newnans Lake and was sampled directly downstream of Waldo Road (SR 24). FWA results were weak positive and moderate positive at LHATWALDO. This is to be expected since this site is downstream of the permitted discharge of effluent from the Brittany Estates Water Reclamation Facility and FWAs are not removed in the treatment process. This site served as a positive control to confirm that FWAs can be detected in flowing waters with a known source of FWAs.

During week one (varied stormflow) and week two (stormflow) the fecal coliform, *Enterococcus* spp., *E. coli*, and *C. perfringens* counts were generally lower than the counts at the three sites on Lake Forest Creek. Most of the LHATWALDO values were one to two orders of magnitude lower than the Lake Forest Creek values. Except for the Brittany Estates Mobile Home Park, the area upstream of LHATWALDO is mostly low density residential and upland forest (silviculture) land uses. Due to budget limitations additional MST efforts were not applied to LHATWALDO.

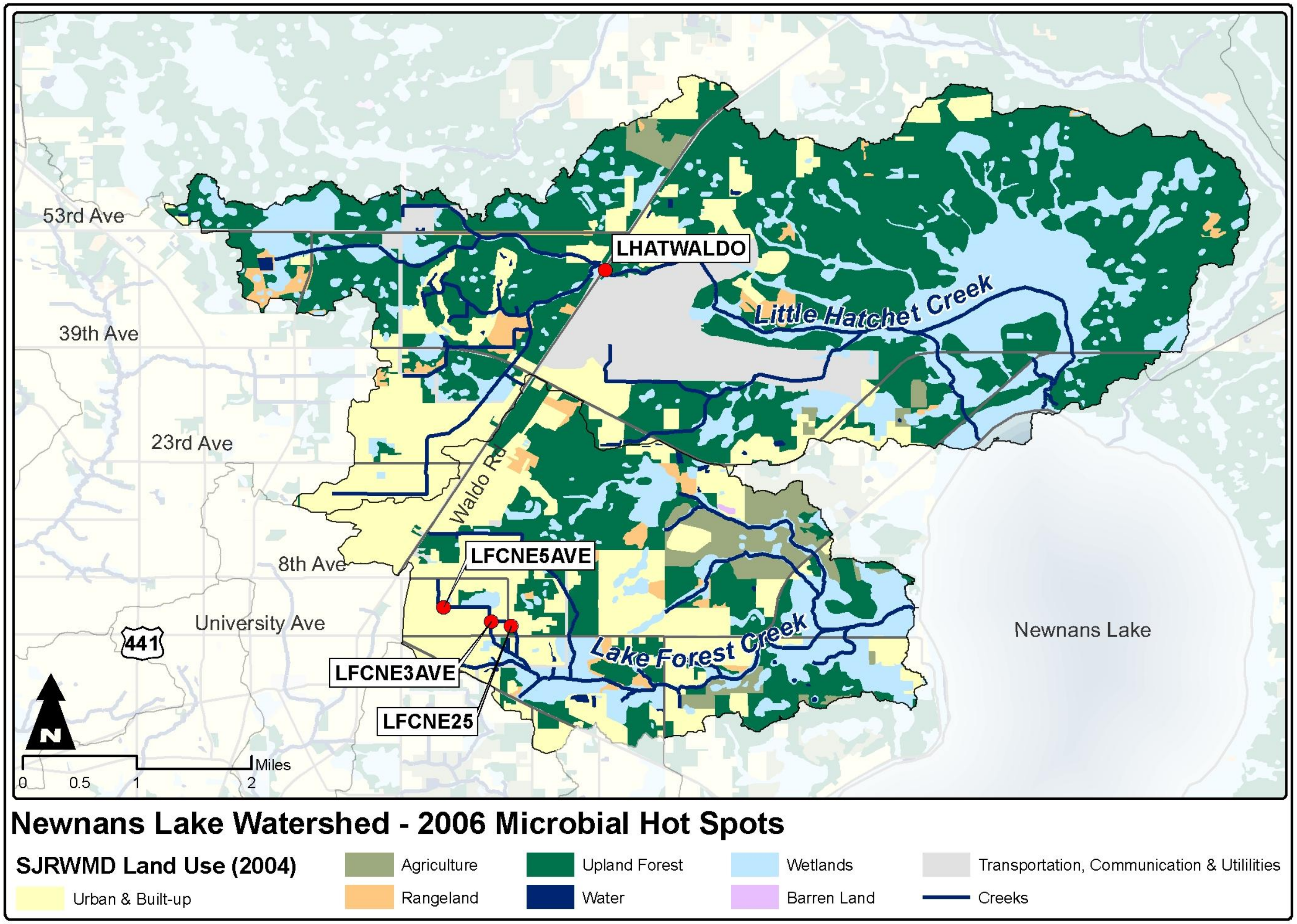


Figure 4-6. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, Alternative Indicators, and Microbial Source Tracking Sample Sites in the Newnans Lake Watershed during April and May 2006

Table 4-10. Summary of Fecal Coliform, Alternative Microbial Indicators, Fluorescent Whitening Agents, and Microbial Source Tracking Data for the Newnans Lake Watershed, April through May 2006

Date	Sample Site	Days with no rain prior to sampling	Rainfall for last 5 days (inches)	Fecal Coliform[a] (CFU/100 mL)	*Enterococcus* spp.[b] (CFU/100 mL)	*E. coli*[c] (CFU/100 mL)	*C. perfringens*[d] (CFU/100 mL)	FWA[e]	MST[e, f]
4/25/2006	LFCNE3AVE	2	0.7	**4,800**	**3,300**	**5,100**	31	NM	NM
	LFCNE5AVE	2	0.7	**2,100**	**900**	**1,400**	43	NM	NM
	LFCNE25	2	0.7	**8,400**	**6,800**	**6,800**	8	NM	NM
	LHATWALDO	2	0.7	710	**480**	**310**	43	NM	NM
5/2/2006	LFCNE3AVE	4	0.35	**3,700**	**1,300**	**1,200**	**111**	ND	Negative
	LFCNE5AVE	4	0.35	**2,500**	**1,200**	**1,900**	**76**	ND	NM
	LFCNE25	4	0.35	**2,600**	**3,200**	**1,800**	24	ND	NM
	LHATWALDO	4	0.35	800	**2,500**	**1,600**	12	Weak Positive/ Moderate Positive	NM
5/9/2006	LFCNE3AVE	0	0.06	**43,000**	**17,000**	**5,200**	**96**	NM	Negative
	LFCNE5AVE	0	0.06	**19,000**	**22,000**	**6,800**	**200**	NM	NM
	LFCNE25	0	0.06	**62,000**	**24,000**	**5,900**	**176**	NM	NM
	LHATWALDO	0	0.06	400	**1,800**	**800**	**98**	NM	NM

a. Bold values exceed the FDEP one time maximum standard of 800 CFU/100 mL for fecal coliform (FDEP 2006)
b. Bold values exceed the FDOH recreational water standard for fresh waters of 61 CFU/100 mL for *Enterococcus* spp. (FDEP 2006)
c. Bold values exceed the USEPA criteria for bathing in recreational freshwaters of 235 CFU/100 mL for *E. coli* (USEPA 1986)
d. Bold values exceed the standard recommended by Fujioka and Shizumura of 50 CFU/100 mL for C. perfringens (1985)
e. NM = Not Measured; ND = Not Detected; Pos = Positive
f. Negative result indicates that three human specific DNA markers (*esp* gene, *Bacteroides* spp, and polyomaviruses) were not detected

4.4 Summary and Conclusions

Combining the results of the various indicators helped narrow the location and origin of the sources of fecal contamination at the "Hot Spots" investigated. Analyzing samples for *E. coli*, *C. perfringens*, and *Enterococcus* spp. did not provide enough additional information to justify the continued inclusion of all the alternative indicators in the ambient fecal coliform monitoring program or the added expense. Additionally, there are currently no applicable FDEP standards to compare the values against. Currently, there is a FDOH standard for bathing areas in fresh waters for *Enterococcus* spp. and proposed standard for *E. coli* (FDOH, 2008).

E. coli levels were lower than fecal coliform, but generally followed the trends for fecal coliform. Harwood (2008) recommended adding *E. coli* to the ACEPD ambient surface water monitoring program since a strong correlation between *E. coli* and fecal coliform suggests a recent source. ACEPD plans to include *E. coli* in future ambient surface water monitoring as a companion indicator to fecal coliform. This new sampling protocol will be implemented in early 2008.

Enterococcus spp. levels were relatively high in all watersheds, with median levels exceeding median fecal coliform levels in the Lake Forest Creek watershed. CH2MHILL (2007) found consistently high *Enterococcus* spp. levels in their Gainesville urban creek monitoring conducted in 2004 through 2006. Hardwood (2008) hypothesized that the shaded nature of the urban creeks, with a well developed tree canopy allowing little natural sunlight penetration, increased the persistence of *Enterococcus* spp. in the surface waters and the sediments. This persistence may contribute to widespread elevated levels of *Enterococcus* spp. in the urban creeks. Given the consistently high levels of *Enterococcus* spp. in the urban creeks, ACEPD does not believe it serves as a useful indicator of fecal pollution in the Gainesville urban creeks and will not include it in future sampling.

Ambient levels of *C. perfringens* were two orders of magnitude lower than the levels of fecal coliform, *E. coli,* and *Enterococcus* spp. This was consistent with expectations. The *C. perfringens* levels in untreated wastewater were also several orders of magnitude lower than the levels of fecal coliform, *E. coli,* and *Enterococcus* spp. for sequential samples. Fecal coliform, *E. coli*, *Enterococcus* spp., and *C. perfringens* levels were generally highest after rainfall events. There were minimal differences in *C. perfringens* levels among watersheds. ACEPD does not plan to include *C. perfringens* in its ambient surface water quality monitoring program.

Positive FWAs and MST results point to human sources in the Sweetwater Branch and Elizabeth Creek watersheds. The results in the Tumblin Creek Watershed were less clear, but this area may also be impacted by human fecal pollution. Additional source tracking work was conducted in the "Hot Spots" in 2007 and is described in Section 5.0.

5.0 Fluorescent Whitening Agents, Fecal Coliform, and Microbial Source Tracking at "Hot Spots" April 2007

5.1 Introduction

Through its ambient monitoring program, Alachua County Environmental Protection Department (ACEPD) has identified fecal coliform "Hot Spots" (Section 1.2). Fluorescent whitening agents (FWAs) and microbial source tracking (MST) studies were conducted in 2005 and 2006 (Sections 3.0 and 4.0) in hopes of identifying the sources of elevated fecal coliform. In 2007 additional work was conducted in the "Hot Spot" areas to build upon the results of past studies; results are presented in Appendix VIIIa.

Sample sites near the "Hot Spots" were chosen based on the results of ACEPD's 2005 and 2006 studies combined with Gainesville Regional Utilities (GRU) input. In a study conducted for GRU, CH2MHILL (2007) identified Possum Creek near NW 34th Street and Sweetwater Branch near SW 8th Street as additional "Hot Spots". ACEPD added sampling stations in these areas.

5.2 Materials and Methods

Throughout this study samples were obtained in proximity to all fecal coliform "Hot Spots". All of the sites were sampled for three consecutive days beginning 4/24/2007 (except SWBLF and LHATNB which could not be accessed on 4/24/2007). The order the sites were sampled varied from day to day, to increase the chances of picking up on episodic discharges associated with times of peak water use. All water column samples were collected from approximately mid-depth in flowing water. Fecal coliform samples were collected daily at each site and were analyzed according to SM 9222D (APHA, 1998) by Advanced Environmental Laboratories (AEL) in Gainesville, FL. Water column samples from 4/25/2007 were also analyzed for three human specific DNA markers: *Enterococcus faecium* (surface protein *esp* gene), *Bacteroides* spp. (16S rRNA), and human polyomaviruses (JC virus and BK virus) by Biological Consulting Services, Inc. (BCS) of Gainesville, FL. MST laboratory methods are further explained in Appendix VII. All water column samples were delivered to the laboratories within six hours of collection time.

Sediment samples were collected from each site on 4/25/2007 and were analyzed for general *Bacteroides* spp. (non-human specific) by BCS. *Bacteroides* spp. will not re-grow in aerobic environments therefore their presence is thought to be more indicative of recent pollution (Scott and Lukasik 2007). Sediment samples were collected in the stream channel and were often from deeper pools where the water velocity is slower. Sediment samples were processed by adding a phosphate-buffered solution to 1-5 grams (wet weight) of sediment. The samples were shaken for 30 minutes and then following 30 minutes of settling time, the supernatant was filtered and analyzed. All sediment samples were delivered to the laboratory within six hours of collection time.

Two FWAs samplers were deployed at each site on 4/24/2007 and were collected on 4/26/2007 (FWAs were deployed on 4/25/2007 at SWBLF and LHATNB since these sites were inaccessible on 4/24/2007). Care was taken to place the samplers in shaded areas or in culverts, to decrease photodegradation. The samples were processed by Ozark Underground Laboratory, Inc. as described in Section 3.2. Water velocity was measured on 4/25/07 with a Marsh McBirney Flow Mate 2000 and used for flow calculations. Site characteristics were recorded for each day.

Several of the sites (LFCNE25, HOGGHSN, RATUSHOG, ELIZARBOR, and ELIZUNIVUS) were re-visited on 5/16/2007 to collect samples for a full suite of surface water indicators. These sites are not routinely monitored as part of ACEPD's ambient surface water monitoring program, so there is little historical data. The samples were analyzed by AEL for many parameters, including nutrients (Appendix VIIIb). Field parameters (dissolved oxygen, temperature, specific conductance and pH) were measured with a YSI556 Multi-Probe System field multi-parameter meter and water velocity was measured with a Marsh McBirney Flow Mate 2000 for flow calculations (Appendix VIIIc). Additional MST samples were collected on 8/7/07 in the Little Hatchet Creek Watershed (BEUPSTREAM, BEINFLUENT, BEEFFLUENT, LHATWALDO, and LHATARBOR) to further explore the initial results. Water column samples were analyzed by BCS for the three human specific DNA markers (Appendix VIIId).

In an attempt to qualitatively describe the wildlife populations in the "Hot Spot" locations, ACEPD conducted biological assessments between 7/17/2007 and 8/6/2007. The "Hot Spot" stream segments were walked by two ACEPD staff members using a detailed map and a corresponding legend to mark the location and/or condition of animals, tracks, scat, food scraps, pipes, riparian zones, exotic vegetation, transient camps, and other pertinent information. Staff paid close attention to nearby yards for bird feeders, outside pets, pet waste or any other unusual sightings. Fecal coliform samples were collected during the surveys. The results of the surveys are discussed in a separate report (Appendix IX).

5.3 Results and Discussion

April was a dry month and all sites were under baseflow conditions for the duration of the study. Fecal coliform counts exceeded the 800 CFU/100 mL one time maximum state water quality standard for 27 out of the 85 samples collected (32%). The median value was the greatest for Elizabeth Creek, 2,760 CFU/100 mL, and lowest for Lake Forest Creek, 122 CFU/100 mL (Table 5-1). This watershed is one of the most rural of those sampled and has considerable wetlands and natural areas buffering the creeks.

Table 5-1. Fecal Coliform Statistics for 4/24/2007 through 4/26/2007

Watershed	Median (CFU/100 mL)	Geometric Mean (CFU/100 mL)	Minimum (CFU/100 mL)	Maximum (CFU/100 mL)	Number (n)
Tumblin Creek	273	215	40	580	12
Sweetwater Branch	268	230	28	1,120	14
Rosewood Branch	604	825	56	9,200	9
Little Hatchet Creek	180	211	140	344	5
Lake Forest Creek	122	125	110	144	3
Hogtown Creek	745	535	26	3,200	18
Possum Creek	280	450	184	1,170	9
Elizabeth Creek	2,760	2,420	790	8,100	9
Rattlesnake Branch	187	232	138	880	6

FWAs were only detected at the two sites (SWB331 and LHATWALDO) downstream of permitted water reclamation facility effluent discharges, which serve as positive control sites. Even at these sites the FWAs were detected at low concentrations (weak positive and slight positive). The samplers were deployed for only three days, compared to the seven day deployment used in the 2005 and 2006 studies. The sample cloths still became coated by a growth noted in the prior studies. It is possible that the growth interfered with the adsorption of FWAs.

Eighteen sites were positive for at least one human specific DNA marker in which FWAs were not detected. Had FWAs been used as a screening tool, these sites would not have been sampled for the MST markers. Conversely, SWB331 had a slight positive FWA result and was negative for the three human specific DNA markers. This site is downstream of the GRU Main Street Water Reclamation Facility and the survival and persistence of host specific markers in the environment and through the treatment process is unknown (Field and Scott, 2007), but is likely to be poor. FWAs did not prove to be an effective screening tool for deciding which sites to sample for MST markers.

Out of the 29 sampled sites, 19 of them were positive for at least one human specific DNA marker in water column samples. Seven sites were positive for only one marker, ten sites were positive for two markers, and two sites were positive for all three markers. *Bacteroides* spp. (general) was detected in 19 of the 29 sediment samples. The sediment general *Bacteroides* spp. test is not a human specific DNA marker, but detects a gene present in all *Bacteroides* spp. The presence of the sediment general *Bacteroides* spp. may indicate recent fecal contamination, but not necessarily from a human source. SWBSE1 and ROSEUNIV were the only positive sediment samples with corresponding water samples which were negative for all three human specific DNA markers.

Median fecal coliform levels were highest in Elizabeth Creek followed by Rosewood Branch and Hogtown and Possum Creek (Table 5-1). Tumblin Creek samples showed the lowest presence of human markers. The *esp* gene was the most frequently detected marker in water samples: 43% of the analyzed samples were positive for *esp* gene. Qualitative analyses for general *Bacteroides* in the sediments indicated that they were most prevalent in the Hogtown Creek Watershed, but each watershed had at least one positive sediment sample (Table 5-2). The relatively low number of MST samples obtained at each of the sites and low sampling frequency preclude trend analysis for these constituents in any of watersheds. However, these data are useful in developing future sampling plans and developing strategies for further assessment of the “Hot Spots.”

Table 5-2. Summary of Microbial Source Tracking Results by Watershed, 2006 and 2007

Watershed	**Water Samples (n)**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma-viruses ID PCR (+/-)**	**Total Human Markers (+/-)**	**Sediment Samples (n)**	**General *Bacteroides* spp. PCR (+/-)**
Tumblin Creek	7	1/6	0/7	2/5	3/18	3	1/2
Sweetwater Branch	7	4/3	2/5	2/5	8/13	5	2/3
Rosewood Branch	4	1/3	0/4	0/4	1/11	4	2/4
Hogtown Creek*	8	4/4	1/7	4/4	9/15	6	5/1
Rattlesnake Branch	2	2/0	1/1	1/1	4/2	2	2/0
Possum Creek	3	2/1	1/2	0/3	3/6	3	2/3
Elizabeth Creek	7	3/4	1/6	1/6	5/16	3	1/2
Lake Forest Creek	1	0/1	0/1	0/1	0/3	2	1/1
Little Hatchet Creek	5	2/3	2/3	2/3	6/9	1	1/0

*Central portion of the watershed from NW 8th Avenue to NW 23rd Avenue

By combining all of the indicators for an area, one can gain a better understanding of the sources of fecal coliform at the "Hot Spots". Scott and Lukasik (2007) have created a matrix for interpreting MST results. One of their core assumptions is that the presence of human specific DNA marker for *Bacteroides* spp. is indicative of recent human fecal contamination due to its inability to persist in the environment. A white paper on their interpretation of the 2007 study is included as Appendix VII. In the following sections the 2007 MST results are examined in the context of the 2006 results for the "Hot Spots" addressed in this study.

5.3.1 Tumblin Creek

Human specific DNA markers were not detected in the Tumblin Creek Watershed during the 2006 study. TUMSW5 is the sample site the most upstream in the watershed and TUMPORTERS is a stormwater pipe exiting the Porters neighborhood (Figure 5-1). In the 2007 study TUMPORTERS was positive for polyomaviruses but negative for the other human specific DNA markers, while TUMSW5 was positive for *esp* gene (Table 5-3). These results suggest residual, or non-recent, sources at these sites (Scott and Lukasik 2007). Further downstream, TUMSW11 was positive for human *Bacteroides* spp**.** and *esp* gene, which indicates there may have been a recent source of human fecal contamination at this site. However, at the furthest downstream site (TUM441) the only human specific DNA marker detected was for polyomaviruses. These results demonstrate the difficulty in interpreting snap shot sampling events in flowing waters, but also strongly suggest the presence of human fecal contamination in these waters.

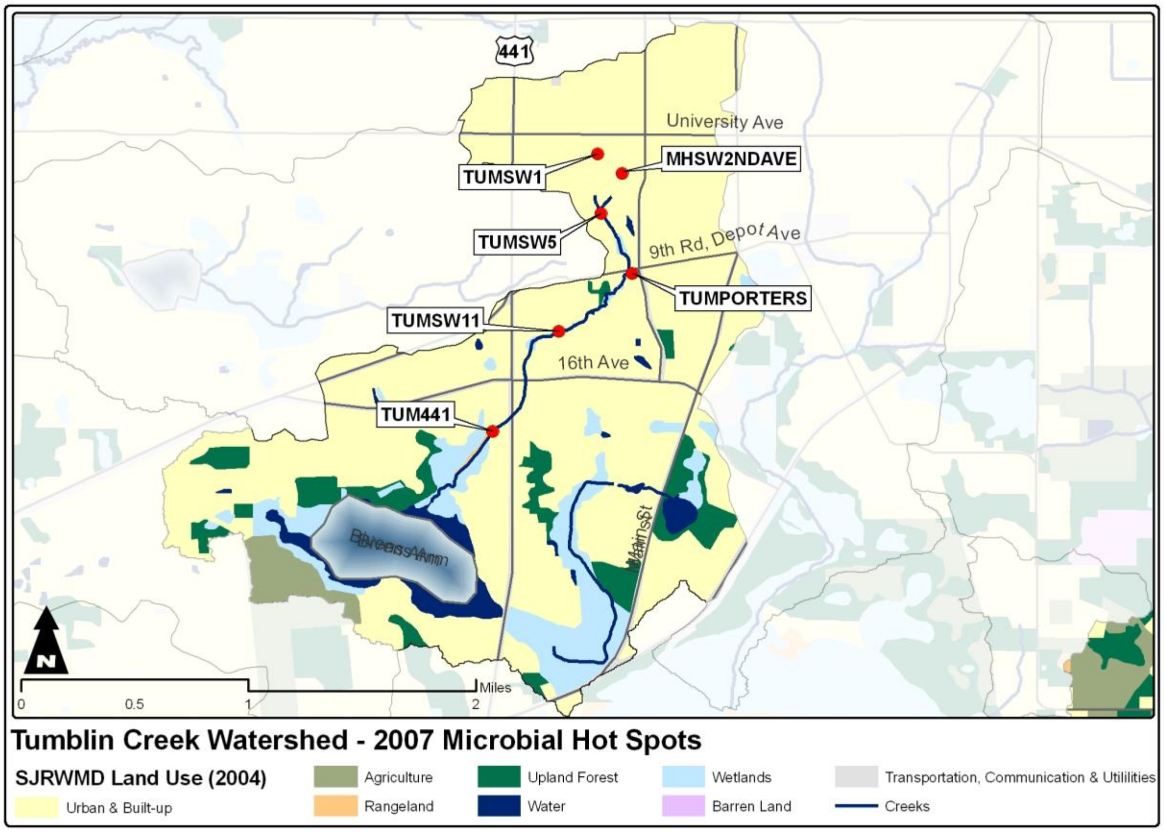


Figure 5-1. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, and Microbial Source Tracking Sample Sites in the Tumblin Creek Watershed, April 2007

Table 5-3. Summary of Microbial Source Tracking Human Markers and Fluorescent Whitening Agents in Tumblin Creek, 2006 and 2007

Sample Site	**Sample Date**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma -viruses ID PCR (+/-)**	**Sediment General *Bacteroides* spp. PCR (+/-)**	**FWA**
TUMSW1	5/4/2006	Negative	Negative	Negative	NS	Weak Positive/ Moderate Positive
TUMSW1	5/11/2006	Negative	Negative	Negative	NS	NS
MHSW2NDAVE	5/4/2006	NS	NS	NS	NS	Weak Positive/ ND
TUMSW5	5/4/2006	Negative	Negative	Negative	NS	Weak Positive/ ND
TUMSW5	5/11/2006	Negative	Negative	Negative	NS	NS
TUMSW5	4/25/2007	Positive	Negative	Negative	Positive	ND
TUMPORTERS	5/4/2006	NS	NS	NS	NS	ND
TUMPORTERS	4/25/2007	Negative	Negative	Positive	Negative	ND
TUM441	4/25/2007	Negative	Negative	Positive	Negative	ND
TUMSW11	4/25/2007	Positive	Positive	Negative	Positive	ND

NS = Not Sampled; ND = Not detected

5.3.2 Sweetwater Branch

The most upstream site, SWBNE10 (Figure 5-2), was negative for all of the human specific DNA markers in 2007 but was positive for all human specific markers in 2006 (Table 5-4). Downstream at the Sweetwater Branch Park (SWBSE1) the human specific markers were not detected in the water column in 2007 but general *Bacteroides* spp. was detected in the sediments. This park is frequented by the local homeless population and toilet paper is often observed along the banks. It is surprising that human markers were not observed in the water column at this site, perhaps the fecal loading to this site is at such low concentrations that it cannot be detected by the methods employed. However, the sediment results suggest there is a recent source of fecal contamination, but not necessarily human. Further downstream at SWBSE8ST, polyomaviruses and *esp* gene were detected which indicates a residual human impact. Further downstream, SWBLF was positive for human *Bacteroides* spp**.** and *esp* gene which indicates there may have been a recent human source of fecal contamination at this site. This site is directly downstream of an area where a large number of homeless people are known to live. It is likely that their "bush bathrooms" are the source of contamination at this site. SWB331 is the furthest downstream site and is located downstream of the permitted GRU Water Reclamation Facility (WRF) discharge. The discharge from the GRU WRF increases the flow substantially, and under baseflow conditions it comprises approximately 75% or more of the flow in Sweetwater Branch at this location. The only positive indicator detected was the slight positive FWAs, which would be expected since FWAs are not removed from wastewater during the treatment processes.

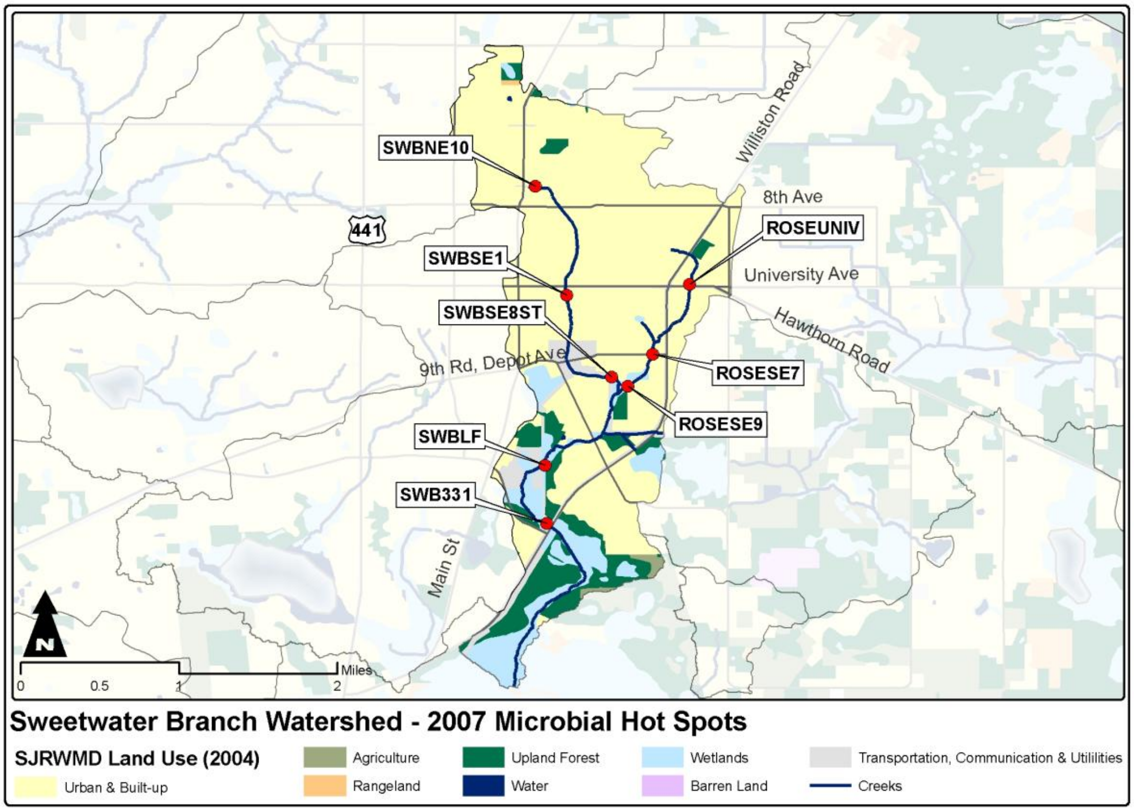


Figure 5-2. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, and Microbial Source Tracking Sample Sites in the Sweetwater Branch Watershed, April 2007

Table 5-4. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in Sweetwater Branch, 2006 and 2007

Sample Site	**Sample Date**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma-viruses ID PCR (+/-)**	**Sediment General *Bacteroides* spp. PCR (+/-)**	**FWA**
SWB331	4/25/2007	Negative	Negative	Negative	Negative	Slight Positive
SWBLF	4/25/2007	Positive	Positive	Negative	Positive	ND
SWBNE10	5/2/2006	Positive	Positive	Positive	NS	Weak Positive
SWBNE10	5/9/2006	Positive	Negative	Negative	NS	NS
SWBNE10	4/25/2007	Negative	Negative	Negative	Negative	ND
SWBSE1	4/25/2007	Negative	Negative	Negative	Positive	ND
SWBSE8ST	4/25/2007	Positive	Negative	Positive	Negative	ND

NS = Not Sampled; ND = Not detected

5.3.3 Rosewood Branch

Rosewood Branch samples were not analyzed for human specific DNA markers in 2006 based on the absence of FWAs, which were used as a screening tool. In 2007 ROSEUNIV, the furthest upstream sample site (Figure 5-2), was negative for all three human specific DNA markers although general *Bacteroides* spp. was detected in the sediment (Table 5-5). Further downstream at ROSESE7 all of the indicators were not detected in the water column or sediments. However, there was a high fecal coliform count (9,200 CFU/100 mL) at this site on 4/24/2007. This spike in fecal coliforms could have been caused by a human source which was no longer present in the flowing waters of the site on 4/25/2007 when the MST samples were collected. Upstream of the confluence with Sweetwater Branch (ROSESE9), *esp* gene was detected in the water column and general *Bacteroides* spp. was detected in the sediments.

Table 5-5. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in Rosewood Branch, 2007

Sample Site	**Sample Date**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma-viruses ID PCR (+/-)**	**Sediment General *Bacteroides* spp. PCR (+/-)**	**FWA**
ROSESE7	4/25/2007	Negative	Negative	Negative	Negative	ND
ROSESE9	4/25/2007	Positive	Negative	Negative	Positive	ND
ROSEUNIV	4/25/2007	Negative	Negative	Negative	Positive	ND

NS = Not Sampled; ND = Not detected

5.3.4 Hogtown Creek

All of the sediment samples from the Hogtown Creek sites except HOGNW8 were positive for the general *Bacteroides* spp. The presence of *Bacteroides* spp. is thought to be indicative of recent fecal sources (Scott and Lukasik 2007). HOGNW23, HOGNW16 and HOGUSRAT are all in the main channel of Hogtown Creek (Figure 5-3) and were all positive for *esp* gene and polyomaviruses, indicating a residual human source (Table 5-6). HOGCETRIB was positive for the polyomaviruses and negative for the remaining human specific DNA markers. This site is a small tributary in the Creeks Edge Condominiums which converges with the main channel of Hogtown Creek downstream of HOGNW23. On 2/24/2007 there was a sanitary sewer overflow from the private wastewater collection system at the condos which resulted in an untreated sewage discharge to the tributary. This event could be influencing these results. HOGGHSN was positive for human B*acteroides* spp. and *esp* gene in the water column, which suggests a recent human source. This site is located on a small tributary that converges with Hogtown Creek downstream of HOGNW23 and upstream of HOGNW16. In 2006, the human specific DNA markers were not detected in this tributary, and it was assumed that the prevalence of feral cats and raccoons were responsible for the high fecal coliform counts. None of the human specific DNA markers were detected at the furthest downstream site (HOGNW8), which makes one question how far downstream from the original source can a human source signature be detected.

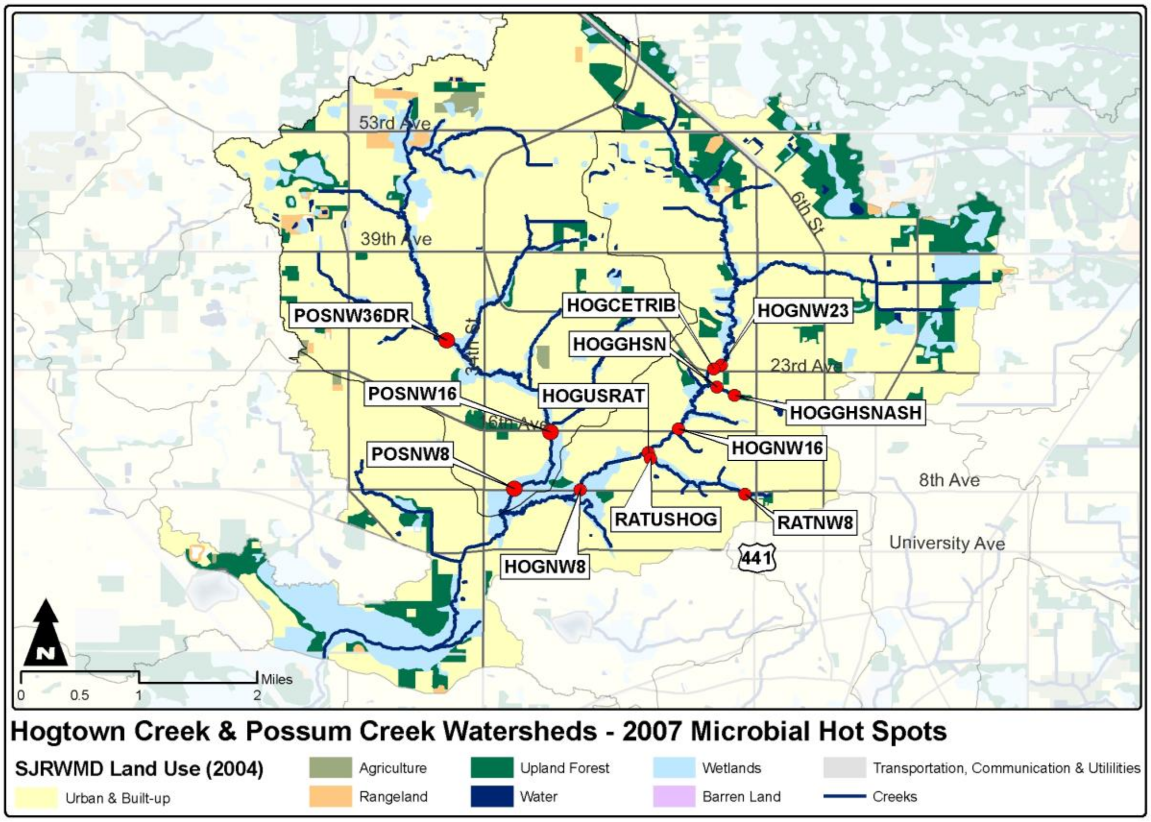


Figure 5-3. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, and Microbial Source Tracking Sample Sites in the Hogtown Creek Watershed, April 2007

Table 5-6. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in Hogtown Creek, 2006 and 2007

Sample Site	Sample Date	*esp* gene ID PCR (+/-)	*Bacteroides* spp. ID PCR (+/-)	polyoma-viruses ID PCR (+/-)	Sediment General *Bacteroides* spp. PCR (+/-)	FWA
HOGGHNASH	5/3/2006	Negative	Negative	Negative	NS	ND
HOGGHNASH	5/9/2006	Negative	Negative	Negative	NS	NS
HOGCETRIB	4/25/2007	Negative	Negative	Positive	Positive	ND
HOGGHSN	4/25/2007	Positive	Positive	Negative	Positive	ND
HOGNW16	4/25/2007	Positive	Negative	Positive	Positive	ND
HOGNW23	4/25/2007	Positive	Negative	Positive	Positive	ND
HOGNW8	4/25/2007	Negative	Negative	Negative	Negative	ND
HOGUSRAT	4/25/2007	Positive	Negative	Positive	Positive	ND

NS = Not Sampled; ND = Not detected

5.3.5 Rattlesnake Branch

Rattlesnake Branch was not sampled for human specific DNA markers in 2006 due to the absence of FWAs used as a screening tool. RATNW8 is the site furthest upstream in this residential watershed, and the water column samples were positive for *esp* gene and human B*acteroides* spp. (Table 5-7). The presence of *Bacteroides* spp. may suggest a recent human source (Scott and Lukasik, 2007). Further downstream at RATUSHOG, upstream of the confluence with Hogtown Creek, *esp* gene and polyomaviruses were detected. Fecal coliform concentrations were relatively low, ranging from 138 to 880 CFU/100 mL during the three day study. A gravity wastewater collection system runs adjacent to this creek, and sewage odors are often reported by citizens. It is possible that domestic wastewater is entering this creek from the collection system, which includes private laterals to the houses along the creek.

Table 5-7. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in Rattlesnake Branch, 2007

Sample Site	Sample Date	*esp* gene ID PCR (+/-)	*Bacteroides* spp. ID PCR (+/-)	polyoma-viruses ID PCR (+/-)	Sediment General *Bacteroides* spp. PCR (+/-)	FWA
RATNW8	4/25/2007	Positive	Positive	Negative	Positive	ND
RATUSHOG	4/25/2007	Positive	Negative	Positive	Positive	ND

ND = Not detected

5.3.6 Possum Creek

Possum Creek was not sampled as part of the 2006 MST study and was added to this study at the request of GRU. POSNW36DR is the site furthest upstream in the watershed and was positive for *esp* gene, human *Bacteroides* spp., and general *Bacteroides* spp. in sediment (Table 5-8). The presence of human *Bacteroides* spp. may indicate a recent human source. *Esp* gene was detected in the water column further downstream at POSNW16 which is one of ACEPD’s ambient surface water monitoring stations. This site may be impacted by a residual, or non-recent, human source which may be the same source as detected upstream at POSNW36DR. POSNW8 is the most downstream site and is located just upstream of the confluence with Hogtown Creek. This site was negative for all of human specific DNA markers and general *Bacteroides* spp. in sediments. It is likely that this site is far enough downstream from the detected source that the human specific indicators are too diluted to be detected. Fecal coliform concentrations were relatively low during this study, ranging from 189 to 1,170 CFU/100 mL, again demonstrating that fecal coliform does not correlate well with MST results.

Table 5-8. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in Possum Creek, 2007

Sample Site	**Sample Date**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma-viruses ID PCR (+/-)**	**Sediment General *Bacteroides* spp. PCR (+/-)**	**FWA**
POSNW36DR	4/25/2007	Positive	Positive	Negative	Positive	ND
POSNW16	4/25/2007	Positive	Negative	Negative	Positive	ND
POSNW8	4/25/2007	Negative	Negative	Negative	Negative	ND

ND = Not detected

5.3.7 Elizabeth Creek

There were three sample sites in the Elizabeth Creek Watershed. ELIZARBOR is on a tributary coming from a residential area (dominated by University of Florida students) west of the president's house (Figure 5-4). ELIZNW23 is on a tributary coming from the residential area north of University Avenue and east of the president's house. ELIZNW7DS is downstream of the convergence of these tributaries and just upstream of the confluence with Hogtown Creek. In 2007 the Elizabeth Creek Watershed had high fecal coliform concentrations with counts ranging from 790 to 8,100 CFU/100 mL. FWAs were not detected at any of the sites during the 2007 sampling (Table 5-9). ELIZARBOR was negative for all three human specific DNA markers and general *Bacteroides* spp. in sediments in 2007. *Esp* gene and sediment general *Bacteroides* spp. were detected at ELIZNW23. Further downstream at ELIZNW7DS, no markers were detected. Future efforts will be concentrated on the tributary ELIZNW23 is located on, since there may be a human source impacting this tributary, as documented in the 2006 study.

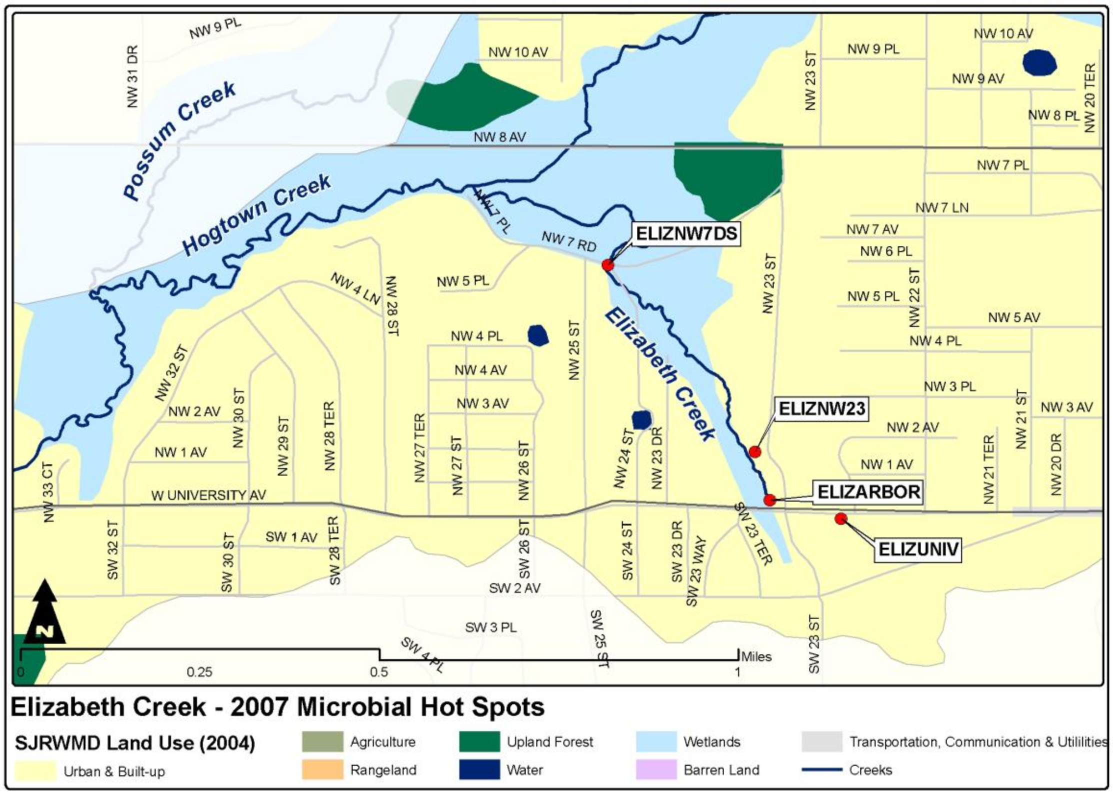


Figure 5-4. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, and Microbial Source Tracking Sample Sites in the Elizabeth Creek Watershed, April 2007

Table 5-9. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in Elizabeth Creek, 2006 and 2007

Sample Site	**Sample Date**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma-viruses ID PCR (+/-)**	**Sediment General *Bacteroides* spp. PCR (+/-)**	**FWA**
ELIZUNIV	5/3/2006	Positive	Positive	Negative	NS	Weak Positive
ELIZUNIV	5/10/2006	Positive	Negative	Positive	NS	NS
ELIZARBOR	5/3/2006	Negative	Negative	Negative	NS	Weak Positive/ND
ELIZARBOR	5/10/2006	Negative	Negative	Negative	NS	NS
ELIZARBOR	4/25/2007	Negative	Negative	Negative	Negative	ND
ELIZNW23	4/25/2007	Positive	Negative	Negative	Positive	ND
ELIZNW7DS	4/25/2007	Negative	Negative	Negative	Negative	ND

NS = Not Sampled; ND = Not detected

5.3.8 Newnans Lake Watershed

There was one sample site on Lake Forest Creek (LFCNE25) and two on Little Hatchet Creek, with LHATWALDO being upstream of LHATNB (Figure 5-5). Both of these creeks are located in the less developed eastern side of Gainesville and eventually discharge to Newnans Lake. For report writing and data analysis, samples from these sites were combined and labeled as the Newnans Lake Watershed.

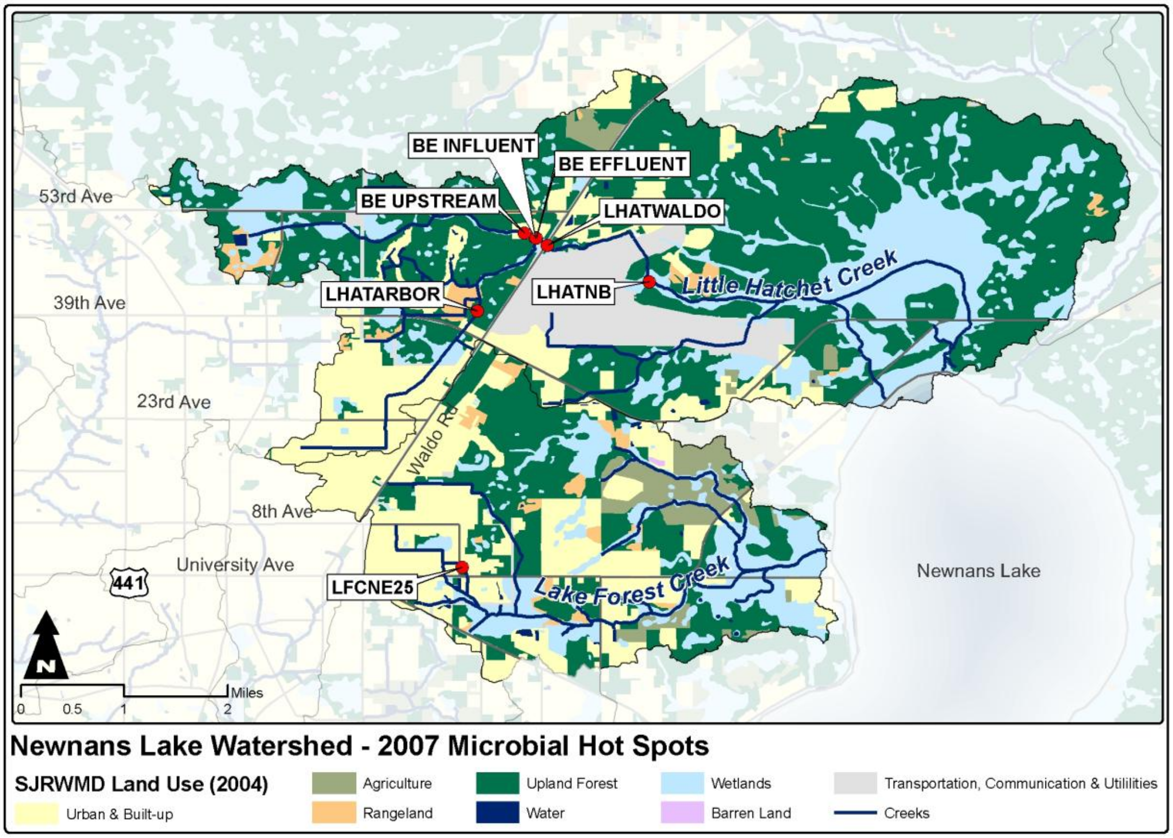


Figure 5-5. Locations of Gainesville Urban Creek Fluorescent Whitening Agents, Fecal Coliform, and Microbial Source Tracking Sample Sites in the Newnans Lake Watershed, April 2007

As in the 2006 study, LFCNE25 was negative for all of the human specific DNA markers and FWAs (Table 5-10). Fecal coliform concentrations were very low (144, 110, and 122 CFU/100 mL) at this site during the three day 2007 study and mirror levels of un-impacted surface waters. FWAs were detected (weak positive and moderate positive) at LHATWALDO but not at LHATNB. LHATWALDO is approximately a quarter of a mile downstream of the permitted discharge of effluent from the Brittany Estates Mobile Home Park Water Reclamation Facility. LHATWALDO serves as a positive control to demonstrate that FWAs are detected downstream of a known source. It is likely that the FWAs became too diluted, photodegraded, or masked by dissolved organics in the water column to be detected downstream at LHATNB. Both of these sites were positive for all three human specific DNA markers in 2007 which is a strong signal of recent human fecal pollution. It is interesting that the fecal coliform counts were low at these sites ranging from 140 to 344 CFU/100 mL. These counts are not indicative of high levels of fecal contamination.

Table 5-10. Summary of Microbial Source Tracking and Fluorescent Whitening Agents in the Newnans Lake Watershed, 2007

Sample Site	**Sample Date**	***esp* gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**	**polyoma-viruses ID PCR (+/-)**	**Sediment General *Bacteroides* spp. PCR (+/-)**	**FWA**
LFCNE25	4/25/2007	Negative	Negative	Negative	Negative	ND
LHATNB	4/25/2007	Positive	Positive	Positive	Positive	ND
LHATWALDO	4/25/2007	Positive	Positive	Positive	Positive	Weak Positive/ Moderate Positive
LHATWALDO	8/7/2007	Negative	Negative	Negative	NS	NS
BEUPSTREAM	8/7/2007	Negative	Negative	Negative	NS	NS
LHATARBOR	8/7/2007	Negative	Negative	Negative	NS	NS
BEINFLUENT	8/7/2007	Positive	Positive	Positive	NS	NS
BEEFFLUENT	8/7/2007	Positive	Positive	Positive	NS	NS

NS = Not Sampled; ND = Not detected

LHATWALDO was re-sampled on August 8/7/2007; field parameters and lab results are presented in Appendix VIIId. An additional site directly upstream of the water reclamation facility (BEUPSTREAM) and a site from another tributary (LHATARBOR) which converges with Little Hatchet Creek upstream of LHATWALDO were also sampled along with the influent and effluent of the water reclamation facility. As would be expected, the influent was positive for all three human specific DNA markers. The reclaimed water effluent was also positive for all three markers, even though the water was treated and chlorinated. The reclamation facility is well maintained and discharges approximately 0.03 million gallons per day to Little Hatchet Creek. The final stages of wastewater treatment are chlorination with sodium hypochlorite tablets and then de-chlorinated with sodium thiosulfate tablets. ACEPD inspects the Brittany Estates Wastewater Reclamation Facility and occasionally notes chlorine levels below the FDEP permit threshold prior to dechlorination. It is likely that the effluent was the source detected in the April sampling. These results differ from the results presented in section 5.3.2 for Sweetwater Branch, where water samples were negative for all three human specific DNA markers downstream of the GRU Main Street Water Reclamation Facility. Harwood (2208) and Scott (2008) were surprised to see human markers detected in treated wastewater.

The three sample sites in Little Hatchet Creek (LHATWALDO, LHATARBOR, and BEUPSTREAM) were negative for all three human specific DNA markers during the August 2007 sample event. It is interesting that a residual signal was not detected at LHATWALDO. However, three months had passed since the original samples were collected. In the future, efforts will be made to conduct monthly monitoring, which will provide adequate data for detecting trends.

5.4 Summary and Conclusions

Fecal coliform counts did not correlate well with the human specific DNA marker results, which supports the case against fecal coliform as an indicator of human fecal contamination. FWAs did not prove to be an effective screening tool for choosing sites to run MST analyses and ACEPD will discontinue their use. MST efforts provided the most information on source of fecal contamination, but still had their weaknesses. It is unclear how long an intermittent source can be detected and at what spatial distribution. Additionally, frequent monitoring for MST human markers must be conducted to provide a sufficient data set for analyses. The flowing water of the urban creeks makes pinpointing sources more difficult since the evidence is always flowing with the water. Another drawback is that the markers are not quantitative, but yield only a presence or absence result.

The frequency of MST data was insufficient to evaluate trends for specific sites. Harwood (2008) suggests that monthly sampling at a single site or group of sites for 10 to 12 months would be optimum. Using this sampling scheme, four or five samples that were consistently positive for human markers would be needed to definitively identify a human source of fecal contamination. This sampling would be very costly; ACEPD is evaluating the option of targeted fecal coliform and *Escherichia coli* sampling to more accurately define the “Hot Spot” areas before further MST work is conducted.

Median fecal coliform levels were highest in Elizabeth Creek followed by Hogtown Creek and Rosewood Branch. At least one human marker was detected in each watershed, except for Lake Forest Creek. The *esp* gene was the marker most frequently detected in water samples. Qualitative analyses for general *Bacteroides* spp. in the sediments indicated that they were most prevalent in the Hogtown Creek Watershed.

In the Paynes Prairie Watershed, Tumblin Creek samples showed the lowest concentrations of fecal coliform and the lowest presence of positive human markers; potentially indicating a source other than human fecal matter as the origin of fecal coliform bacteria in Tumblin Creek. Fecal coliform levels in Sweetwater Branch were higher than in Tumblin Creek, and human markers were detected at three sites in the watershed. Rosewood Branch had elevated fecal coliform. The limited MST sampling during one sampling event in 2007, indicted the *esp* gene at one (ROSESE9) of the three sample sites.

In the Hogtown Creek Watershed, the central portion of the main channel of the creek and several tributaries were found to have both elevated fecal coliform levels and the presence of human markers. Hogtown Creek was found to have elevated fecal coliform bacteria levels, in addition to the repeated presence of human markers in the water and general *Bacteroides* spp. in the sediments. Rattlesnake Branch had relatively low levels of fecal coliform, but was not frequently monitored as part of this study. The limited MST analyses for Rattlesnake Branch showed water samples to be positive for two of the three human markers at both sample sites in April 2007; sediments at both sample sites were also positive for general *Bacteroides* spp. Based on the limited data set for Rattlesnake Branch, this area requires further investigation. Fecal coliform levels in

Possum Creek were lower than in Hogtown Creek, and some human markers were detected as part of the limited sampling conducted. Elizabeth Creek, another tributary of Hogtown Creek, had the highest levels of fecal coliform (median value of 2,760 CFU/100 mL) of all the streams sampled as part of this 2007 study. Additionally, human markers were detected in this watershed in 2006 and 2007.

Lake Forest Creek and Little Hatchet Creek in the Newnans Lake Watershed both had areas of elevated fecal coliform bacteria. Lake Forest Creek was negative for all human markers analyzed as part of the MTS work. Little Hatchet Creek was positive for human markers in the water and general *Bacteroides* spp. in the sediments in April 2007, but negative for the markers in August 2007. Both of these “Hot Spot” areas require additional work; it appears from the limited data that the source(s) for elevated levels in Lake Forest Creek may be non-human.

Sources of fecal pollution in the “Hot Spot” watersheds will be further evaluated in 2008 by ACEPD in partnership with GRU, the Alachua County Health Department, the City of Gainesville Public Works Department, and the Florida Department of Environmental Protection. The partnership will evaluate each “Hot Spot” independently which will allow for concentrated sampling in a location over a specified time period. Collectively, this work will include septic tank system surveys and inspections, integrity testing of the stormwater collection and wastewater collection systems and additional sampling to narrow in on the sources of contamination. A correction action plan will be developed if applicable.

6.0 High Frequency Short Duration Fecal Coliform Monitoring, Tumblin Creek Pilot Project December 2006

6.1 Introduction

Numerous strategies have been employed for baseflow fecal coliform monitoring, including monthly, every other month, and quarterly sampling. The results have significant variability and it has been difficult, if not impossible, to identify trends. In an effort to address the difficulties in interpreting fecal coliform data, Alachua County Environmental Protection Department (ACEPD) investigated an alternative sampling frequency recommended by the Florida Department of Environmental Protection (FDEP). This sampling protocol was used as an attempt to reduce temporal variability by high frequency (daily) and short duration (one to two weeks) sampling.

6.2 Materials and Methods

To decrease the effects of temporal variability, samples were collected at a high frequency over a short period of time. Fecal coliform samples were collected daily for four consecutive days for two consecutive weeks. To address the heterogeneous nature of fecal coliform colonies, three replicate grab samples (field duplicates) were collected from approximately mid-depth in flowing water at each sample site during each sampling event. The grab samples were collected one after the other and are referred to as replicates in this document. Samples were brought to Advanced Environmental Laboratories, Inc. in Gainesville, Florida and they were analyzed using the SM9222D membrane filtration method (APHA, 1998). Water velocity was measured with a Marsh McBirney FlowMate 2000 for flow calculations and sample location characteristics were recorded for each site.

Tumblin Creek was selected for a pilot study to evaluate the high frequency sampling technique. Four sample sites (Figure 6-1) were selected in the Tumblin Creek Watershed including TUMSW5, which has historically had high fecal coliform concentrations. All samples were collected in the dry season under very low flow conditions. To assess the variation between the three replicate samples collected at each site each day, the coefficient of variation was calculated. The coefficient of variation represents the ratio of the standard deviation to the mean, and is a useful statistic for comparing the degree of variation from one data series to another, even if the means are drastically different from each other. When the coefficient is greater than one there is high variance, and there is little variance when the coefficient is less than one.

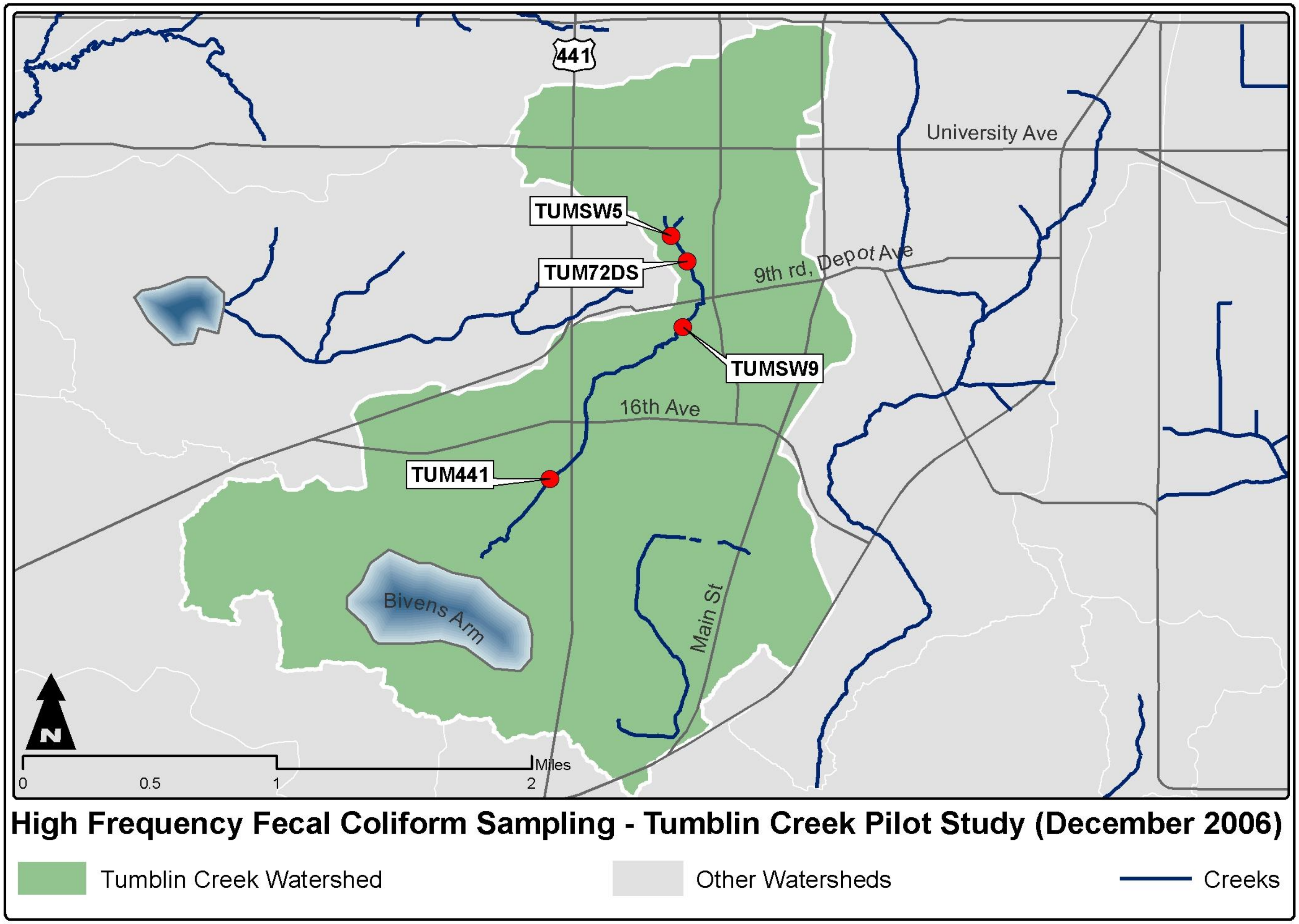


Figure 6-1. Locations of High Frequency Fecal Coliform Monitoring Sites for Tumblin Creek Pilot, December 2006

6.3 Results and Discussion

The first sampling event was conducted on December 11, 2006. Extended dilutions were run on all samples collected, except those obtained the first day. All values for those initial samples were reported as too numerous to count. Extended dilutions were run for all of the other sampling events, to ensure the fecal coliform counts could be quantified.

Fecal coliform counts ranged from 40 to 1,990 CFU/100 mL (Appendix Xa). The coefficient of variation for the three site samples per day ranged from 0.01 to 0.49. Since all of the values were less than one, this indicates that there was little variability among the replicate samples, which gave confidence in the laboratory analyses and our sample collection methods. The median, arithmetic mean and geometric mean of the replicates were also very close to each other. In earlier work ACEPD found median values to closely track the geometric mean, both measures of central tendency that are less influenced by extreme values. For comparison purposes arithmetic mean, median and geometric mean values are presented in Figure 6-2.

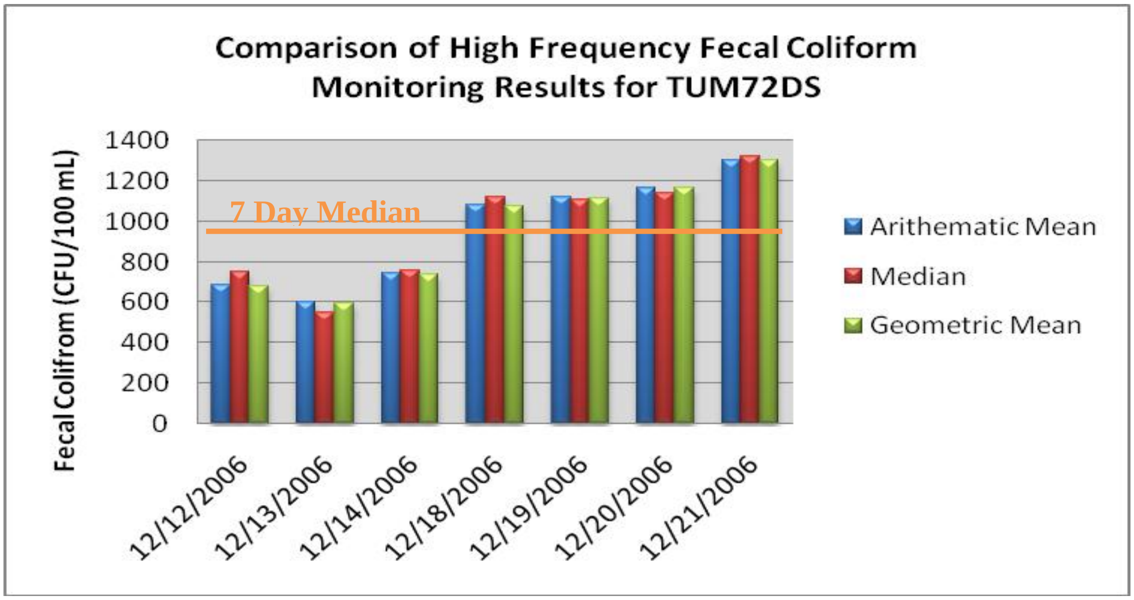


Figure 6-2. Arithmetic Mean, Median, and Geometric Mean Values for Fecal Coliform Samples Collected at TUM72DS, December 2006

Fecal coliform concentrations at each sampling site tended to vary on a daily basis. Average concentrations increased over time at TUM72DS. This site is a deeper pool directly downstream of a 72-inch stormwater culvert servicing the Gainesville urban area near the University of Florida. This site is upstream of the confluence with the main channel of Tumblin Creek. Average concentrations also increased over time at TUMSW5. This site is downstream of where Tumblin Creek surfaces from under the parking lots of Shands at Alachua General Hospital (AGH) and upstream of the confluence with water from the 72-inch culvert. Fecal coliform concentrations tended to decrease at TUMSW9 and TUM441, as the water levels continued to drop at these sites over time. Both of these sites are further downstream in the watershed. A steady increase in baseflow fecal coliform concentrations at the two sites where the creek emerges from the stormwater collection system, may indicate an illicit discharge or cross connection upstream in the system, however the goal of this study was to investigate sampling methods not sources.

One goal of this project was to characterize the fecal coliform levels under baseflow conditions at the sample sites. There was variability at all of the sites with TUMSW5 showing the greatest range, with a minimum value of 460 and a maximum value of 1,990 CFU/100 mL. To evaluate variance (dispersion) among replicate samples, the coefficient of variation (the ratio of the standard deviation to the mean) was calculated for each site for the overall sampling period. The coefficient of variation was less than one for each of the sites, indicating that the variability is low (Table 6-1). All of the replicates from each day were combined to obtain a site median (Figure 6-3). Combining values from a high frequency, short duration sampling event provides data which integrates the conditions at the site over a short period of time. When the site averages and/or medians were compared, it was clear that fecal coliform concentrations increased as one moved up the watershed during this time period. This may be due, in part, to increased flow in the creek as elevations decrease and springs and seeps from the surficial and intermediate aquifer systems enter the stream.

Table 6-1. Summary of All Samples Collected During Pilot Study

Site Name	Arithmetic Mean (CFU/100 mL)	Median (CFU/100 mL)	Geometric Mean (CFU/100 mL)	Coefficient of Variation
TUMSW5	1,007	890	924	0.42
TUM72DS	958	970	915	0.29
TUMSW9	594	550	508	0.55
TUM441	348	300	270	0.71

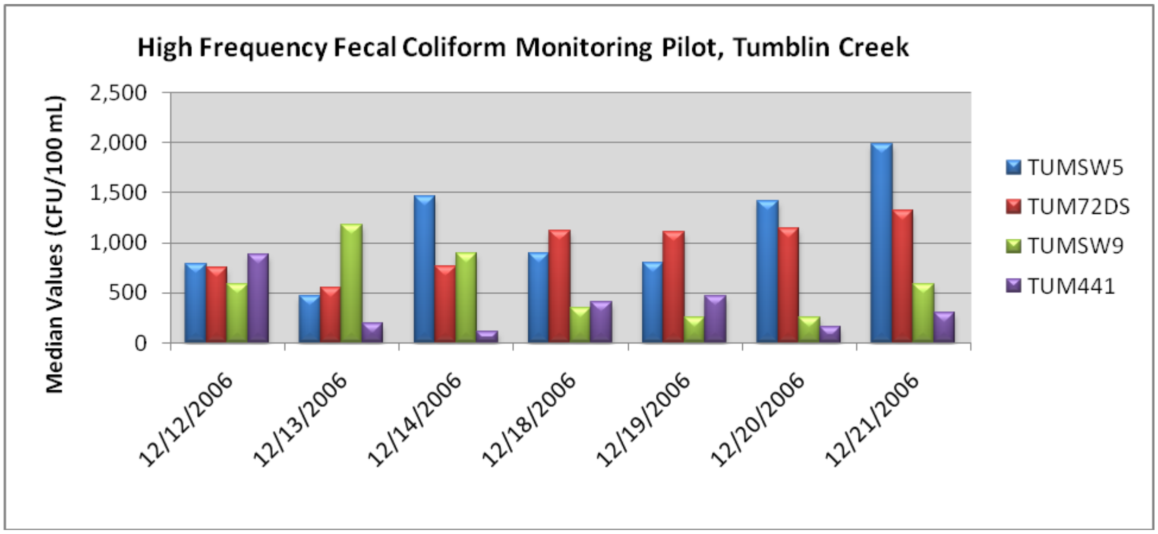


Figure 6-3. Median Fecal Coliform Values for the Tumblin Creek High Frequency Monitoring Pilot Project, December 2006

6.4 Summary and Conclusions

The replicate values had relatively low standard deviations and coefficients of variation, indicating that grab samples are representative of site conditions. The high precision of the replicates validates the laboratory analyses and sample collection techniques.

Replicate samples can confirm sampling anomalies and increase the accuracy of a sampling program. Fecal coliform concentrations varied widely at each individual site over the sampling period, which makes relying on one single observation unadvisable. However, it is not always economically feasible to collect replicate samples and laboratories can not always accommodate the high volume of samples within the specified holding time.

Collecting samples at a high frequency over a short period of time provides extensive data on site conditions during the duration of the study. These values may be more valuable then single day grab samples spread out over a year. This data set clearly characterizes fecal coliform concentrations in Tumblin Creek under baseflow conditions. This study should be repeated during the rainy season to compare the sites under differing weather conditions and expanded to the Sweetwater Branch and Hogtown Creek watersheds.

7.0 High Frequency Fecal Coliform Sampling and Sediments Analysis, January 2007

7.1 Introduction

Studies have shown that members of the fecal coliform group, *Escherichia coli, Enterococcus* spp., and *Clostridium perfringens* bacteria may be persisting and undergoing re-growth in the environment (Pisciotta et al., 2002 and Scott et al., 2002). If indicator organisms do not require the intestines of warm blooded animals for reproduction, then their presence is not indicative of recent fecal pollution. The sediments of water bodies in warm climates may be serving as reservoirs for bacteria detected in water samples. To address these possibilities, Alachua County Environmental Protection Department (ACEPD) explored the issue of bacteria persistence in the sediments by collecting sediments and water column samples during a high frequency fecal coliform sampling event in the Hogtown Creek, Tumblin Creek, and Sweetwater Branch watersheds.

7.2 Materials and Methods

To decrease the effects of temporal variability, water column samples were collected at a high frequency over a short period of time. Eight of ACEPD's routine sample sites were visited for four consecutive days starting January 2, 2007 (Figure 7-1). To address the heterogeneous nature of fecal coliform colonies, three replicate samples were collected at each sample site during each sampling event. Water column samples were collected at approximately mid-depth in flowing water and were analyzed for fecal coliform by Advanced Environmental Laboratories, Inc. in Gainesville, Florida using the SM9222D membrane filtration method (APHA, 1998). Water velocity was measured with a Marsh McBirney FlowMate 2000 for flow calculations and sample location characteristics were recorded for each site.

On January 2, 2007 sediment samples were collected at six sites within the Sweetwater Branch, Hogtown Creek, and Tumblin Creek watersheds (Figure 7-2). Surface sediments in pools and slower moving segments of the creeks were collected using a 125 mL sample container. The lid of the bottle was used to push the sediments into the bottle. All sediment analyses were conducted by Biological Consulting Services (BCS), Inc. in Gainesville, Florida. BCS added a phosphate-buffered solution to 1-5 grams (wet weight) of sediment. The samples were shaken for 30 minutes and then following 30 minutes of settling time, the supernatant was filtered and analyzed. The supernatant were analyzed for fecal coliform, *Enterococcus* spp., and *E. coli* on a CFU/1 gram wet weight basis. The samples were also analyzed for two human specific DNA markers: *Enterococcus faecium* (surface protein *esp* gene) and *Bacteroides* spp. (16S rRNA).

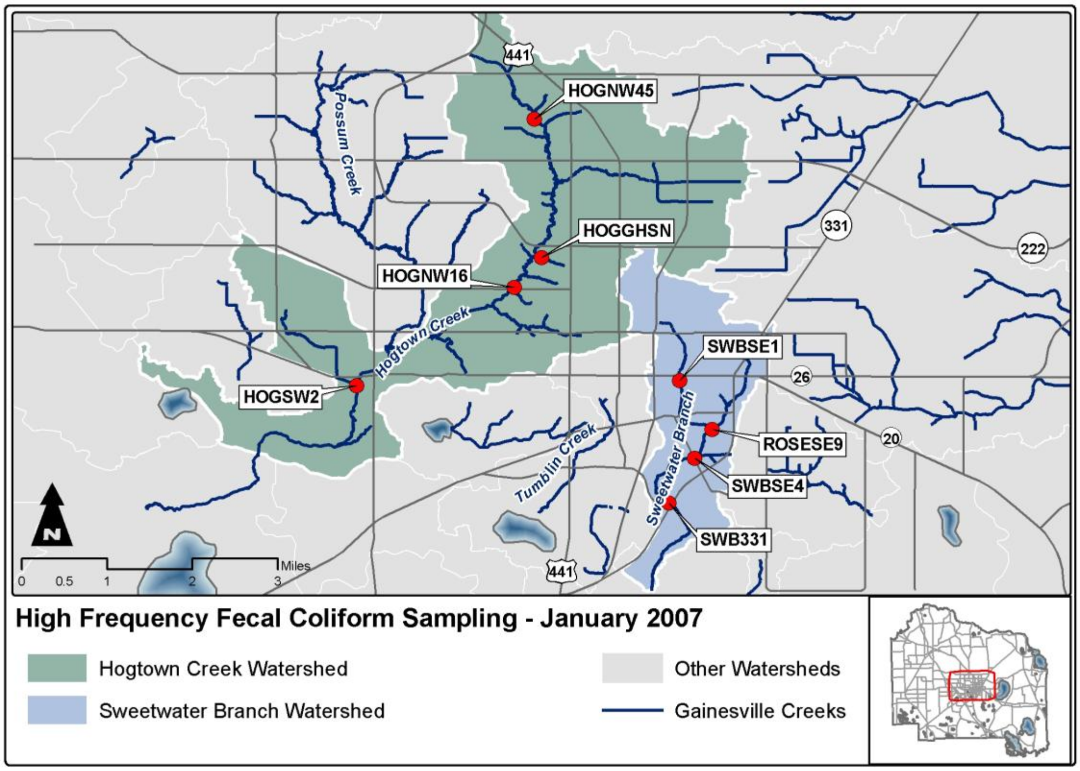


Figure 7-1. Locations of High Frequency Fecal Coliform Monitoring Sites for Water Samples Collected in January 2007

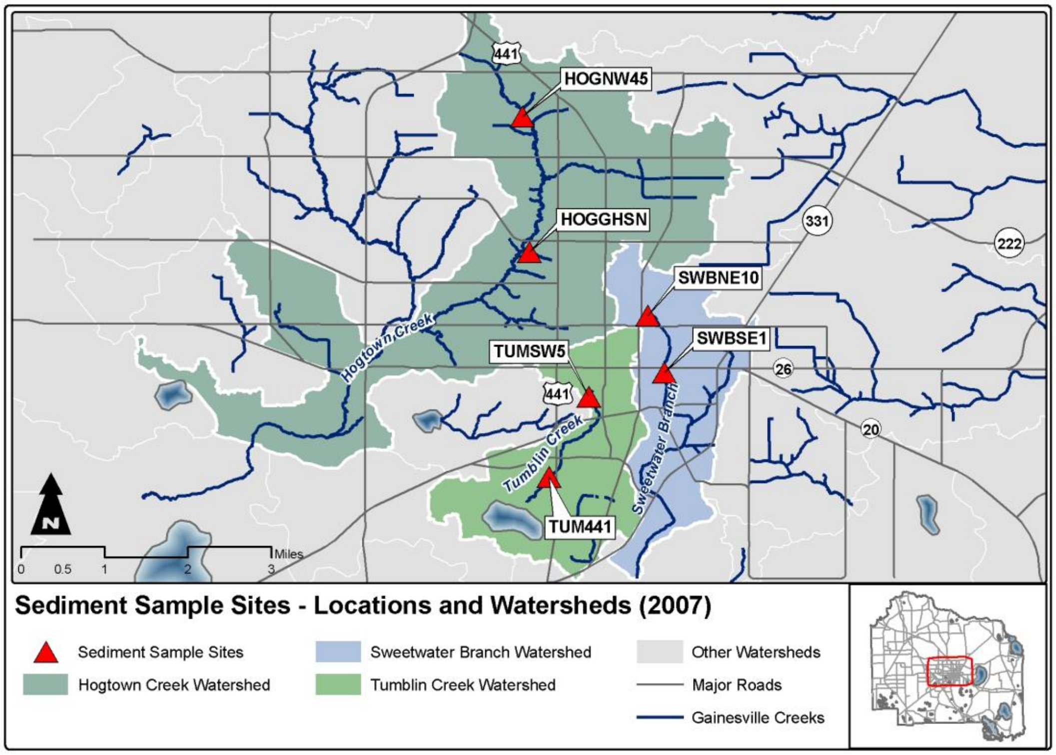


Figure 7-2. Locations of High Frequency Fecal Coliform Monitoring Sites for Sediment Samples Collected in January 2007

7.3 Results and Discussion

Similar to the results of the pilot study in Tumblin Creek (Section 6.0), the coefficient of variation was less than one for each set of replicates indicating low variability (Appendix Xb). The high precision of the replicates validates laboratory and sample collection techniques.

Fecal coliform concentrations in the water column were the highest at each site on January 3, 2007 (Figure 7-3 and Figure 7-4). This is likely due to the 0.39 inch rain event that occurred during the morning of the sampling event. On this date, 88% of the samples exceeded the fecal coliform one time maximum state water quality standard of 800 CFU/100 mL. On January 2, 2007 (one day after a 0.98 inch rain event), 50% of the samples exceed the state standard. Baseflow conditions may better be represented by the samples collected on 1/4/07 and 1/5/07, when only 17% and 29% of the samples exceeded the standard respectively.

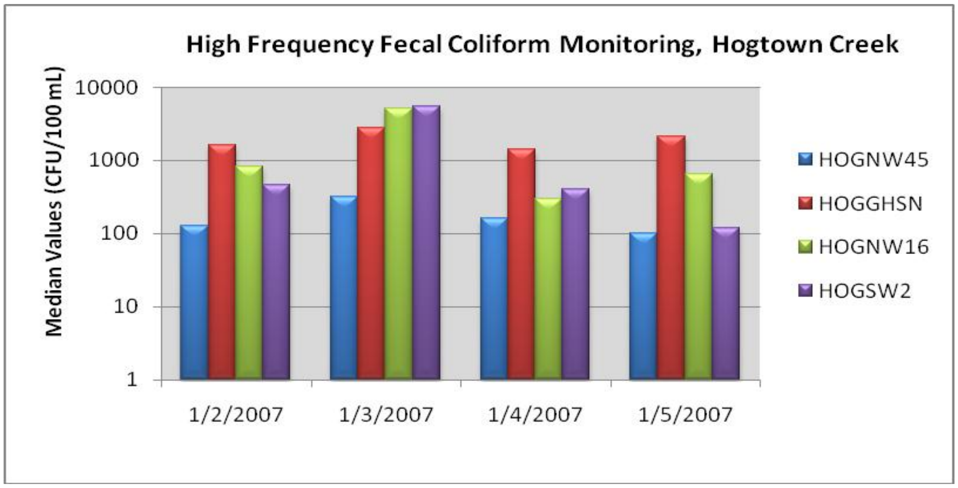


Figure 7-3. Median Fecal Coliform Values for Hogtown Creek Water Column Samples Collected in January 2007

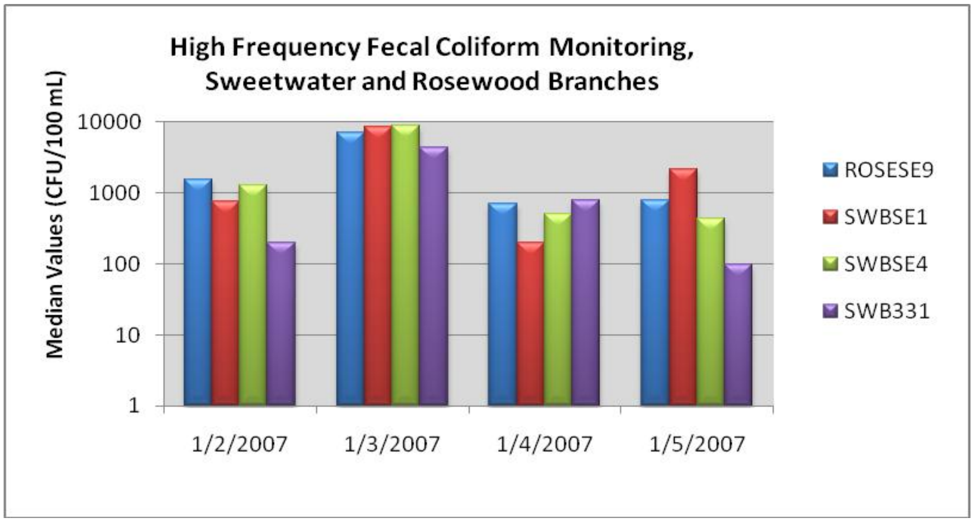


Figure 7-4. Median Fecal Coliform Values for Water Column Samples Collected in January 2007 in Sweetwater and Rosewood branches

The replicates from the four sample dates were averaged for each site and the coefficient of variation was calculated. Out of the eight sites, only two of them (HOGNW45 and HOGGHSN) had coefficients of variation less than one (Table 7-1). This indicates that the majority of the sites had significant variability from day to day. This is not surprising, since it is known that fecal coliform concentrations increase during and following rain events. These results suggest there is temporal variation at sites and that collecting samples on a single day may not give a complete picture of site conditions.

Table 7-1. Summary of All Samples Collected During High Frequency Monitoring, January 2007

Site Name	**Arithmetic Mean (CFU/100 mL)**	**Median (CFU/100 mL)**	**Geometric Mean (CFU/100 mL)**	**Coefficient of Variation**
ROSESE9	2,440	960	1,484	1.12
SWBSE1	2,858	1,440	1,313	1.18
SWBSE4	2,778	925	1,269	1.32
SWB331	1,377	430	517	1.33
HOGNW45	196	160	174	0.53
HOGGHSN	1,980	1,850	1,904	0.29
HOGNW16	1,742	660	910	1.24
HOGSW2	1,583	460	579	1.45

All three indicator bacteria were present in every sediment sample (Table 7-2). It is difficult to compare the concentrations in the sediment to the concentrations in the water column because the sediments have varying surface areas and the concentrations are reported per 1 gram of wet weight.

Table 7-2. Summary of Fecal Coliform, *Enterococcus* spp., *E coli*, *esp* gene, and *Bacteroides* spp. Results for Sediment Samples Collected on January 2, 2007

Site	**Fecal Coliform (CFU/1 g wet wt)**	***Enterococcus* spp. (CFU/1 g wet wt)**	***E. coli* (CFU/1 g wet wt)**	esp **gene ID PCR (+/-)**	***Bacteroides* spp. ID PCR (+/-)**
SWBNE10	150	6	64	Negative	Negative
SWBSE1	1,100	53	530	**Positive**	**Positive**
TUM441	2,000	34	110	Negative	Negative
TUMSW5	100	36	79	Negative	Negative
HOGNW45	80	79	32	**Positive**	Negative
HOGGHSN	210	70	19	Negative	Negative

Harwood (2008) recommends multiplying the data by 100 to convert it to per 100 gram of wet weight, so that it can be more easily compared to water column data (which is reported per 100 mL of water). The converted values were compared to the water column data from 2006 and 2007 (Table 7-3) for the sites in which data was available. Fecal coliform water samples were collected as part of the January 2007 sampling at SWBSE1, HOGNW45, and HOGGHSN. At these sites, fecal coliform concentrations were an order of magnitude greater in the sediments, indicating that sediments may be a significant source of bacteria.

Table 7-3. Fecal Coliform, *Enterococcus* spp., and *E coli* Results for Sediment Samples Collected on January 2, 2007 Compared to Water Column Medians

Site	**Fecal Coliform**		***Enterococcus spp.***		***E. Coli.***	
	Water Column (CFU/ 100 mL)	**Sediments (CFU/ 100 g wet wt)**	**Water Column (CFU/ 100 mL)**	**Sediments (CFU/ 100 g wet wt)**	**Water Column (CFU/ 100 mL)**	**Sediments (CFU/ 100 g wet wt)**
SWBNE10	5,600[a]	15,000	550 [a]	600	2,300 [a]	6,400
SWBSE1	1,440 [b]	110,000	NA	5,300	NA	53,000
TUM441	NA	200,000	NA	3,400	NA	11,000
TUMSW5	29,000 [a]	10,000	12,000 [a]	3,600	4,100 [a]	7,900
HOGNW45	160 [b]	8,000	NA	7,900	NA	3,200
HOGGHSN	1,850 [b]	21,000	9,900 [a]	7,000	7,500 [a]	1,900

a. median (n=3) of the samples collected in April 2006 (includes stormflow and baseflow)
b. median (n=12) of the samples collected during the week of January 2, 2007
NA = fecal coliform data not available for these time periods

The sediments at SWBSE1 had high concentrations of indicator bacteria and were positive for the *esp* gene and *Bacteroides* spp. human specific DNA markers. The presence of *Bacteroides* spp. can be indicative of recent contamination by human fecal matter (CH2MHILL, 2007). The source could be the homeless population in the area, a leak from the wastewater collection system, or an illicit discharge.

In the headwaters of Hogtown Creek, HOGNW45 was positive for the *esp* gene but negative for the *Bacteroides* spp. human specific DNA marker. This combination of results suggests there may be a residual (non-recent) source (CH2MHILL, 2007) of human sewage. Water column fecal coliform concentrations were low at this site ranging from 80 to 400 CFU/100 mL. Sediment *Enterococcus* spp. concentrations were the greatest at this site (79 CFU/1 g wet wt) compared to the other sites (6 to 70 CFU/1 g wet wt). *Enterococcus* spp. populations from a past septic tank failure or wastewater collection system release may be persisting in the sediments at this site, as indicated by the high concentrations of indicator bacteria.

7.4 Summary and Conclusions

The replicate samples collected during each sampling event had low coefficients of variation, indicating that grab samples are representative of site conditions. The temporal variability observed suggests that sites should be sampled under differing hydrologic conditions to characterize overall site conditions. The fecal coliform levels increased during storm events; which demonstrates the importance of providing auxiliary data when reporting coliform data. Not all of the values collected during this study represent baseflow conditions.

In the Hogtown Creek watershed, the station furthest upstream in the watershed (HOGNW45) had the lowest concentration of fecal coliform. Measures of central tendency for this site were all below 200 CFU/100 mL indicating water quality criteria for Class III recreational waters were met at this location. These data indicate that fecal pollution sources further downstream are responsible for elevated concentrations of bacteria in Hogtown Creek. Inflows and tributaries downstream should be evaluated in greater detail, including Springstead Creek and the east branch of Hogtown Creek. Levels of fecal coliform in Sweetwater Branch were highest in the upstream reaches, decreasing (likely due to dilution from increased flow) downstream of the Gainesville Regional Utilities (GRU) Main Street Water Reclamation Facility. Based on the variation in the overall data set, it is clear the some samples show the influence of stormflow conditions.

Indicator bacteria were present in all of the sediment samples. It is difficult to directly correlate the sediment concentrations with the water column concentrations to determine if the sediments are a source. By converting the units of measure, the sediment data can be more readily compared to water column data. However, one must recognize that the data is not a direct comparison because the surface area and grain size of the sediments is not taken into account. Human specific DNA markers were found in the sediments at two of the six sites where sediment samples were collected. Since water column samples were not analyzed for the human specific DNA markers, it is difficult to determine if the source was the sediments or an external source.

8.0 Wet Season High Frequency Short Duration Fecal Coliform Monitoring, July 2007

8.1 Introduction

In the relatively dry months of December 2006 (Section 6.0) and January 2007 (Section 7.0) high frequency short duration fecal coliform monitoring events were conducted by Alachua County Environmental Protection Department (ACEPD). The goal of the projects was to minimize temporal variability and to characterize fecal coliform concentrations at the sample sites during the dry season. In July 2007 the sample sites were revisited using the high intensity short duration monitoring strategy; results are presented in Appendix Xc. The goal was to compare the results obtained during the wet season to those of the dry season.

8.2 Materials and Methods

Most of the sites sampled in the previous high frequency short duration studies were also sampled for this study (Figure 8-1). Some additional sites were added based on the 2007 microbial source tracking (MST) study (Section 5.0). Fecal coliform samples were collected at each site for three consecutive days beginning on 7/24/2007. To address the heterogeneous nature of fecal coliform colonies, two replicate samples were collected at each sample site during each sampling event. Three replicates were collected in the previous study, but due to the increase in the number of sites the laboratory could not process three samples per site for this study. Water column samples were collected at approximately mid-depth in flowing water and were analyzed for fecal coliform by Advanced Environmental Laboratories, Inc. in Gainesville, Florida within six hours of collection using the SM9222D membrane filtration method (APHA, 1998). Turbidity was measured with a Hach2100P turbidimeter. Water velocity was measured with a Marsh McBirney FlowMate 2000 for flow calculations and sample location characteristics were recorded for each site.

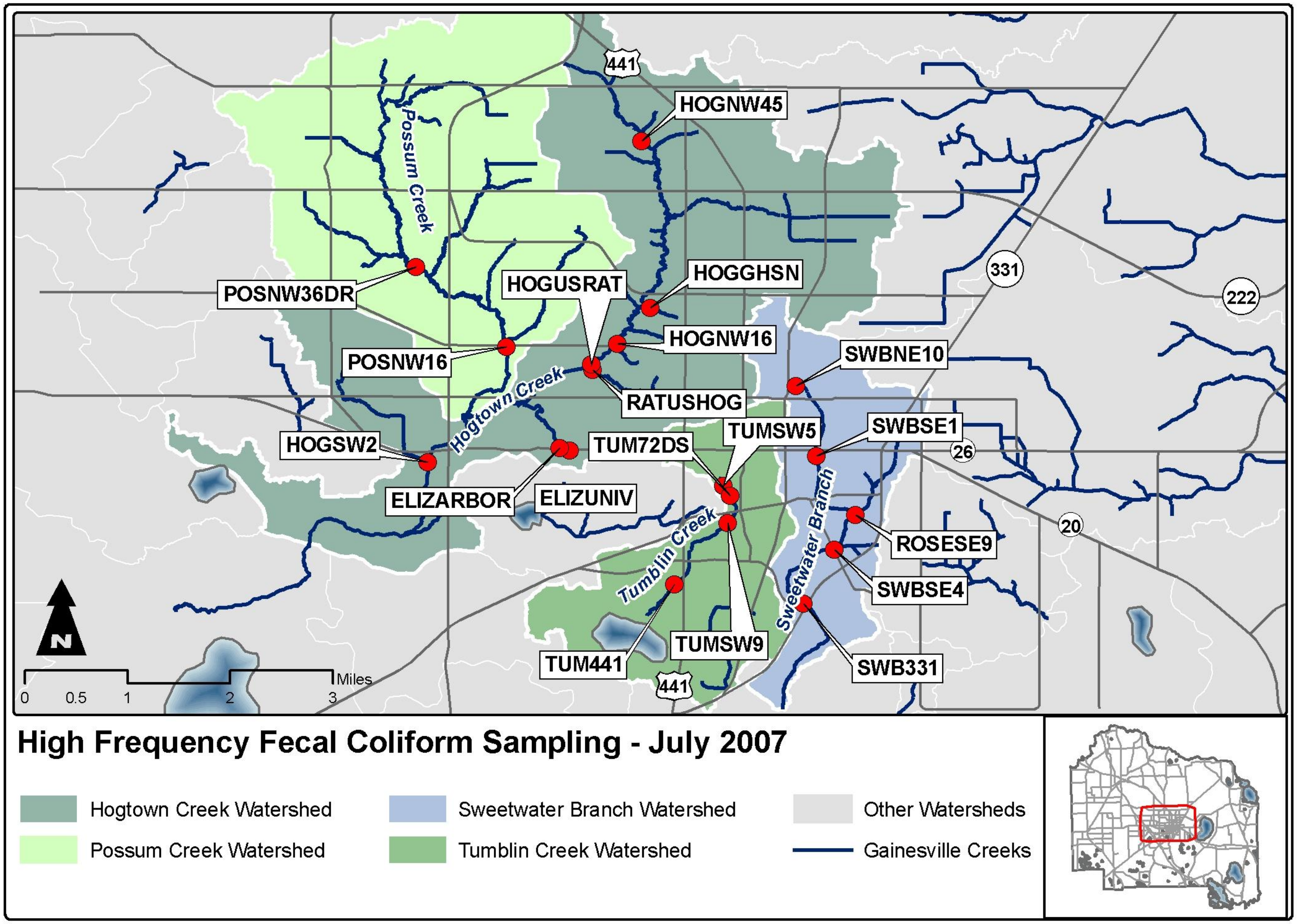


Figure 8-1. Locations of High Frequency Fecal Coliform Monitoring Sites for July 2007

8.3 Results and Discussion

There was a rain event on 7/24/2007 prior to the collection of the samples. The sites appeared to be under stormflow conditions with turbidity values ranging from 0.50 to 28.88 NTU. Fecal coliform concentrations were the highest at most sites on this day (Table 8-1 and Figure 8-2). The sites appeared to be back under baseflow conditions on 7/25/2007 with turbidity values ranging from 0.63 to 1.88 NTU. There was a rain event after the sampling on 7/25/2007 which appears to have variably affected the watersheds during the 7/26/2007 sampling. Turbidity values ranged from 0.78 to 4.63 NTU. Tumblin Creek and some areas of Hogtown and Possum creeks had elevated fecal coliform concentrations. The summer rains are variable, some parts of town will receive considerable rainfall while other areas will be unaffected. Rain gages would be needed in each watershed to compare rainfall data and its affects on the creeks.

Table 8-1. Median Fecal Coliform Values for All Samples Collected within Each Watershed for July 2007

Watershed	**7/24/2007**		**7/25/2007**		**7/26/2007**	
	Fecal Coliform (CFU/100 mL)	**Coefficient of Variation**	**Fecal Coliform (CFU/100 mL)**	**Coefficient of Variation**	**Fecal Coliform (CFU/100 mL)**	**Coefficient of Variation**
Tumblin Creek	19,700	0.20	3,400	0.97	11,300	0.57
Sweetwater Branch	5,850	0.64	1,100	0.64	930	1.66
Hogtown Creek	2,600	0.99	1,100	0.79	6,153	0.63
Elizabeth Creek	17,750	0.09	10,950	0.18	8,550	0.08
Possum Creek	560	0.44	695	0.80	1,600	0.60

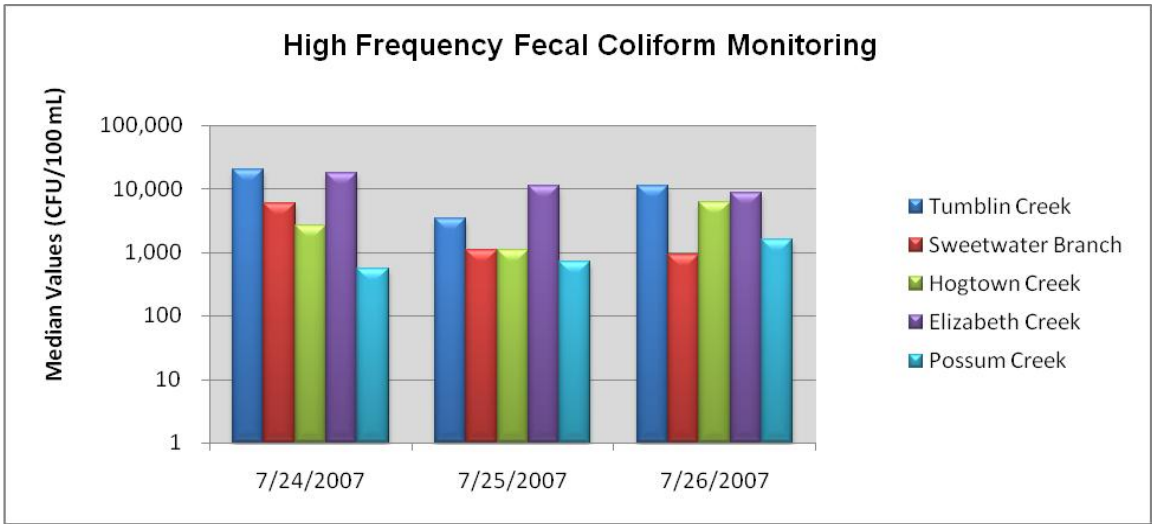


Figure 8-2. Median Fecal Coliform Values for All Samples Collected within Each Watershed for July 2007

The coefficients of variation of the sites within each watershed for each day were calculated (Table 8-1). Almost all of the coefficients were less then one, suggesting that there is not much variability among the sites within each watershed. Sweetwater Branch

(7/26/2007 under varied rainfall conditions) and Hogtown Creek (7/24/2007 under stormflow conditions) had coefficients of 1.66 and 0.99 respectively, indicating variability among the various sample site locations. The variability in Sweetwater Branch is likely due to the high fecal coliform value (13,400 CFU/100 mL) at SWBNE10 on 7/26/2007. This site often has higher levels than the other Sweetwater Branch sites and may be impacted by intermittent sources. An un-named tributary (HOGGHSN) of Hogtown Creek was sampled as part of the Hogtown Creek Watershed and the resulting elevated fecal coliform levels at this site (15,300 and 11,800 CFU/100 mL) contribute to the watershed variability.

The wet season fecal coliform site medians collected in this study were compared to the dry season data (Figure 8-3 and Table 8-2). The dry season Tumblin Creek data was collected under very low baseflow conditions and each median represents a sample set of 21 samples. The dry season Rosewood and Sweetwater Branch and Hogtown Creek sites were affected by a storm event. However, since median values are reported, the data was not strongly influenced by the event. Each median was calculated using the twelve samples collected over the four days at each site. The wet season data was influenced by storm events and each median was calculated using the six samples collected over the three days at each site.

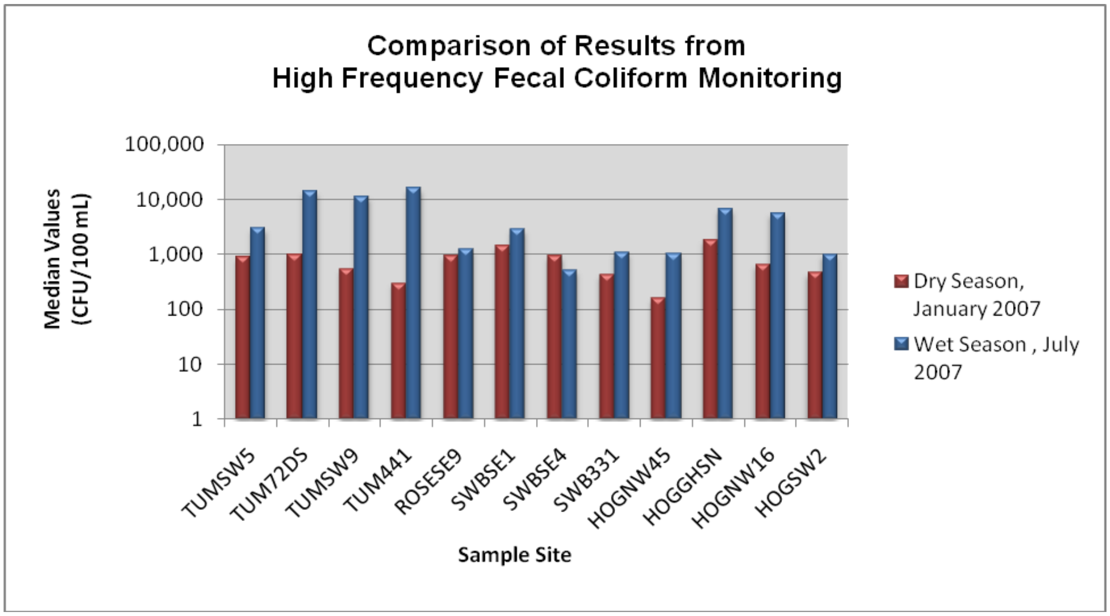


Figure 8-3. Comparison of Median Fecal Coliform Values for Wet and Dry Season Sampling Events in 2007

Table 8-2. Comparison of Median Fecal Coliform Values for Wet and Dry Season Sampling Events in 2006 and 2007

	Dry Season		Wet Season	
Site ID	**Median (CFU/100 mL)**	**Number (n)**	**Median (CFU/100 mL)**	**Number (n)**
TUMSW5	890	19	3,000	6
TUM72DS	970	21	14,000	5
TUMSW9	550	21	11,300	6
TUM441	300	21	15,850	6
ROSESE9	960	12	1,220	6
SWBSE1	1,440	12	2,850	6
SWBSE4	925	12	520	6
SWB331	430	12	1,100	6
HOGNW45	160	12	1,045	6
HOGGHSN	1,850	12	6,700	6
HOGNW16	660	12	5,650	6
HOGSW2	460	12	1,000	6

On 7/26/2007 an illicit discharge of chlorinated pool water was located directly upstream of TUM441. The chlorine residual in the creek was measured as being greater than 2.2 mg/L using a hand held Hach colorimeter 46700-11. Fecal coliform was not detected in the water column samples on this day, due to the high concentration of chlorine.

The wet season fecal coliform counts were elevated compared to the dry season values. The Tumblin Creek Watershed showed the greatest increase. This is likely due to the urban nature of this creek and the large volume of stormwater it receives. Sweetwater Branch is also a highly urbanized watershed and it is surprising that the coliform counts were not much higher during the wet season study. The predominant landuse in the Hogtown Creek Watershed is low density residential. The elevated wet season fecal coliform counts in this watershed could be due to pet wastes flushed from lawns during the storm events.

8.4 Summary and Conclusions

Fecal coliform counts were elevated during the wet season high frequency short duration sample events compared to the dry season values. At several of the stations, the wet weather results were an order of magnitude greater than the baseflow results. The wet weather results tended to be especially high in the urbanized Tumblin Creek Watershed. However, it is important to note that the sample size was lower in the wet season study. It was helpful to have the turbidity values to aid in distinguishing between baseflow and stormflow samples. ACEPD plans to collect turbidity samples for each site during future fecal coliform monitoring events.

Possum Creek had the lowest concentrations of fecal coliform bacteria in the wet season in 2007. Sweetwater Branch showed the least difference between dry and wet season levels of fecal coliform among the watersheds. Tumblin Creek showed the greatest difference in dry and wet season fecal coliform. Wet season levels of fecal coliform exceeded those observed under dry conditions at all sites, except for SWBSE4.

The relatively low coefficients of variation for each watershed, suggest there is not much variation among the sites. The number of sites in each watershed could be decreased and the cost savings could be used to sample the reduced list of sites more frequently. A disadvantage of this monitoring scheme is that it may be more difficult to locate areas affected by intermittent discharges if the number of sample locations is decreased.

9.0 Summary and Recommendations

9.1 Summary

Alachua County Environmental Protection Department (ACEPD) has utilized many of the human specific tools in the microbial source tracking (MST) toolbox to determine the sources of fecal coliform contamination at the identified "Hot Spots" in Tumblin Creek, Sweetwater and Rosewood branches, Hogtown and Possum creeks, Rattlesnake Branch, Elizabeth Creek, and Lake Forest and Little Hatchet creeks (Figure 1-2). Although it is still not completely clear as to which sources are affecting each "Hot Spot", much has been learned from the studies described in this report.

Using fecal coliform data alone, one cannot determine the sources of fecal coliform contamination. ACEPD has been monitoring fecal coliform concentrations since the 1970's, and it is important to continue to monitor long term fecal coliform trends as long as this group is recognized as the indicator organism for microbial water quality in Florida. The Florida Department of Environmental Protection (FDEP) has set pollutant loading targets, also called Total Maximum Daily Loads (TMDLs), for fecal coliform bacteria on Tumblin Creek, Sweetwater Branch, and Hogtown Creek. The TMDLs will be met through the implementation of the Orange Creek Basin Management Action Plan (BMAP). As part of the BMAP requirements, ACEPD will monitor the urban creeks for fecal coliform, turbidity, total suspended solids, and total organic carbon. Sites are sampled four to five times a year under flowing conditions. Additional parameters, including nutrients, are collected at some sites. For a list of sampling stations and parameter coverage see Appendix XI.

ACEPD utilized fluorescent whitening agents (FWAs) in three studies (Sections 3, 4, and 5) as a qualitative indicator of the presence of laundry wash water at the fecal coliform "Hot Spots." FWAs were detected at the positive control sites downstream of the permitted discharge of effluent from water reclamation facilities. However, FWA results did not correlate well with MST results and fecal coliform concentrations. It may be that the laboratory analysis is not sensitive enough to detect FWAs at the concentrations present in the urban creeks. FWAs did not prove to be an effective screening tool for identifying candidate sites for MST tests, and ACEPD will discontinue their use as a possible fecal contamination indicator.

Clostridium perfringens was found to be in low concentrations when compared to the other indicators throughout the watersheds, as one would expect. *Enterococcus* spp. results were relatively high in all of the watersheds, with no specific trends identified. As a whole, these additional bacteriological indicators did not provide additional information into the sources of elevated fecal coliform bacteria at the "Hot Spots." ACEPD will not include *C. perfringens* and *Enterococcus* spp. in its ambient surface water monitoring network, but will continue to monitor for fecal coliform since there is an applicable state standard. ACEPD will also collect samples for *E. coli,* based on Dr. Harwood's (2008) advice that comparing fecal coliform and *E. coli* concentrations from monthly sampling may help distinguish recent discharges to the environment from residual sources.

MST results indicate that there are human sources of fecal contamination in all of the "Hot Spot" watersheds (Sections 4 and 5) except for Lake Forest Creek. However, combining the MST results with fecal coliform data, FWAs, and alternative microbial indicators often led to uncertainty. As illustrated in Figure 9-1, considerable microbial source tracking has been conducted in some watersheds while there is minimal data for others. Looking at the ACEPD MST data alone, one would likely explore non-human sources in the Lake Forest Creek, Rosewood Branch, and Possum Creek watersheds and human sources in the remaining watersheds. The positive human specific DNA marker results in the Little Hatchet Creek Watershed are thought to be a result of the discharge of the permitted effluent from the Brittany Estates Water Reclamation Facility. Little Hatchet Creek was re-sampled in August 2007 and results were negative for the human markers.

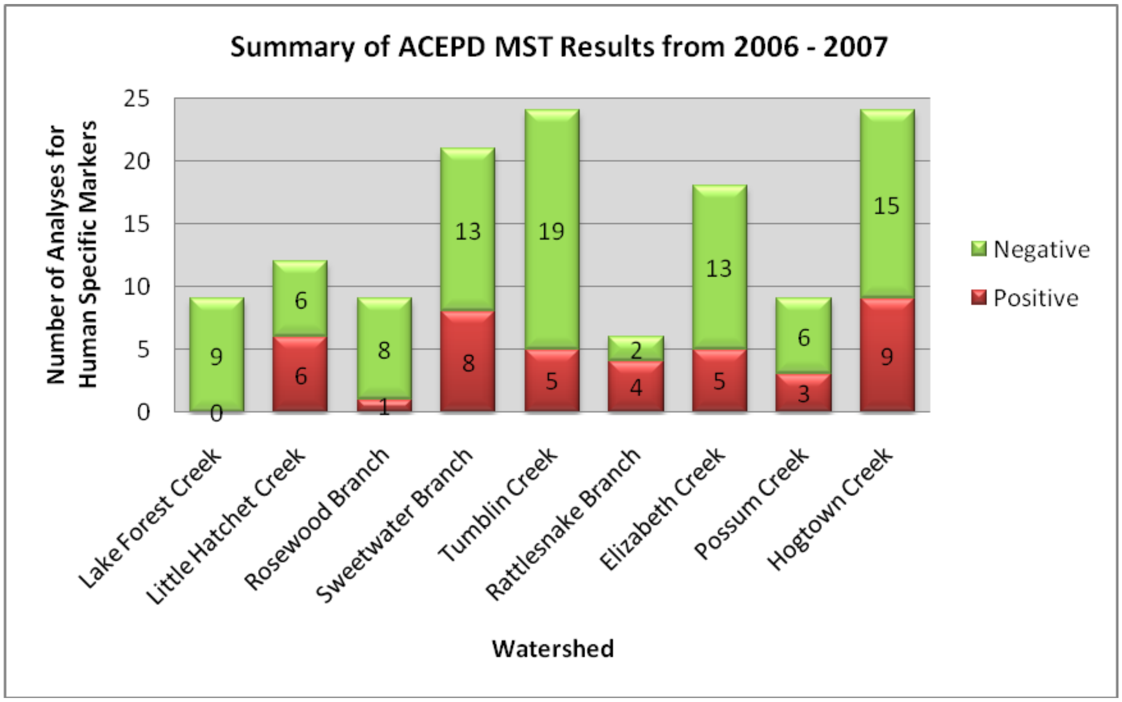


Figure 9-1. Summary of ACEPD MST Results from 2006 and 2007

To increase the sample set size, ACEPD and Gainesville Regional Utilities (GRU) MST results (CH2MHILL 2007) were combined (Figures 9-2 through 9-7). The GRU study was a three phased sampling program that incorporated innovative MST techniques. During the first phase "Hot Spots" were located by sampling for fecal coliform, total coliforms, and *Enterococcus* spp. A detailed MST pilot was implemented at three high priority sites for phase two. The final phase was a focused MST sampling of 10 "Hot Spots". The human specific indicators included in the following figures include *Enterococcus* spp., polyomaviruses, *Bacteroides* spp., and the enteric virus. Appendix XII contains comprehensive tables, including results from MST analyses conducted by ACEPD and GRU and septic tank surveys conducted by ACHD.

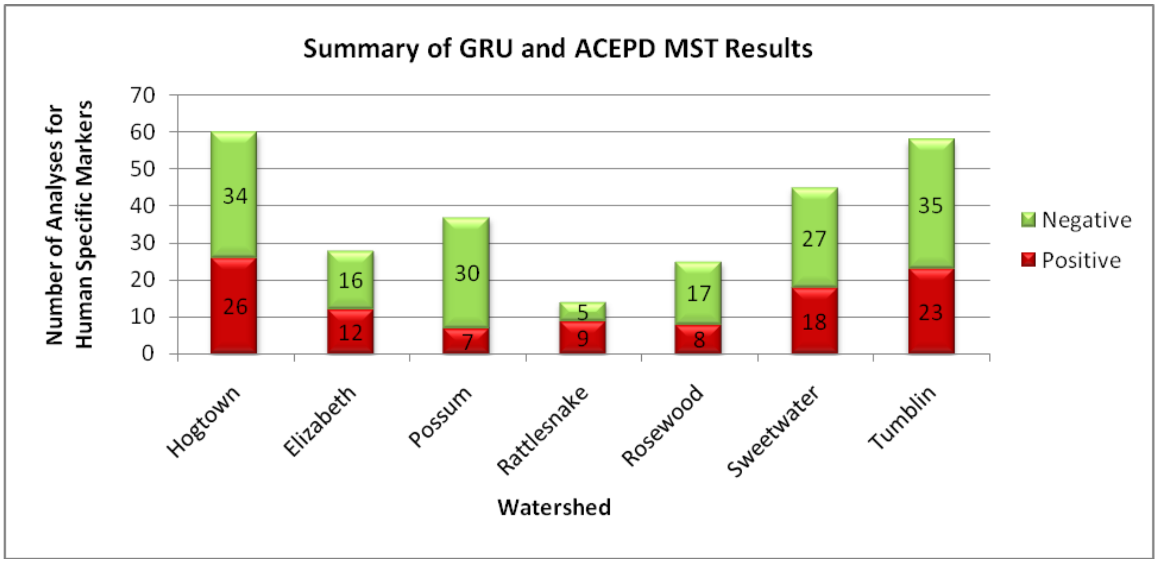


Figure 9-2. Summary of MST Results from GRU and ACEPD

It is important to recognize that MST is an emerging field that continues to develop with time, and interpretation of results may not be straightforward. Human specific DNA markers only represent a single gene, constituting a very narrow view of occurrence of human fecal contamination in the environment. Furthermore, very small samples are analyzed relative to the large volume that constitutes a water body, therefore representative sampling is problematic. It is important to note that a negative result for a human marker may only mean that the individual marker tested for was not detected, not that there is no risk of human fecal contamination of the waterbody. Due to the expense of MST analyses, it was not feasible in these studies to run duplicate samples or to continuously sample in one location to verify results. That being said, MST methods and a well designed sampling plan may be the best available analytical techniques for determining sources of fecal contamination at this point in time.

Several limitations of the MST studies conducted have been recognized by ACEPD. The use of validation strategies (Stoeckel and Harwood, 2007) and negative and positive controls are essential in development and implementation of MST sampling plans. There are numerous variables that must in some way be accounted for in the field sampling and laboratory analyses: hydrologic conditions, episodic discharges, spatial variability, DNA recovery efficiency, and PCR amplification efficiency. All of these variables were recognized by Stoeckel and Harwood (2007) to have a profound influence on the interpretation of MST results. Furthermore, current presence/absence methods for PCR cannot provide information about the proportional contribution of various sources to overall loading of fecal contaminants.

ACEPD experimented with a high frequency short duration sampling regime (Sections 6.0 and 7.0) to characterize site conditions. The robust data set associated with this sampling regime was more useful for statistical analysis then periodic grab samples. However, it is expensive and time consuming to collect such a high volume of samples. One goal of ACEPD's ambient monitoring program is to detect illicit discharges. By evaluating monthly *E. coli* and fecal coliform sampling, ACEPD is hoping to increase the likelihood of detecting illicit discharges. Additionally, ACEPD will collect surface water samples for nutrients and selected inorganic indicators four to five times per year to monitor trends in water quality.

The high precision of replicate samples (Sections 6.0, 7.0, and 8.0) validates the laboratory analyses and sample collection techniques. Replicate samples can confirm sampling anomalies and increase the accuracy of a sampling program, but ACEPD can not justify the expense of collecting duplicate samples at every site. Additionally, the high volume of samples generated when duplicate samples are collected is problematic for the laboratory conducting the analyses. Duplicate samples will likely be collected in the future when sampling is required to verify illicit discharges or support enforcement actions.

ACEPD explored the concentration of indicators in sediments (Sections 5.0 and 7.0) since it has been suggested that sediments may be serving as a reservoir of bacteria. Indicator bacteria were present in the sediments and human specific DNA markers were detected in several of the samples. It was difficult to correlate sediment data with elevated fecal coliform concentrations in the water column. Although sediments are transported downstream in storm events, it is likely that sediment sample results are less transient than corresponding water column samples from continuously flowing creeks. However, ACEPD did not collect duplicate sediment samples or continuously sample in one location to verify results. Similar to water column samples, sediment samples are heterogeneous and there may be great spatial variability. ACEPD does not plan to continue to analyze sediment samples as part of the ambient monitoring program.

ACEPD recognizes the shortcomings of the various indicators of fecal pollution; however, the studies conducted did provide valuable insight into the possible sources. The following discussion details ACEPD's source hypotheses for each of the "Hot Spots." It is important to recognize all of the MST results are from single grab samples and further investigations would be necessary to confirm results and hypotheses.

9.1.1 Tumblin Creek

In Tumblin Creek, human specific markers were detected in 23 out of 58 samples (40%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-3). It is suspected that the main sources of fecal contamination may be from wastewater collection systems and/or waste from domestic and wild animals in the watershed. There are private wastewater collection systems along with the Gainesville Regional Utilities (GRU) collection system in this watershed. ACHD identified two suspect (horizontal setback to creek less than 75 feet and/or vertical setback of less than 24 inches to the seasonal high water table) septic systems adjacent to Tumblin Creek (Alachua County Health Department, 2007). Other likely sources of fecal contamination are the birds, feral cats, raccoons, and dogs frequently observed in this watershed. It is interesting that human markers were detected in only one out of 13 samples at TUMSW5, since fecal coliform concentrations are often elevated at this site. The detection of human specific DNA markers appears to increase downstream of Depot Ave (TUMDEPOT, TUMSW9, TUMPKYonge and TUMSW11), however additional sampling is needed to narrow in on the sources in this watershed.

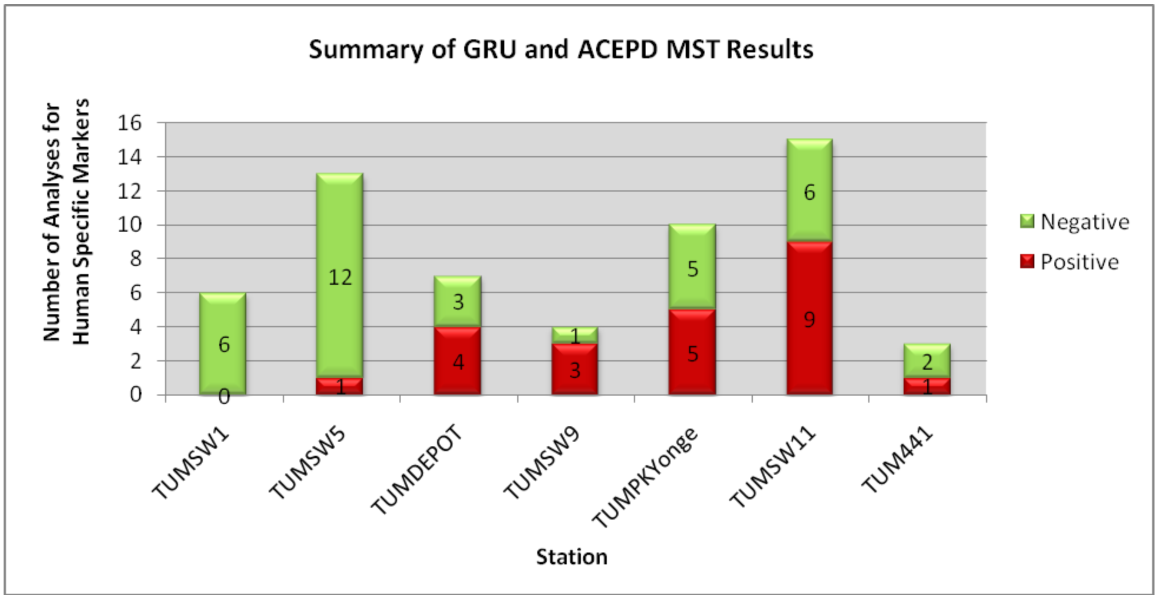


Figure 9-3. Summary of MST Results from GRU and ACEPD for Tumblin Creek
*The station IDs are ACEPD's designations. Some of GRUs sites were slightly upstream or downstream of ACEPD's sites, but were combined here for comparison purposes.

9.1.2 Sweetwater Branch

In Sweetwater Branch human specific DNA markers were detected in 18 out of 45 samples (40%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-4), which indicates that human fecal matter is likely a source of contamination. One possible source is the aging wastewater collection system along the creek, of which several sanitary sewer overflows have been reported over the years. Another potential source is the transient human population that camps along the banks of the creek north of NW 14th Avenue and in Sweetwater Park (SWBSE1). Discarded toilet paper has been observed along the banks in these areas. ACHD identified 11 suspect (horizontal setback to creek less than 75 feet and/or vertical setback of less than 24 inches of the seasonal high water table) septic systems adjacent to Sweetwater Branch upstream of SWBLF (Alachua County Health Department, 2007). Additionally, hydrodynamic separators have been installed along the creek to trap sediments. These moist sediments may serve to harbor bacteria and promote growth in the warm dark environment. Several samples downstream of the hydrodynamic separators have shown elevated fecal coliform bacteria levels.

In Rosewood Branch human markers were detected in 8 out of 17 samples (32%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-4). There are known septic tanks in this area, and a faulty one was located by the Alachua County Health Department and repaired in 2006. It is likely that septic systems and/or wastewater collection systems are a source of fecal contamination in this watershed. However, the *esp* gene was often detected in samples. The presence of the *esp* marker indicates that the source may be originating from a source other then septic systems, since this marker is not normally detected in the effluent of septic systems (Harwood, 2008).

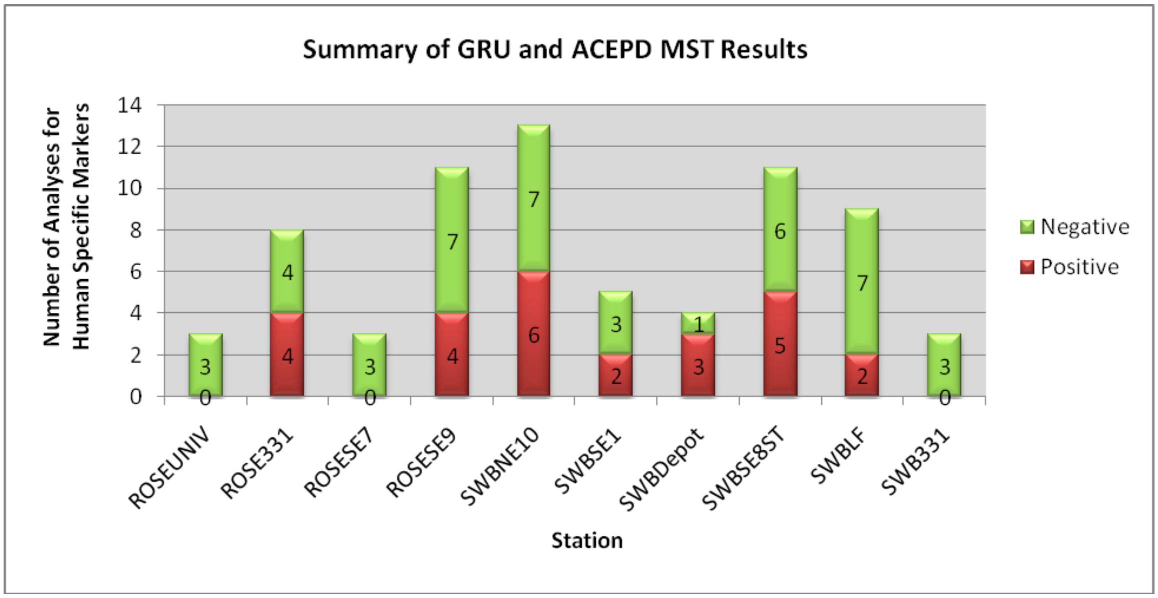


Figure 9-4. Summary of MST Results from GRU and ACEPD for Sweetwater and Rosewood branches

*The station IDs are ACEPD’s designations. Some of GRUs sites were slightly upstream or downstream of ACEPD’s sites, but were combined here for comparison purposes.

9.1.3 Hogtown Creek

In Hogtown Creek human specific DNA markers were detected in 26 out of 60 samples (43%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-5), indicating there are human sources in the watershed. Parts of the centralized wastewater collection system are located adjacent to the creek and may be compromised as the creek erodes the surrounding banks; additionally there are private wastewater collection systems in the Hogtown Creek Watershed. ACHD identified 13 suspect (horizontal setback to creek less than 75 feet and/or vertical setback to the seasonal high water table less than 24 inches) septic systems adjacent to Hogtown Creek between HOGNW53 and HOGNW8 (Alachua County Health Department 2007). Human markers appear to be more prevalent at HOGNW23, HOGNW16, and HOGUSRAT. However, these sites were also sampled at a greater frequency than the remaining sites. The unnamed tributary north of Gainesville High School often has fecal coliform concentrations indicative of an illicit discharge. It is interesting that there was only one positive human marker result out of the nine samples collected in this tributary (HOGGHSN and HOGGHSNASH). Feral cats and other wild animals observed in this watershed may be a dominant source of bacteria.

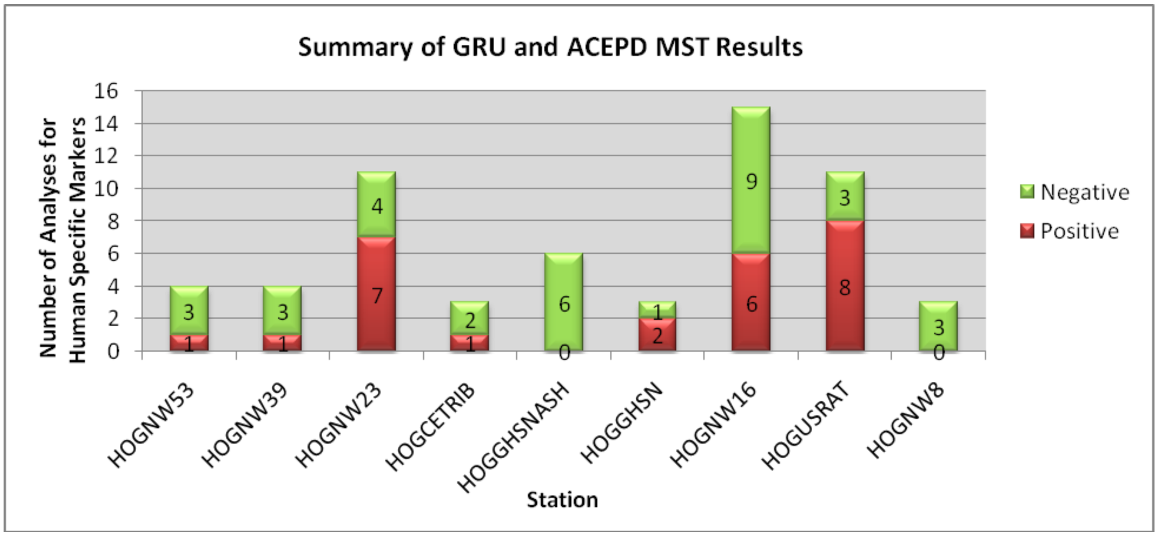


Figure 9-5. Summary of MST Results from GRU and ACEPD for Hogtown Creek
*The station IDs are ACEPD's designations. Some of GRUs sites were slightly upstream or downstream of ACEPD's sites, but were combined here for comparison purposes.

9.1.4 Possum Creek

In Possum Creek human specific DNA markers were detected in 7 out of 37 samples (19%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-6), indicating non-human sources may be the major contributor of fecal coliform bacteria. Like all of the urban creeks, Possum Creek provides refuge for wildlife which may be a source of bacteria. Pet waste from surrounding residential areas is another likely source of fecal contamination.

In Rattlesnake Branch human specific markers were detected in 9 out of 14 samples (64%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-6), indicating that human sources are very prevalent in this watershed. The centralized wastewater collection system in this area is located along the creek and crosses it in several locations. Sewage odors are often reported in this watershed and are attributed to gas released from manholes. It is possible that this infrastructure and/or private sewer connections in the watershed are the source of fecal coliform contamination. ACHD identified 2 suspect (horizontal setback to creek less than 75 feet and/or vertical setback to seasonal high water table less than 24 inches) septic systems adjacent to Rattlesnake Branch (Alachua County Health Department 2007). However, the *esp* gene was present in every sample it was analyzed for, indicating septic systems are likely not the source of human fecal contamination.

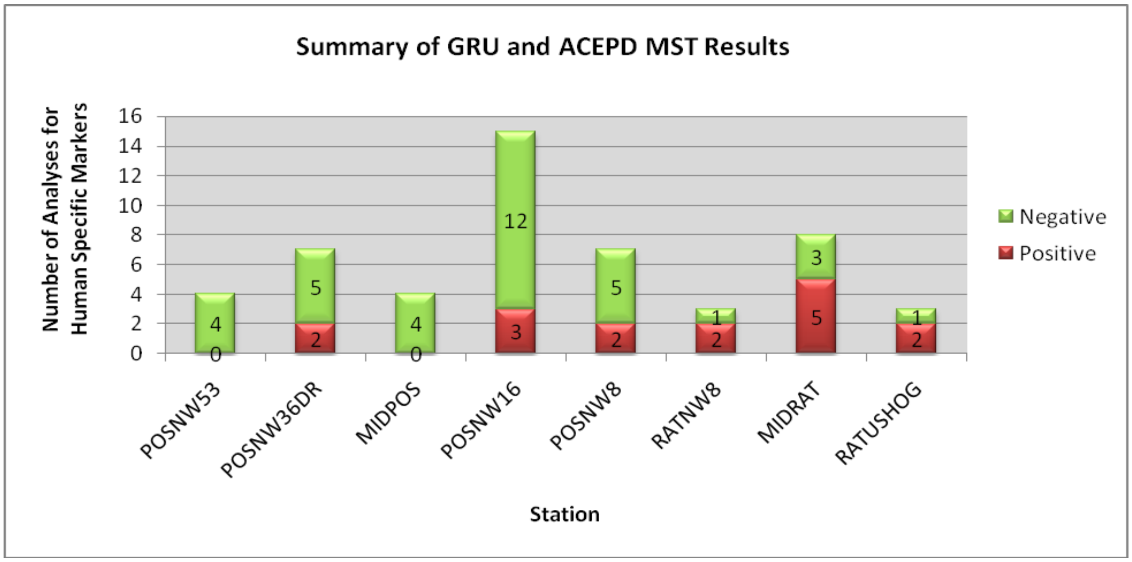


Figure 9-6. Summary of MST Results from GRU and ACEPD for Possum Creek and Rattlesnake Branch

*The station IDs are ACEPD's designations. Some of GRUs sites were slightly upstream or downstream of ACEPD's sites, but were combined here for comparison purposes.

9.1.5 Elizabeth Creek

In Elizabeth Creek human specific DNA markers were detected in 12 out of 28 samples (43%) collected by GRU and ACEPD between 2004 and 2007 (Figure 9-7), indicating human sources are present in the watershed. Failing wastewater collection system infrastructure has been a historic problem in the Elizabeth Creek Watershed as indicated by the dated warning sign located at ELIZNW7. Several neighborhoods in this watershed have been converted to student housing. It is possible that sewer lines from housing retrofits have been tied into the stormwater collection system and are a source of fecal coliform bacteria. ACHD did not identify any suspect (horizontal setback to creek less than 75 feet and/or vertical setback to seasonal high water table less than 24 inches) septic systems adjacent to Elizabeth Creek (Alachua County Health Department 2007). However, there are septic systems in this watershed.

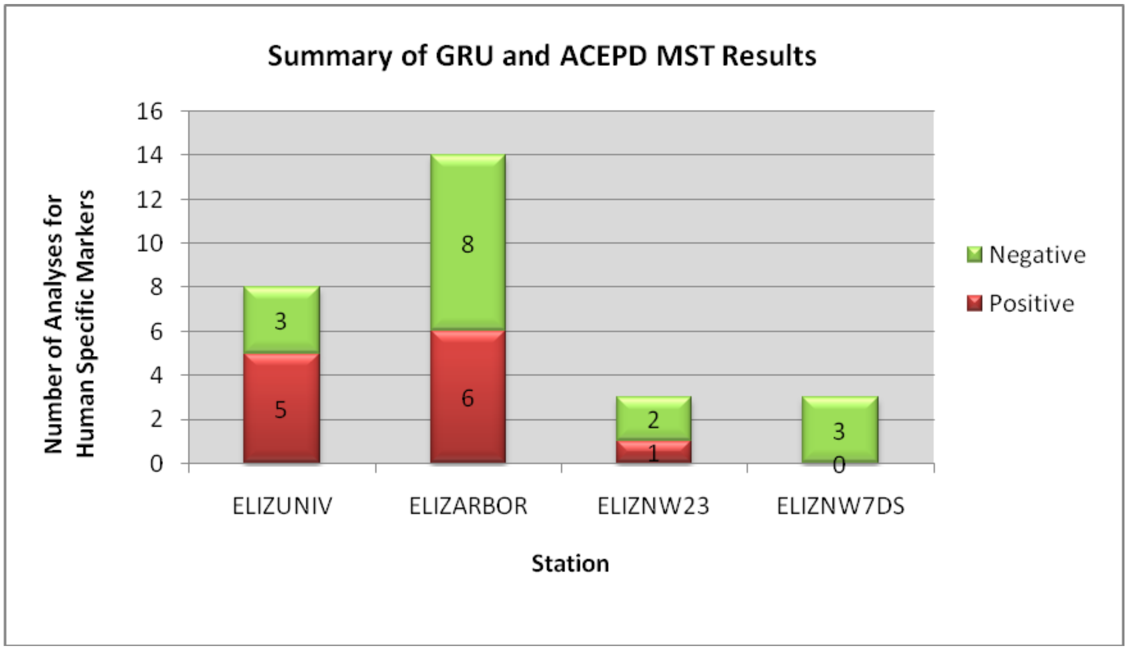


Figure 9-7. Summary of MST Results from GRU and ACEPD for Elizabeth Creek
*The station IDs are ACEPD's designations. Some of GRUs sites were slightly upstream or downstream of ACEPD's sites, but were combined here for comparison purposes.

9.1.6 Little Hatchet and Lake Forest creeks

Little Hatchet Creek is a less urbanized watershed, but has still shown high fecal coliform counts. FWAs were detected at LHATWALDO in all three of the studies utilizing this indicator. This site served as a positive control because it is downstream of the permitted discharge of reclaimed water (treated wastewater effluent) from the Brittany Estates Water Reclamation Facility. All three of the human specific DNA markers were detected in both of the Little Hatchet Creek sites during the April 2007 MST study. The markers were not detected when the sites were re-sampled in August 2007. All of the markers were present in samples collected from the influent and effluent of the wastewater plant during the August 2007 sampling event. This indicates that the markers were not destroyed during the wastewater treatment process at this plant and the effluent is likely the source detected in the earlier sampling.

Human markers and FWAs were not detected in the Lake Forest Creek watershed, although fecal coliform counts were elevated. It does not appear that human sources are an issue in this area. Lake Forest Creek is channelized in this area and is fed by several ditches that flow through neighborhoods. A likely source of fecal coliform is the large population of domestic dogs in this area, as well as other animals and possible bacterial reservoirs in the sediment.

MST technology using human markers provided the most specific source information, but they are also very costly. It is important to recognize that MST is an emerging field and that negative results for a human marker may only mean that the individual marker tested for was not detected, not that there is no risk of human fecal contamination in the waterbody. The repeated absence of human markers in urban areas is likely indicative of wildlife and/or pets and sediment reservoirs as the primary source of fecal coliform contamination, making it helpful in concluding which sites were likely not influenced by human fecal coliform contamination.

9.2 Recommendations and Future Actions

Through conducting these studies, analyzing the data, and compiling the results in this report, ACEPD has learned much about the "Hot Spots" and sampling techniques. It is important to fully evaluate the watershed, including the potential contributing conditions, the land use, and hydrology. There is no one indicator that will accurately track and differentiate sources of fecal pollution. Lasalde (2005) and Harwood (2006) caution in relying on a single type of analyses to differentiate sources because of the genetic heterogeneity observed among bacteria populations. To further our understanding and to improve the quality of future data, ACEPD has taken (or plans to take) the following actions:

- Dr. VJ Harwood with the University of South Florida was contracted to review this report and the collected data along with GRU's MST study (CH2MHILL 2007). Dr. Harwood is working with ACEPD in building upon the results and developing an effective monitoring plan (Appendix XIII).

- A partnership has been formed and includes ACEPD, the Alachua County Health Department, the City of Gainesville Public Works Department, GRU, and FDEP to further evaluate the "Hot Spots." The FDEP has contracted with PBS&J for assistance in reviewing the available data and plans to contract for a pilot study focusing on one "Hot Spot": upper Elizabeth Creek.

- ACEPD plans to conduct comprehensive field surveys of the "Hot Spots". Staff will walk the creeks with maps displaying the locations of stormwater and wastewater infrastructure and septic tank systems. Water and/or sediment samples will be sampled at suspect locations.

- ACEPD will systematically evaluate potential illicit discharges in the "Hot Spots" and other areas using outfall reconnaissance techniques and targeted sampling including indicators such as field parameters (pH, specific conductance, temperature, dissolved oxygen, and turbidity), fecal coliform, surfactants, ammonia nitrogen, and potassium as set forth in EPA's policy and guidance manual for developing an illicit discharge detection program (Brown et al., 2004). This rapid outfall screening will be developed into a more sophisticated monitoring program on a site-specific basis to identify and eliminate illicit discharges.

- To explore the possibility of wildlife and domestic animals as a source of fecal coliform contamination, ACEPD has conducted wildlife surveys in the "Hot Spot" areas (Appendix IX). To expand on this knowledge, ACEPD sent a questionnaire to residents living adjacent to the "Hot Spot" segments of each watershed.

- ACEPD will conduct a pet waste public outreach campaign. Community based social marketing techniques will be employed to increase the success of the campaign and to monitor its success.

- ACEPD will continue its ambient water quality monitoring program. Turbidity samples will be analyzed with every fecal coliform sample to aid in determining if the samples were collected under stormflow or baseflow conditions. *E coli* samples will also be collected to help determine if sources are recent or residual.

- In future studies and monitoring events, ACEPD will employ quality control measures by submitting field blank samples and spiked samples to the laboratories conducting the analyses. When applicable, positive and negative control samples will also be submitted. Samples will be collected in a control watershed, in which minimal impact is suspected.

- ACEPD will take a proactive response to conduct detailed analyses of the stormwater and wastewater infrastructure in the "Hot Spot" areas. Targeted sampling for fecal coliform and *E. coli* will be used to further delineate the "Hot Spots" and narrow the areas that require further investigation.

- ACEPD is conducting a pilot study for inspecting and regulating private collection systems' infrastructure to evaluate the need and feasibility of such a program.

ACEPD plans to continue with a multi-parameter approach to assessing fecal coliform bacteria in the Gainesville urban creeks. Further investigations of the "Hot Spots" areas will include walking the creeks and conducting specific rapid, targeted outfall sampling in these watersheds. The partner agencies will contribute as well with additional septic tank system surveys and inspections and integrity testing of stormwater collection and wastewater collection systems using TV camera surveys, smoke testing, and other investigative methods. The role of wildlife and domestic animals as sources will also be further explored.

10.0 Glossary

Bacteroides spp.

The genus *Bacteroides* is a common bacterial constituent of human and animal feces, contributing to approximately 30% of the intestinal tract flora in humans. A conventional host-specific PCR assay can detect the human specific *Bacteroides* spp. 16S rRNA marker in environmental samples.

Clostridium perfringens

C. perfringens is an anaerobic gram-positive, endospore forming sulfite-reducing bacterium found in human and animal feces. It has been used as a fecal pollution indicator in many areas and is a widely used fecal pollution indicator in Europe. Although *C. perfringens* is an anaerobe, its ability to form endospores may allow it to persist in the environment, reducing its predictive value.

coefficient of variation

This is a relative measure of variation. It is equal to the standard deviation divided by the arithmetic mean.

enteric virus

Also called Enterovirus; genus of viruses of the family Picornaviridae that preferentially inhabit the intestinal tract, with infection usually asymptomatic or mild. Human enteroviruses were originally classified as polioviruses, coxsackieviruses, or echoviruses.

Enterococcus spp.

This genus is a subgroup of the fecal streptococci group and a common inhabitant of the intestinal tract of humans and animals. *Enterococcus* spp. is a smaller group than streptococci, and thought to have greater specificity; it includes *E. faecalis*, *E. faecium*, *E. durans*, *E. gallinarum and E. avium*. Although long-lived, they do not reproduce in the aquatic environment, making them a useful indicator.

Two species of *Enterococcus* (*E. faecalis and E. faecium)* have a human specific DNA marker that makes them potentially useful for detecting human wastes in waters. The *E. faecium* surface protein *esp* gene that can be detected in this bacterium may be used in a data-library independent determination of the presence of human waste.

EPA Method 1600

This method describes a membrane filter (MF) procedure for the detection and enumeration of *Enterococcus* spp. bacteria in water.

EPA Method 1603

This method describes a membrane filter (MF) procedure for the detection and enumeration of *Escherichia coli* bacteria in water.

Escherichia coli

E. coli is one of many species of bacteria living in the lower intestines of mammals. It is a gram-negative, non-sporulating bacterium in the family *Enterobacteriaceae* (Phylum Proteobacteria). It is thought to be a better indicator of fecal pollution and human health risk than the broader group of fecal coliform bacteria.

fecal coliform bacteria

A subset of the total coliform group that are distinguished by their ability to grow and ferment lactose under incubation at 44.5 ^{o}C. Fecal coliform bacteria originate from the digestive track of warm blooded animals; therefore their presence in water is more indicative of fecal pollution than total coliform.

fecal streptococcus group

Consists of a number of species of the genus *Streptococcus* that are found in the gastrointestinal tract of warm-blooded animals. Certain streptococcal species predominate in some animal species and not in others, but it is not possible to differentiate the source of fecal contamination based on the speciation of fecal streptococci.

fluorescent whitening agent (FWA) or optical brightener (OB)

Dyes that are found in most laundry detergents; they adsorb ultraviolet and fluorescent blue light in the visible spectrum. FWAs are highly substituted large aromatic organic compounds, which commonly contain double bonds that are activated by UV light.

geometric mean

The geometric mean is frequently used when comparing or analyzing data that varies over several orders of magnitude and can be thought of as a log-transformation of the data. This makes the geometric mean a better measure of central tendency because, unlike the arithmetic mean, the effects of very high or low values are reduced.

human polyomavirus

Only found in humans and are present in the general population. These viruses are usually asymptomatic within the host and potentially high concentrations are excreted in human urine, therefore it is can be used as a marker for sewage contamination.

linear regression

Analyzes the relationship between two variables and determines the best straight line through the data. The square of the correlation coefficient is known as R^2. The value R^2 is between zero and one, with the value of one indicating that all points lie directly on a straight line and the variables are strongly correlated.

membrane filtration (MF)

A direct plating technique that also allows concentration of bacteria in a water sample. Results are reported as colony counts per unit volume, typically as colony forming units (CFU), and are reported relative to the 100 mL sample.

microbial source tracking (MST)

This term refers collectively to methodologies used to detect and differentiate sources of fecal pollution in waters. MST methods can be divided into broader categories including; chemical methods, microbiological methods, phenotypic library-dependent methods, genotypic library dependent methods, and library independent methods

most probable number (MPN)

Multiple tube fermentation tests include replicate tubes and dilutions and results are reported in terms of the most probable number (MPN). MPN is determined from probability formulas that estimate the average density of coliform bacteria in a sample based on the results of a number of dilutions from a single sample. Results are reported relative to a 100 mL sample.

polymerase chain reaction (PCR)

A technique which is used to amplify the number of copies of a specific region of DNA, in order to produce enough DNA to be adequately tested. This technique can be used to detect and identify microorganisms with high sensitivity and specificity.

SM 9222D

Membrane filter procedure that determines the density of fecal coliform bacteria. Results are given as colony forming units (CFU) per 100 mL.

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