Identification and Quantification of Human Fecal Sources in Alachua County Urban Creeks

Troy M. Scott, Ph.D. and George Lukasik, Ph.D.
BCS Laboratories, Inc., Gainesville, FL

OVERVIEW

The development of total maximum daily load (TMDL) compliance levels and the undertaking of associated risk assessment based on these calculations requires not only that the source(s) of fecal inputs into a watershed be identified but, ideally, that the relative contributions from all sources be quantified. One currently available tool, which can be used to achieve the former of these objectives, is Microbial Source Tracking technology. The evolution of source tracking technologies has included the general shift from library-dependent methods to library-independent methods. The former described methods typically utilize databases containing genotypic or phenotypic "fingerprints" from organisms from known sources to which fingerprints from unknown isolates can be compared. The latter described methods target host-specific microorganisms or genes using various molecular methods, usually polymerase chain reaction (PCR). The appropriate use and correct interpretation of MST technology has been shown to be extremely beneficial in watershed studies. This is especially true, should the investigators follow specific guidelines with regard to data collection and interpretation.

The success (or failure) of a source-tracking project lies in the types of methods employed, the specific approaches taken, and the types of analytical tools used to interpret the results.

- Specific knowledge of the watershed and potential fecal inputs is a critical component of a successful source tracking study. By concentrating only on potential inputs, the methods employed can be specifically targeted to identify these sources.

- All methods should be employed in a tiered fashion and results should be interpreted sequentially so as to implicate or eliminate potential sources one at a time.

- The sampling approach should be well planned and should be conducted during both high flow (wet) and low flow (dry) events. Care should also be taken to perform what is referred to as "targeted sampling". Using this approach, samples are taken both upstream and downstream of potential fecal inputs. If bacterial counts are low upstream and high downstream (and source tracking data indicates a strong correlation with the suspected source(s)), then this is a strong indication that the source has been identified. Conversely, this approach can also eliminate suspected sources and redirect attention to other potential impact sites.

- End-users are encouraged to adopt a "toolbox" approach to any source tracking study. By using multiple methods that target and identify different source indicators, more comprehensive conclusions can be drawn. The most important aspect of the toolbox approach is that individual results can be confirmed and substantiated. Conclusions regarding potential inputs should NEVER

be drawn due to the results of a single test on a single water sample. On the contrary, multiple positive results using a variety of methods are a strong indicator that the source(s) of the pollution have been identified.

- If budgetary constraints are present, end users are encouraged to conduct their source tracking studies in a piecemeal fashion. It is better to target one or two potential sources with several tests, rather than to try to pinpoint all the potential sources with more broad tests. Most source tracking projects do not produce meaningful results because too many sources of fecal pollution are targeted simultaneously. By limiting the number of potential targets, the end user can work by a process of elimination towards the likeliest sources of contamination.

METHODS

Isolation and characterization of *Enterococcus spp.*. Enterococci were concentrated by membrane filtration. Filters were incubated for 24 hours on mEI agar (Difco), and processed according to the methodology outlined in USEPA Method 1600 and Scott et al. (2005).

Preparation of template DNA for PCR reactions. Filters containing enterococci (from above step) were lifted and DNA extraction was performed directly from the filter using a Qiagen Stool DNA extraction kit with slight modification (Qiagen, Inc.). Previous research in our laboratory has indicated that there is no significant bias introduced to the qPCR quantification by this culturing step. Total gene copy numbers increase, but relative ratios remain stable.

Concentration of Bacteroidetes DNA. Bacteroidetes were concentrated by membrane filtration and DNA extraction was performed directly from filter membranes using a modification of the Qiagen Stool DNA extraction kit.

Concentration of Human Derived Polyoma Virus (HPyV), DNA Extraction and Amplification. HPyVs were concentrated from 200 ml of water onto nitrocellulose filters by the methodology described in McQuaig et al. (2006). Viral particles were eluted from the filters using 3% beef extract (pH 9.3), and DNA was extracted using QIAamp DNA Blood Midi Blood Kit. The final volume of DNA eluted from the column was 90µl. A positive PCR reaction confirms the presence of human polyomaviruses in the sample, and indicates the contamination of the sample with human-derived urine material.

PCR assays. Assays for the human enterococcus, human bacteroides, and human polyomavirus assays were performed according to methodology outlined in Scott et al. (2005), Bernhard and Field (2000), and McQuaig et al. (2006), respectively. The individual characteristics of each method are outlined in Table 1.

Table 1. Characteristics of host specific molecular markers of human fecal pollution

Type of Genetic Marker	Marker Specificity	Fate in the Environment	Potential for Regrowth
Human Polyoma virus	**Very highly specific**	**Persistence in groundwater and surface water up to 21 days. Usually indicative of recent contamination**	**Does not re-grow in environment due to lack of host.**
Human *Enterococcus*	**Very highly specific**	**Can persist in surface water for 10 to 21 days.**	**Regrowth in surface waters and sediments has been documented**
Bacteroides	**Very highly specific**	**Usually rapid die-off in aerobic surface waters. Can persist for up to 21 days**	**Will not re-grow in aerobic environments**

RESULTS and DISCUSSION

Interpreting Human Molecular Marker Data for Submitted Samples

The results of the human specific DNA marker testing are summarized in Table 2. Tables 3 and 4 are supplementary tables. Table 3 depicts the presence of Bacteroides DNA in sediments and Table 4 summarize sites positive for at least one molecular marker of human fecal pollution, respectively. Appendix A is a map showing the sample site locations.
The results of the DNA marker analyses performed above have additional value based on qualitative interpretation by qualified staff at BCS Laboratories, Inc. Generally, there are two major conclusions that can be drawn from patterns and/or presence/absence of the DNA MST host specific markers.

- A positive result for the Human Enterococcus marker in the absence of any other human fecal marker should be interpreted as residual or trace fecal contamination
- The presence of the Human Enterococcus marker in conjunction with either the Human Bacteroides or Human Polyomavirus, or both, should be interpreted as a recent contamination event.

The interpretations of possible DNA marker results are summarized in Table 5. For purposes of this report, the presence of Bacteroides DNA in the submitted sediment samples is used to indicate the recent deposition of fecal material, regardless of source.

Sample Name/Type	Sampling Date	Volume analyzed	Human Enterococcus ID PCR (+/-)	Human Bacteroides ID PCR (+/-)	Human Quan. Enterococcus ID PCR (Ratio)**	Human Quan. Bacteroides ID PCR (copies/100ml)	Human Polyomavirus ID PCR (+/-)
PositiveControl	NA	NA	POSITIVE	POSITIVE	NA	NA	POSITIVE
Field Blank		NA	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
Pos NW 16	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD*	NEGATIVE
Rose Univ.	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Tum 441	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
Tom Porters	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
Hog NW 8	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Hog NW 16	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
RatusHog	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
Eliz NW 7	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
SWB LF	4/25/07	100 ml	POSITIVE	POSITIVE	0.11%	1.25×10^7	NEGATIVE
LFC NE 25	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Hog GHS N	4/25/07	100 ml	POSITIVE	POSITIVE	0.23%	5.94×10^6	NEGATIVE
Eliz Arbor	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
LHAT NB	4/25/07	100 ml	POSITIVE	POSITIVE	0.60%	2.79×10^3	POSITIVE
SWB NE10	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
SWB SE8	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSTIIVE
Pos NW 8	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Rose SE9	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
HogCETrib	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
Tum SW 5	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Hog NW 23	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
Tum SW 11	4/25/07	100 ml	POSITIVE	POSITIVE	0.33%	4.65×10^4	NEGATIVE
Pos NW36	4/25/07	100 ml	POSITIVE	POSITIVE	0.45%	7.24×10^6	NEGATIVE
Eliz NW23	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
HogusRat	4/25/07	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE
Rose SE7	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
SWB331	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
LHATWaldo	4/25/07	100 ml	POSITIVE	POSITIVE	0.64%	4.9×10^7	POSITIVE
SWB SE1	4/25/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Rat NW8	4/25/07	100 ml	POSITIVE	POSITIVE	Below LOD	2.64×10^6	NEGATIVE
Sewage	4/25/07	10 ml	POSITIVE	POSITIVE	1.5%	2.5×10^8	POSITIVE
BEUpstream	8/7/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
BE Influent	8/7/07	100 ml	POSITIVE	POSITIVE	1.2%	1.6×10^7	POSITIVE
BE Effluent	8/7/07	100 ml	POSITIVE	POSITIVE	1.1%	1.1×10^4	POSITIVE
LHATWaldo	8/7/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
LHATArbor	8/7/07	100 ml	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE

Table 2. Results of human specific molecular marker testing on Gainesville creeks
***Limit of Detection**Ratio of 1.5% corresponds to raw sewage**
Purple: Triple Positive, High Probability human fecal pollution; results conclusive; resample to confirm
Yellow: Double Positive, High to Moderate Probability human fecal pollution; results conclusive; resample to confirm
Grey, Single Positive, Low probability recent human fecal pollution or diffuse septic source, Results inconclusive; Resample to confirm
White, Triple Negative, Low probability Human Fecal pollution, Results conclusive; Resample to confirm for regulatory purposes

Table 3. Detection of Bacteroides spp. in Sediment by Polymerase ChainReaction as a Proxy for Recent Fecal Pollution

Sample Name	Sample Type	Amount analyzed	General Bacteroides PCR
Positive Control	NA	POSITIVE	POSITIVE
Negative Control	NA	NEGATIVE	NEGATIVE
Pos NW 16	Sediment	10 g	POSITIVE
Rose Univ.	Sediment	10 g	POSITIVE
Tum 441	Sediment	10 g	NEGATIVE
Tom Porters	Sediment	10 g	NEGATIVE
Hog NW 8	Sediment	10 g	NEGATIVE
Hog NW 16	Sediment	10 g	POSITIVE
RatusHog	Sediment	10 g	POSITIVE
Eliz NW 7	Sediment	10 g	NEGATIVE
SWB LF	Sediment	10 g	POSITIVE
LFC NE 25	Sediment	10 g	NEGATIVE
Hog GHS N	Sediment	10 g	POSITIVE
Eliz Arbor	Sediment	10 g	NEGATIVE
LHAT NB	Sediment	10 g	POSITIVE
SWB NE10	Sediment	10 g	NEGATIVE
SWB SE8	Sediment	10 g	NEGATIVE
Pos NW 8	Sediment	10 g	NEGATIVE
Rose SE9	Sediment	10 g	POSITIVE
HogCETrib	Sediment	10 g	POSITIVE
Tum SW 5	Sediment	10 g	POSITIVE
Hog NW 23	Sediment	10 g	POSITIVE
Tum SW 11	Sediment	10 g	POSITIVE
Pos NW36	Sediment	10 g	POSITIVE
Eliz NW23	Sediment	10 g	POSITIVE
HogusRat	Sediment	10 g	POSITIVE
Rose SE7	Sediment	10 g	NEGATIVE
SWB331	Sediment	10 g	NEGATIVE
LHAT Waldo	Sediment	10 g	POSITIVE
SWB SE1	Sediment	10 g	POSITIVE
Rat NW8	Sediment	10 g	POSITIVE
Sewage	Sediment	10 g	POSITIVE

Table 4. Results of sites positive for at least one molecular indicator of human fecal pollution

Sample Name/Type	Human Enterococcus ID PCR (+/-)	Human Bacteroides ID PCR (+/-)	Human Quantitative Enterococcus ID PCR (Ratio)**	Human Quantitative Bacteroides ID PCR (copies/100ml)	Human Polyomavirus ID PCR (+/-)	Sediment General Bacteroides PCR##
Positive Control	POSITIVE	POSITIVE	NA	NA	POSITIVE	POSITIVE
Submitted Blank (-)	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
Pos NW 16	POSITIVE	NEGATIVE	Below LOD	Below LOD*	NEGATIVE	POSITIVE
Rose Univ.	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE	POSITIVE
Hog NW 16	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE	POSITIVE
RatusHog	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE	POSITIVE
SWB LF	POSITIVE	POSITIVE	0.11%	1.25×10^7	NEGATIVE	POSITIVE
Hog GHS N	POSITIVE	POSITIVE	0.23%	5.94×10^6	NEGATIVE	POSITIVE
LHAT NB	POSITIVE	POSITIVE	0.60%	2.79×10^5	POSITIVE	POSITIVE
SWBSE8	100 ml	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE
Rose SE9	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE	POSITIVE
Hog CE Trib	NEGATIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE	POSITIVE
Tum SW 5	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE	POSITIVE
Hog NW 23	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE	POSITIVE
Tum SW 11	POSITIVE	POSITIVE	0.33%	4.65×10^4	NEGATIVE	POSITIVE
Pos NW36	POSITIVE	POSITIVE	0.45%	7.24×10^6	NEGATIVE	POSITIVE
Eliz NW23	POSITIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE	POSITIVE
HogusRat	POSITIVE	NEGATIVE	Below LOD	Below LOD	POSITIVE	POSITIVE
LHAT Waldo	POSITIVE	POSITIVE	0.64%	4.9×10^7	POSITIVE	POSITIVE
SWB SE1	NEGATIVE	NEGATIVE	Below LOD	Below LOD	NEGATIVE	POSITIVE
Rat NW8	POSITIVE	POSITIVE	Below LOD	2.64×10^6	NEGATIVE	POSITIVE
Sewage	POSITIVE	POSITIVE	1.5%	2.5×10^8	POSITIVE	NOT TESTED
BE Influent	POSITIVE	POSITIVE	1.2%	1.6×10^7	POSITIVE	NOT TESTED
BE Effluent	POSITIVE	POSITIVE	1.1%	1.1×10^4	POSITIVE	NOT TESTED

**Ratio of 1.5% corresponds to raw sewage

Purple: Triple Positive, High Probability human fecal pollution; results conclusive; resample to confirm

Yellow: Double Positive, High to Moderate Probability human fecal pollution; results conclusive; resample to confirm

Grey, Single Positive, Low probability recent human fecal pollution or diffuse septic source, Results inconclusive; Resample to confirm

##General *Bacteroides* marker indicates the presence of fecal pollution, regardless of host, and serves as an indicator of recent fecal deposition

Table 5. Possible signature patterns of human specific molecular markers and interpretation

Category	Polyoma Virus	*Enterococcus*	Bacteroides	Characteristics of the Signature		
				Strength Human Signature	Recent or Residual Source	Other
Triple Positive	Positive	Positive	Positive	Very strong positive	Recent	
Double Positive	Positive	Negative	Positive	Very strong positive	Recent	Possible septic tanks
	Positive	Positive	Negative	Very strong positive	Residual	
	Negative	Positive	Positive	Strong positive	Recent	Strong signature based on realistic method sensitivity
Single Positive	Positive	Negative	Negative	Moderate positive	Residual	No evidence of human fecal contamination
	Negative	Positive	Negative	Weak positive	Residual	Low evidence of human fecal contamination; Resample
	Negative	Negative	Positive	Weak/moderate positive	Recent	Possible septic tanks; Resample
No Positive	Negative	Negative	Negative	Very strong negative	No recent human source/impact	

Interperting Human Polyoma Virus results

The human polyomaviruses (HPyVs), JCV and BKV, have similarly structured genomes that show 75% homology. The prevalence of these viruses in the human population is worldwide. Serological studies have shown 60-90% of adults harbor antibodies against human polyomaviruses (HPyVs). A symptomless primary infection occurs during childhood; following which, the viruses establish latent infections in the renal tissue and can persist indefinitely. Asymptomatic viruria can occur occasionally or continuously in infected individuals. Disease is normally associated when the host's immune system becomes suppressed by conditions such as AIDS. In general, polyomaviruses have a low morbidity, latency, and symptom-less reactivation. Approximately one million viral particles can be shed in one milliliter of urine from a healthy individual.

In this study we use human-specific polyomaviruses (HPyVs) as an indicator of human fecal pollution. HPyVs are ubiquitous throughout the human population. They are secreted in the urine in high titers and mostly cause asymptomatic infections. Infected individuals shed viruses throughout their life span.

We have developed and optimized a rapid and sensitive method to concentrate and extract DNA of HPyVs from environmental water samples (McQuaig et al.; 2007). Primers specific for the conserved T-antigen of both JCV and BKV are used in a nested PCR reaction to detect HPyVs. The method is able to detect as little as one microliter of raw sewage added to 100-ml of water.

The detection of the presence of Human polyoma viruses in a water sample indicates the direct contamination of the water sample by a human waste source. This could be either direct contamination (urination) or impact by faulty septic or sewage systems. Though the persistence of the marker has not been documented in studies, our studies and field trials have indicated that it does not persist for extended periods in tropical climates. The presence of this marker in conjunction with the human enterococcus marker indicates a high correlation with human derived sewage contamination. Where as, the presence of this marker in the absence of the human enteroccoccus marker, indicates a high septic system impact.

Interpreting Human Enterococcus Quantification Results

While the presence/absence molecular methods provide valuable, rapid information as to the sources of fecal pollution in a water system, they offer no information regarding relative contributions to a watershed impacted by multiple sources. This difficultly can be addressed through the use of real time quantitative PCR (qPCR). This is a valuable tool that can be used to quantify the amount of DNA in a water sample but its use is limited by a satisfactory means of data interpretation. *Specifically, if the number of gene copy numbers of a specific molecular marker can be quantified in an environmental sample, what does this number mean*? We have approached this question by establishing relative ratios of a host specific gene marker to generic gene markers present in specific bacteria from different host sources. If the fecal pollution present in a watercourse is from a single source, the relative ratios of the specific and generic gene markers should be similar to that seen in the host source. In this project we used a highly sensitive and specific quantitative PCR assay to collect information on the temporal and spatial distribution, as well as relative abundance of specific and generic targets present in specific fecal sources. This information was then used to establish baselines to which unknown samples can be compared. While this approach could conceivably be applied to any molecular target, this study used the Enterococcal Surface Protein gene as a proxy for human fecal contamination (Scott et al., 2005).

The logic behind this approach relies on several assumptions: First, assuming that the total copy number of human specific markers is relatively constant in sewage influent and that the total bacterial populations in sewage influent are relatively constant; the ratio of human marker to total population should be a relatively fixed value. Second, this ratio should be affected predictably by factors such as dilution and inflow of background bacteria from non-target hosts. Specifically, ratios will not be affected as total volume of the system increases but absolute bacterial levels remain the same. Likewise, relative ratios will decrease when background bacterial levels increase but levels of host specific marker remains the same. This approach should also work within systems in which dilution and influx of non-target bacteria occur concurrently. Thus, we have a test that quantifies the total

number of ***bacteria*** (*Ent. Faecium*) that originate from human sources and not an absolute quantification of total human fecal pollution in a system. It is important to make this distinction as it defines the method by quantifying a subset of indicator microorganisms present in sewage, not the sewage itself. This approach should prove useful for calculation of Total Maximum Daily Loads (TMDLs).

Final Interpretation of Human Molecular Marker Data for Submitted Samples

Of the submitted samples, all sites that initially draw concern are depicted in Table 4. Of these, the sites outlined in yellow and purple are of the most concern and are the most likely to have received the impact of human fecal pollution. Based on the interpretation scheme depicted in Table 5, the following sites are likely to have received a recent human fecal contamination event prior to sampling: SWB LF, Hog GHS N, LHAT NB, Tum SW 11, Pos NW36, LHAT Waldo, and Rat NW8.

The remaining flagged sites, Hog NW 16, Rat Vs Hog, Hog NW 23, Hog vs Rat, and SWBSE8 likely contain human fecal material; however, the contamination event probably occurred at a some time long prior to sampling.

Overall, the LHAT NB and LHAT Waldo sites show the most significant human signature and these results are corroborated by the quantitative data shown below.

Final Interpretation of Quantitative Human Enterococcus and Human Bacteroides Data

The final interpretation of the human Enterococcus quantitative assay is inherently difficult due to the high background of enterococci found in the creek system. For the Human Bacteroides Quantitative data, seven sites show significant levels of recent human input and directly mirror the results of the presence/absence data for human fecal pollution: SWB LF, Hog GHS N, LHAT NB, SWBSE8, Tum SW 11, Pos NW36, LHAT Waldo, and Rat NW8.

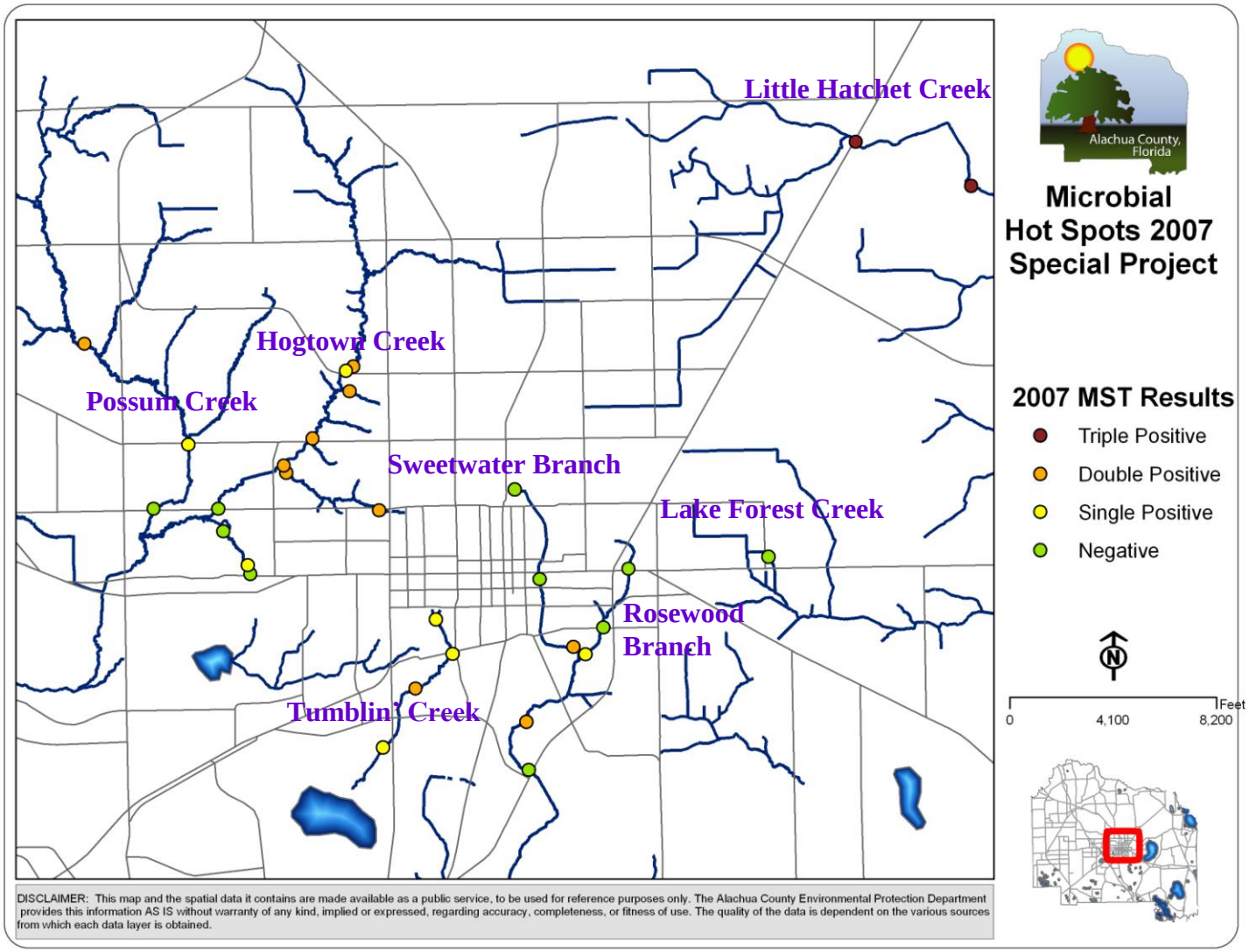
Overall, "LHAT NB" and "LHAT Waldo" warrant further investigation due to the high ratio of human marker to total enterococci observed in these samples as well as the quantifiable presence of the human Bacteroides marker. Also of significance are the sites that contained both markers in quantifiable numbers (SWB LF, Hog GHS N, Tum SW 11, and Pos NW36) although it is difficult to assign a priority level to these sites due to the small number of samples collected.

Summary and Conclusions

- **Three molecular markers of human fecal pollution (*Ent. faecium esp* gene, Human specific 16S rDNA sequence in *Bacteroides spp*., and Human polyomavirus) were used as a proxy for the presence of human fecal contamination in Alachua County creeks.**

- **In general, all markers are highly specific for human fecal pollution. The highest sensitivity is achieved with the esp marker.**
- **None of the markers persist in the environment for extended periods of time. The average maximum retention time of each marker is approximately 21 days. The *esp* marker is particularly useful for detecting domestic sewage and its occurrence in septic systems is sporadic. Therefore, the presence of either human *Bacteroides* or polyomavirus in the absence of *esp* would be an indicator of septic tank impact.**
- **Sites that are negative for human markers but positive for General *Bacteroides* in the sediments should be considered sites of recent fecal deposition (nonhuman).**
- **For samples collected on 8/7/07, two sites (BE influent, BE effluent) were positive for all three markers of human fecal pollution.**
- **More interestingly, the sample collected from just upstream (BE upstream) contained indicator levels several orders of magnitude greater than BE effluent (data not shown) but contained no markers of human fecal pollution.**
- **The Alachua County creeks system contains high levels of bacterial indicators of fecal pollution. The use of more specific, molecular assays enables the targeting of "hot spots" and areas of interest in order to direct more appropriate focus on sites that can be remediated (i.e. sites impacted by human fecal pollution)**

- **Append**


Little Hatchet Creek
Hogtown Creek
Possum Creek
Sweetwater Branch
Lake Forest Creek
Rosewood Branch
Tumblin' Creek
Alachua County, Florida
Microbial Hot Spots 2007 Special Project
2007 MST Results
Triple Positive
Double Positive
Single Positive
Negative
N
0
4,100
8,200
Feet
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