Florida Department of Environmental Protection

Division of Environmental Assessment and Restoration

FDEP's Application of Molecular and Chemical Markers of Fecal Source Contamination in Jacksonville, Florida Area Waters Impaired for Fecal Coliforms

by
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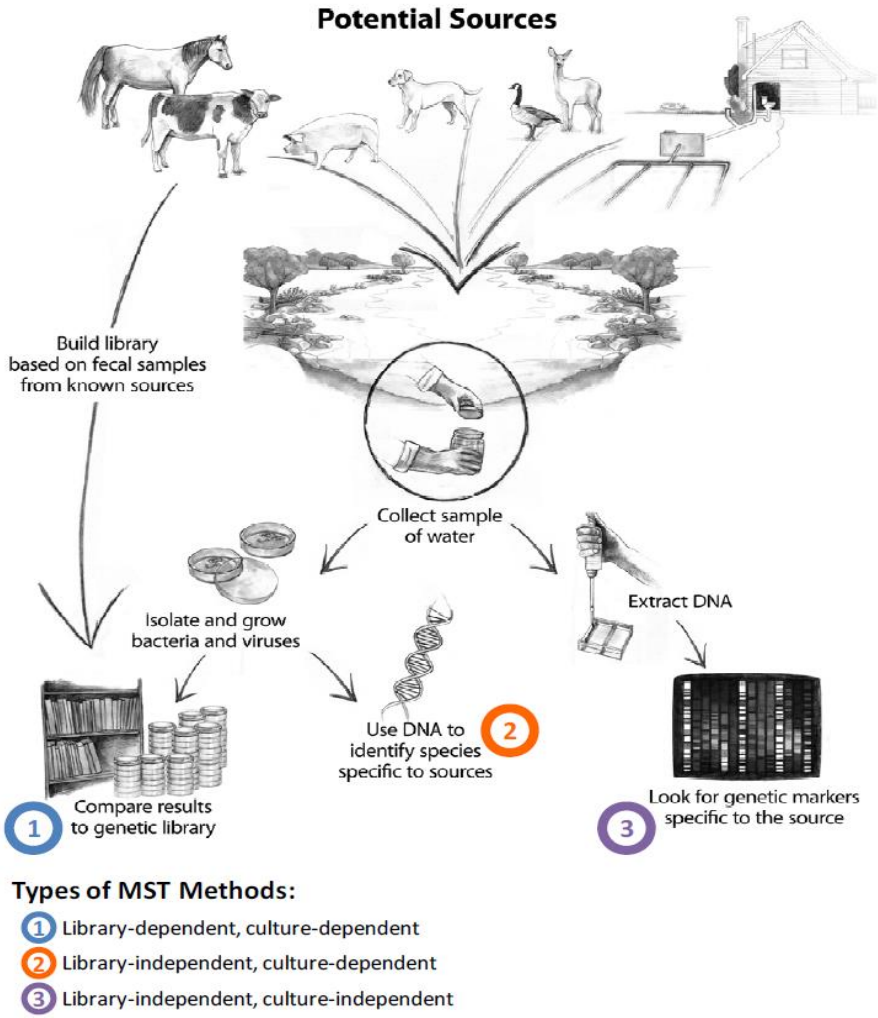
August 15, 2014





Microbial Source Tracking

- Library-dependent, culture-dependent
- Library-independent, culture-dependent
- Library-independent, culture-independent
- Phenotype
- Chemical
- Immunological



Tetra Tech, Inc., 2011. Using Microbial Source Tracking to Support TMDL Development and Implementation. Produced for U.S.E.P.A.

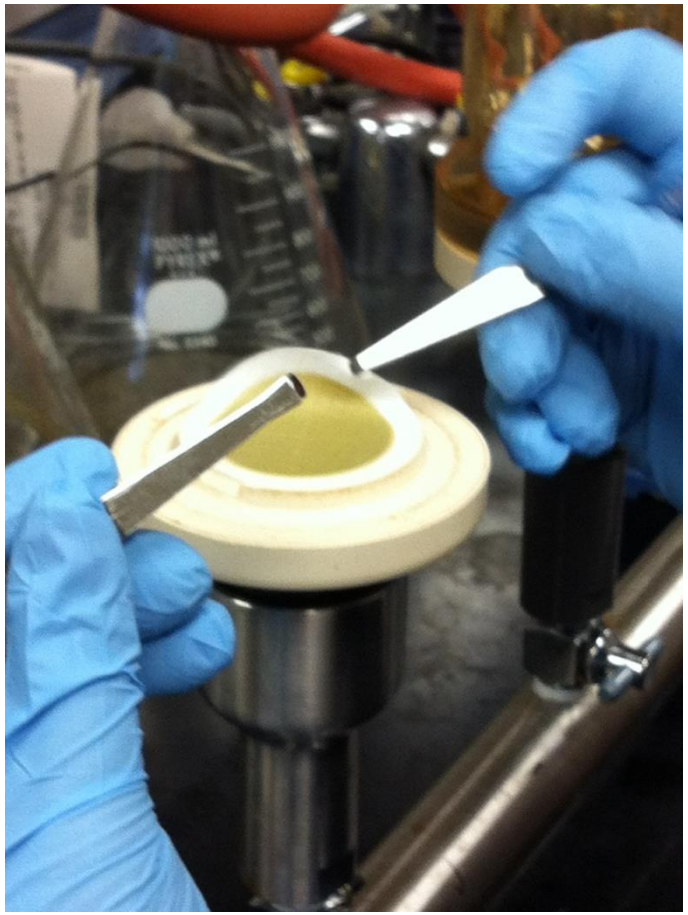
Microbial Source Tracking

- Quantitative Polymerase Chain Reaction (q-PCR)
- Bacterial or viral genetic markers strongly or uniquely associated with particular groups of animals (e.g., human, cattle, dog, pig, chicken…)
- Analytical Chemistry
- Chemical compounds unique to human wastewater sources (e.g., sucralose, galaxolide, tonalide, gadolinium…)

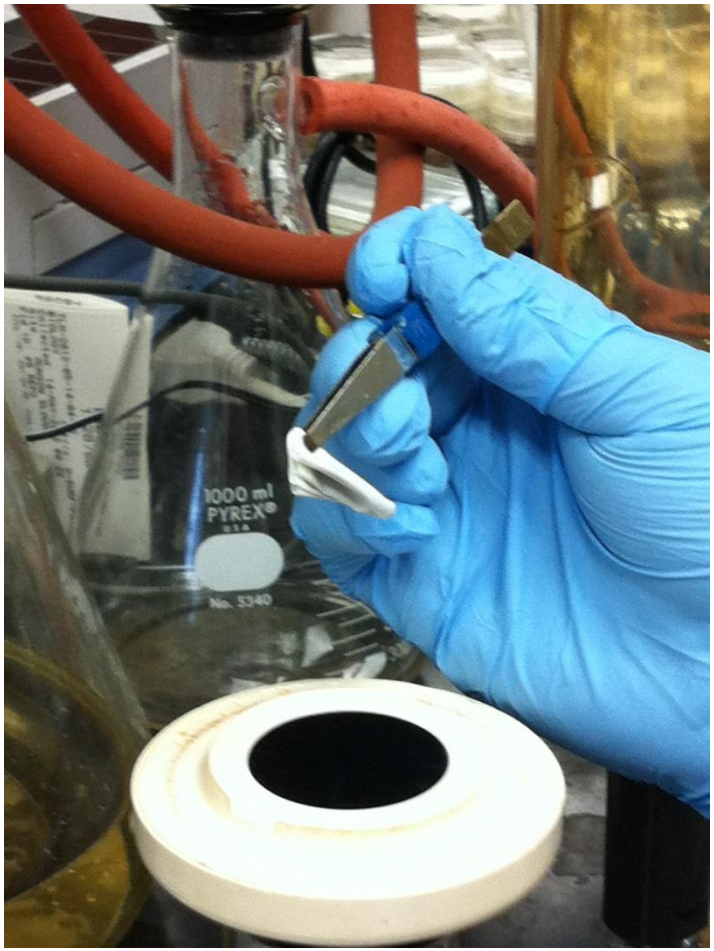
MST Sampling

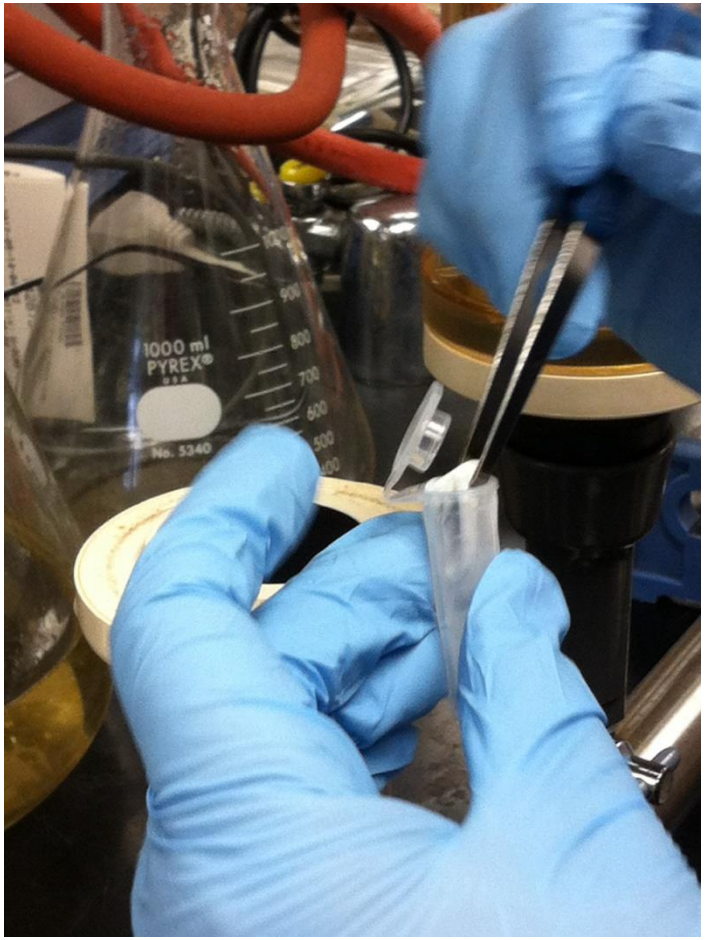
- Fecal indicators, PCR, and chemical marker samples collected at each station
- Samples collected using fresh disposable gloves at each site
- Protecting the sampler from the water, but also protecting the sample from the sampler

MST Sample Processing

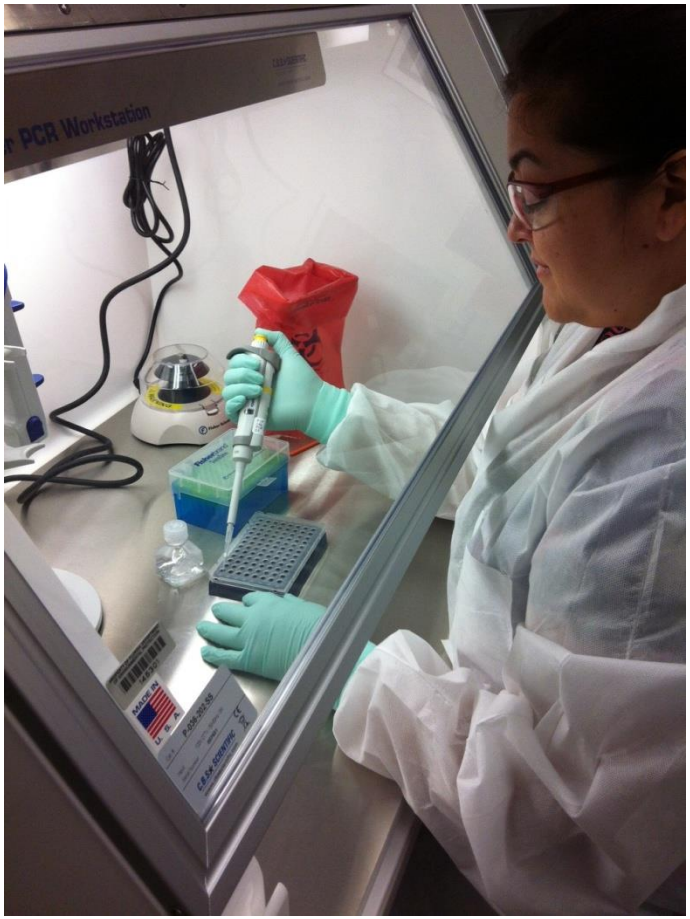


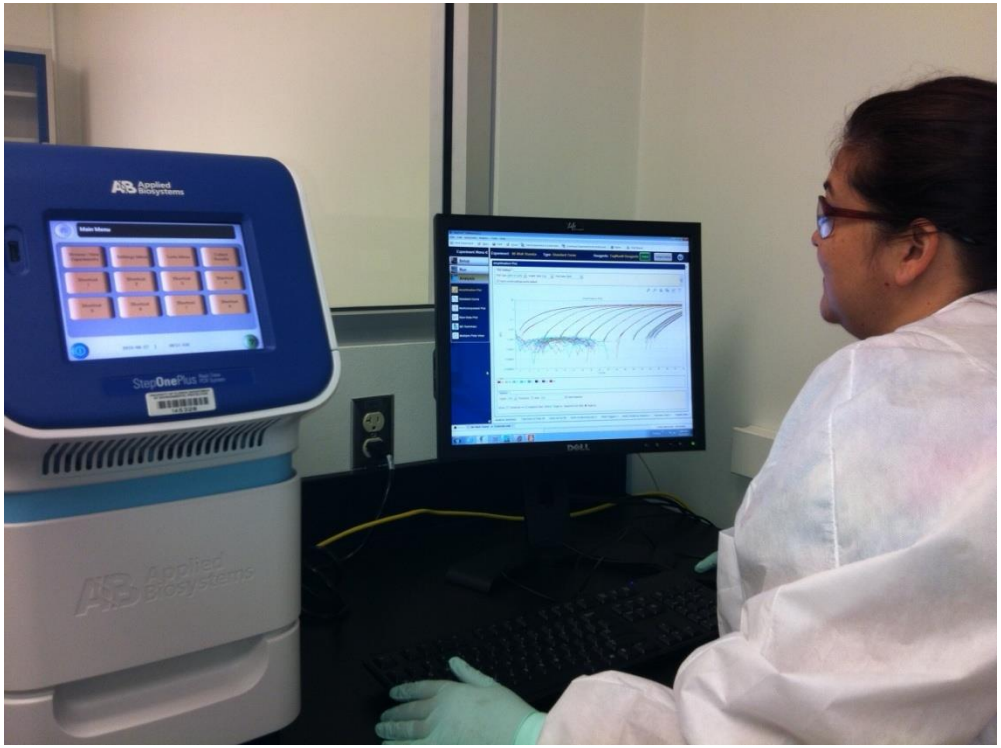
MST Sample Processing





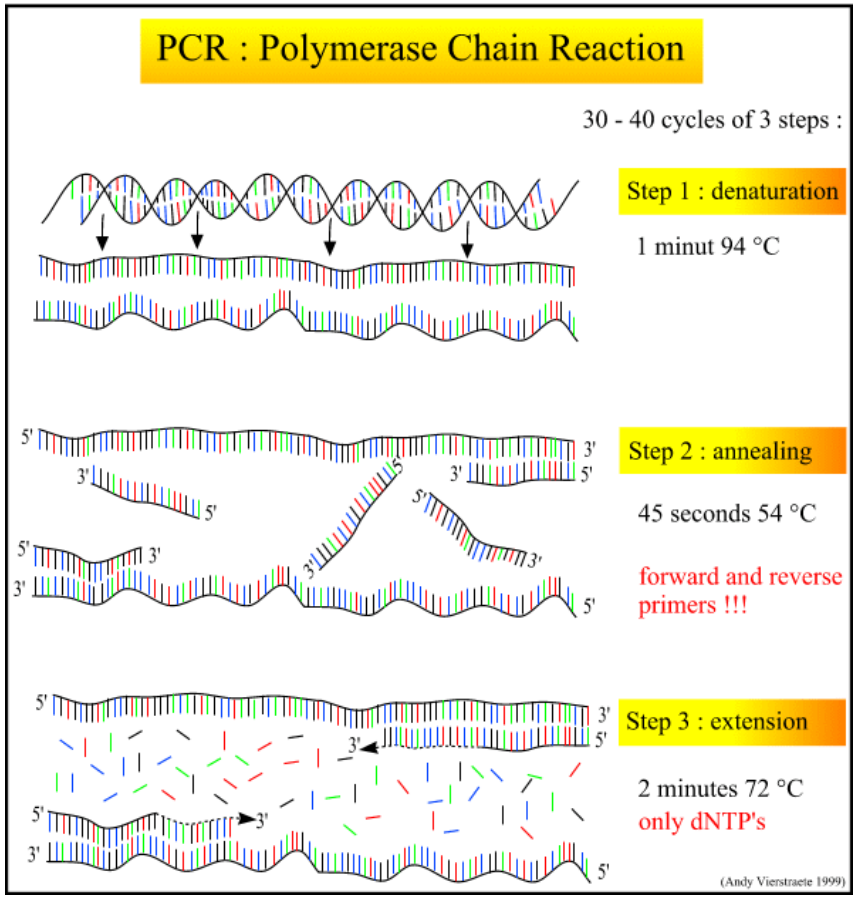
MST Sample Processing



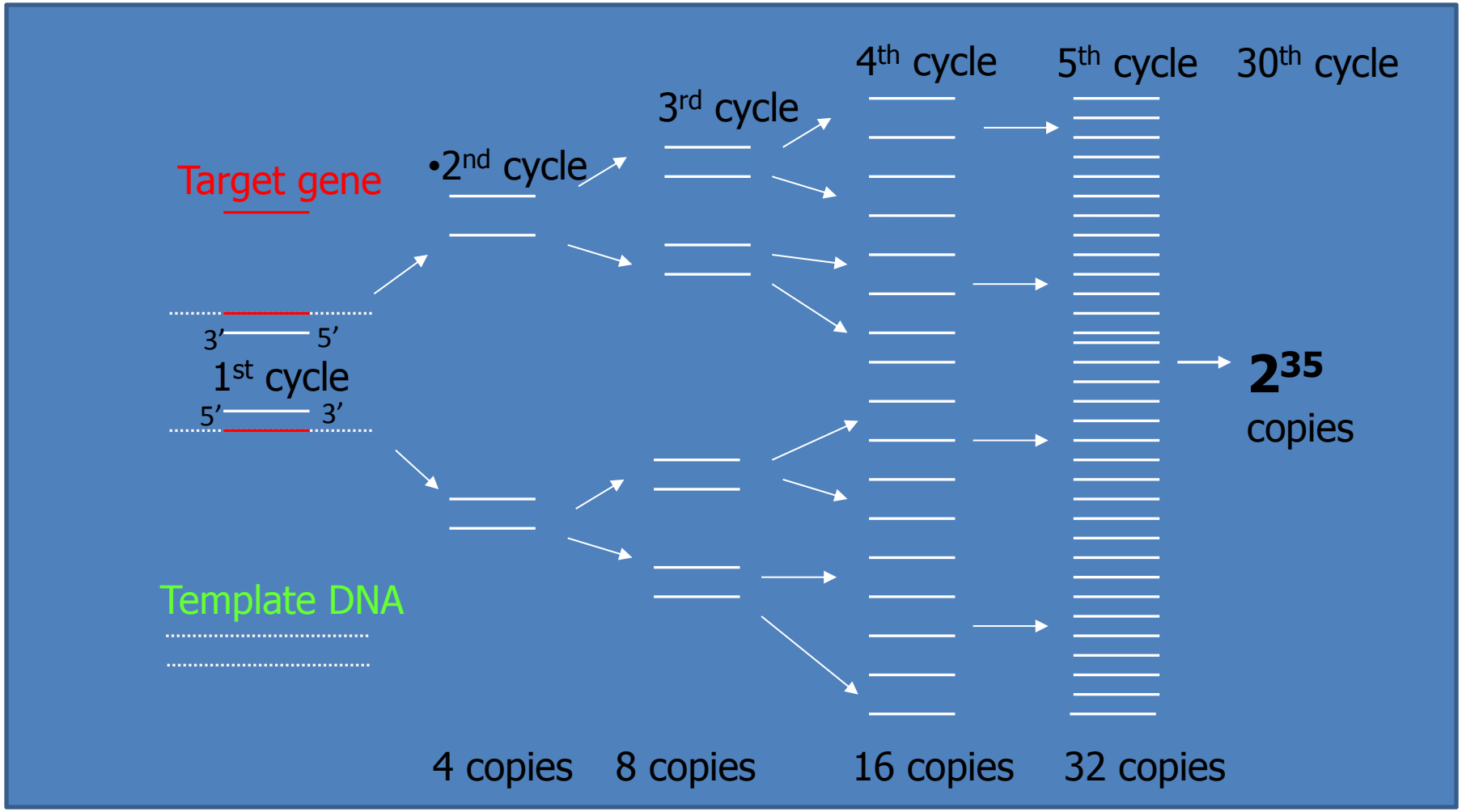


PCR Basics

- Polymerase Chain Reaction (PCR)
- Three basic steps that are repeated over multiple cycles (e.g. 40 cycles)



PCR Reactions are Exponential



Litaker, W. 2013. PCR and qPCR Basic Overview. Presentation at Rapid Methods Workshop, UNC

PCR Data Interpretation

- HF183 human source marker reported as Genomic Equivalent Units (GEUs) per 100 mL of sample
- Raw WWTP influent runs in the 10s of millions GEUs/100 mL
- Treated WWTP effluent highly variable and may run from non-detect to 10s of thousands of GEUs/100 mL depending on level/type of treatment and how well the plant is functioning
- Raw septage runs in the 100s of thousands of GEUs/100 mL

Prioritizing MST Markers

- Initial MST marker being used by the FDEP lab is a human source marker
  - Bacteroidales HF183
  - additional human markers will be included soon
- Additional markers being considered
  - Bird
    - Gull2 (Sinigalliano, 2010)
      - *Catellicoccus marimammalium*
    - GFD (Green, 2011)
      - *Helicobacter sp.*
  - Dog
    - DogBac (Sinigalliano, 2010)
      - *Bacteroidales*
  - Cow
    - Rum2Bac (Mieszkin, 2010)
      - *Bacteriodales*
    - CowM2 (Shanks, 2008)
      - *Bacteroidetes*

Current PCR Limitations

- Specificity of most bacterial markers is not 100%
- Differences in persistence between MST markers in natural waters making it difficult to apportion fecal sources within a watershed with high degree of confidence
- MST is a rapidly developing area of science, with few standardized methods

Analytical Chemistry

Wastewater markers currently used by FDEP

- sucralose
- acetominophen
- carbamazepine
- primadone
- triclosan methyl
- chlorinated flame retardants (i.e., TCEP, TDCPP, TCPP)
- caffeine
- 1,4-dioxane
- gadolinium
- synthetic musks
  - galaxolide
  - tonalide

Analytical Chemistry

Sucralose

- Artificial sweetener
- Basically a chlorinated sucrose molecule
- Is not broken down through digestion or wastewater treatment processes
- In 2012, D/EAR conducted a survey of Florida wastewater treatment plants detected sucralose concentrations between 10 and 40 parts per billion in final effluent
- Detection limit was approximately 10 parts per trillion

Analytical Chemistry

- Sucralose is found in reuse/reclaimed water at concentrations similar to WWTP effluent
- The detection of sucralose does not indicate the presence of untreated wastewater, only that there has been some mixing of wastewater (treated or untreated) and the surface water
- Additional markers that are not so recalcitrant to degradation by common wastewater treatment processes are needed to help discriminate treated from untreated wastewater sources

Analytical Chemistry

Acetaminophen (Tylenol®)

- Is present in WWTP influents in relatively high concentrations similar to sucralose
- Unlike sucralose, acetaminophen is readily removed by common wastewater treatment processes and is therefore often absent from, or significantly reduced in, final effluents

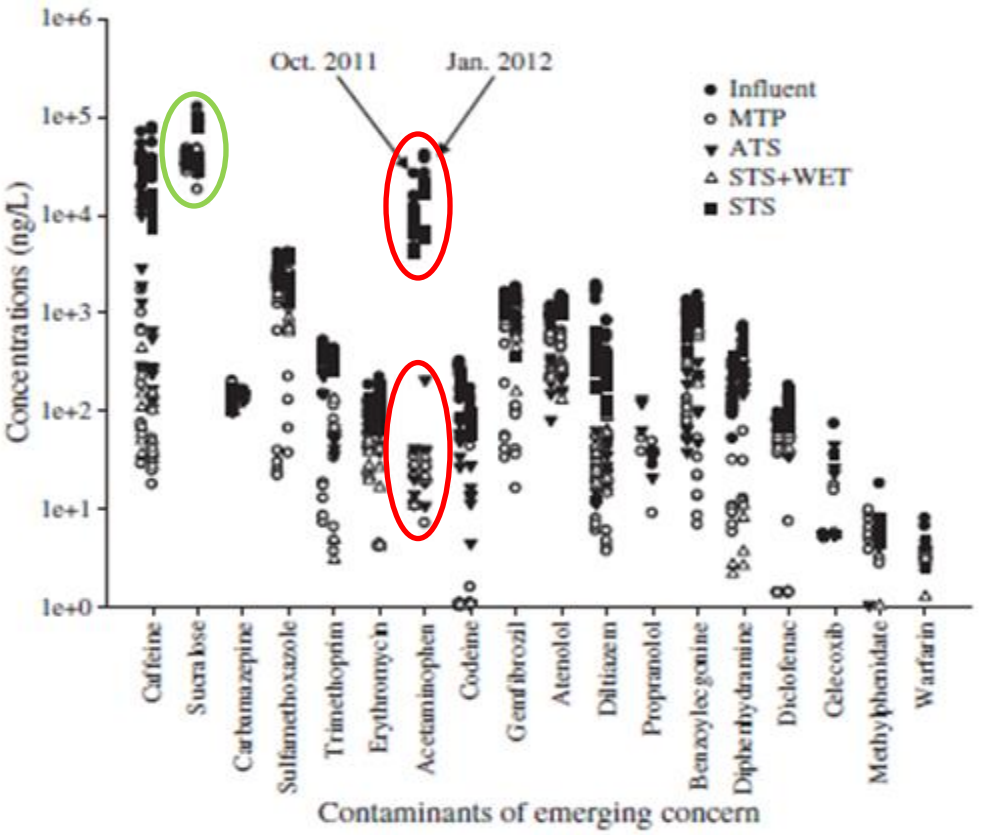


Fig. 2. Detected target contaminants of emerging concern in influent and various effluent discharges by season. MTP = municipal treatment plant; ATS = aerobic treatment system; STS = septic treatment system; WET = wetland.

Kephart, C.M., and Bushon, R.N., 2010, Utility of microbial source-tracking markers for assessing fecal contamination in the Portage River watershed, northwestern Ohio, 2008: U.S. Geological Survey Scientific Investigations Report 2009–5036, 7 p.

Table 1
Concentrations across all 50 effluent samples.

Analyte	CasNumber	Method[a]	RL[b] (ng/L)	Number of measurements	Number of detections	PEC[c] (ng/L)	Mean[d] (ng/L)	Max[d] (ng/L)
10-Hydroxy-amitriptyline	64520-05-4	1	5	50	6	5029	<RL	<RL
Acetaminophen	103-90-2	1	5	50	7	306,955	79 (300)	1500 (4500)
Albuterol	18559-94-9	1	9.7	50	27	471	14	35
Alprazolam	28981-97-7	1	9.1	50	15	103	10	31
Amitriptyline	549-18-8	1	5	50	20	5029	11	110
Amlodipine	111470-99-6	1	5	50	11	94	6.9	18
Amphetamine	51-63-8	1	1.6	50	5	387	3.5	40
Atenolol	29122-68-7	1	6	50	48	4137	940	3000
Atorvastatin	134523-00-5	1	38	48	4	2906	<RL	<RL
Benztropine	86-13-5	1	10	50	0	33	ND	ND
Carbamazepine	298-46-4	1	4.4	50	48	5607	97 (140)	240 (460)
Ciprofloxacin	85721-33-1	2	10	49	30	NA	67 (72)	260 (320)
Clonidine	4205-91-8	1	35	50	0	43	ND	ND
Desmethylsertraline	79902-63-9	1	9.4	50	9	615	9.9 (10)	24
Diltiazem	33286-22-5	1	2.8	49	41	3343	85	340
Diltiazem-desmethyl	130606-60-9	1	1.6	50	34	3343	24	100
Enalapril	76095-16-4	1	1	50	9	369	4.6	38

Table abbreviated

M.S. Kostich et al. / Environmental Pollution 184 (2014) 354–359

Implementing MST Studies

- Waters with elevated fecal indicators would be placed on a Planning List
- MST studies would be initiated on high priority waters (e.g., those perceived as being of greatest risk to human health)
  - human waste contamination
  - cattle waste contamination
  - high potential for exposure through recreational use
- q-PCR, chemistry, land use, and field reconnaissance surveys would be performed to identify likely fecal contamination sources

Implementing MST Studies

- Conditions must be assessed over multiple seasons because site conditions are not static
  - Temperature
  - Rainfall
  - Flow
  - Ground water level
  - Light intensity
  - Land use
    - Agriculture, snow birds, vacationers
  - Wildlife patterns

Implementing MST Studies

- MST studies use a toolbox approach since no one tool is 100% reliable
- Relative source contributions are estimated using a weight of evidence approach
- Results of MST studies are used to direct further Basin Management Action Plan (BMAP) activities

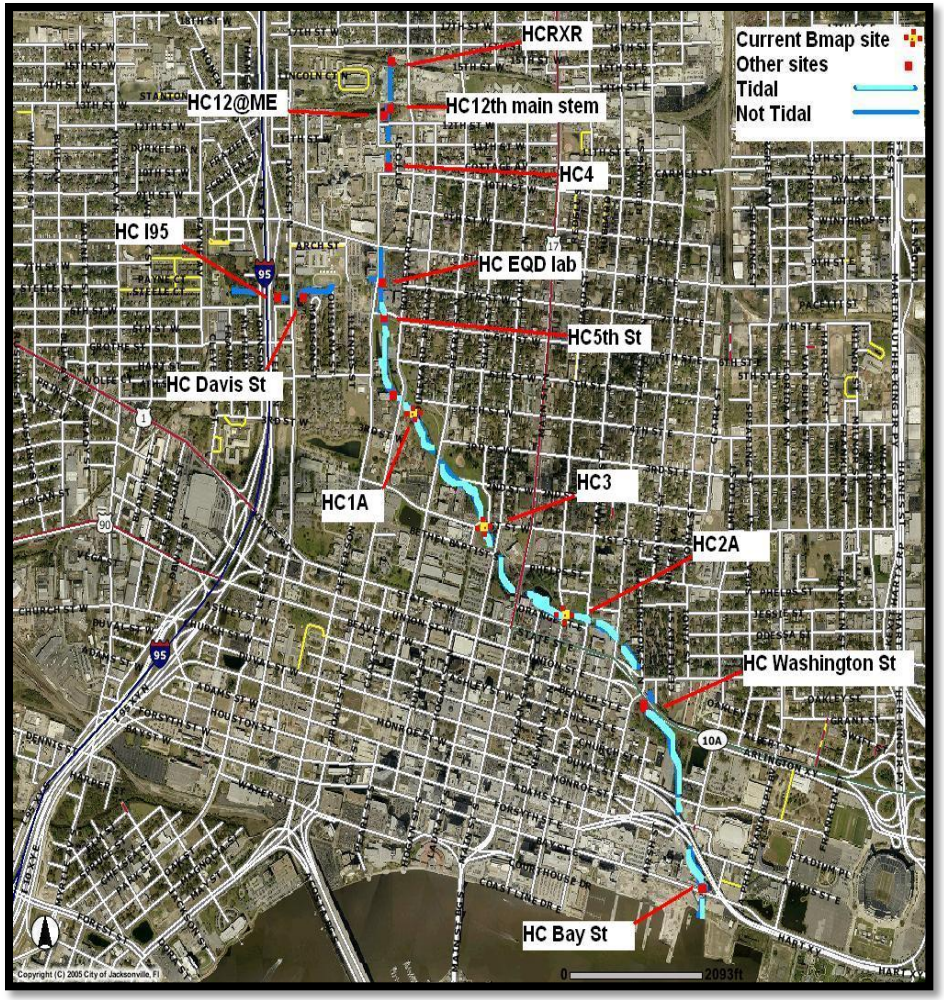
Jacksonville Area MST Studies

WBID Number	WBID Name	TMDL Median (#/100 mL) (1996-2003)	Median (#/100 mL) (2009-2013)	% Reduction	Monitored By
2235	Newcastle Creek	2,500	1,000	60.0%	COJ
2252	Hogan Creek	5,000	1,291	74.2%	COJ
2322	Butcher Pen Creek	2,400	2,600	-8.3%	FDEP
2287	Miller Creek	5,000	4,250	15.0%	COJ
2304	Miramar Creek	7,000	1,100	84.3%	COJ
2280	Big Fishweir Creek	3,000	2,215	26.2%	FDEP
2256	Deer Creek	2,765	440	84.1%	COJ
2204	Terrapin Creek	1,367	700	48.8%	FDEP
2326	Goodbys Creek	3,000	520	82.7%	FDEP
2299	Open Creek	1,000	550	45.0%	FDEP

Jacksonville Area MST Studies

Hogan Creek (2252)

- 3 MST sampling events since 12/2013
- HF183 Human source marker consistently detected throughout stream reach, right up to the culvert at railroad near W.16th St.
- Elevated levels of sucralose (0.24 µg/L) and acetaminophen (0.12 µg/L) detected coming out of RXR culvert
- Similar levels of sucralose and acetaminophen strongly suggest untreated human wastewater source

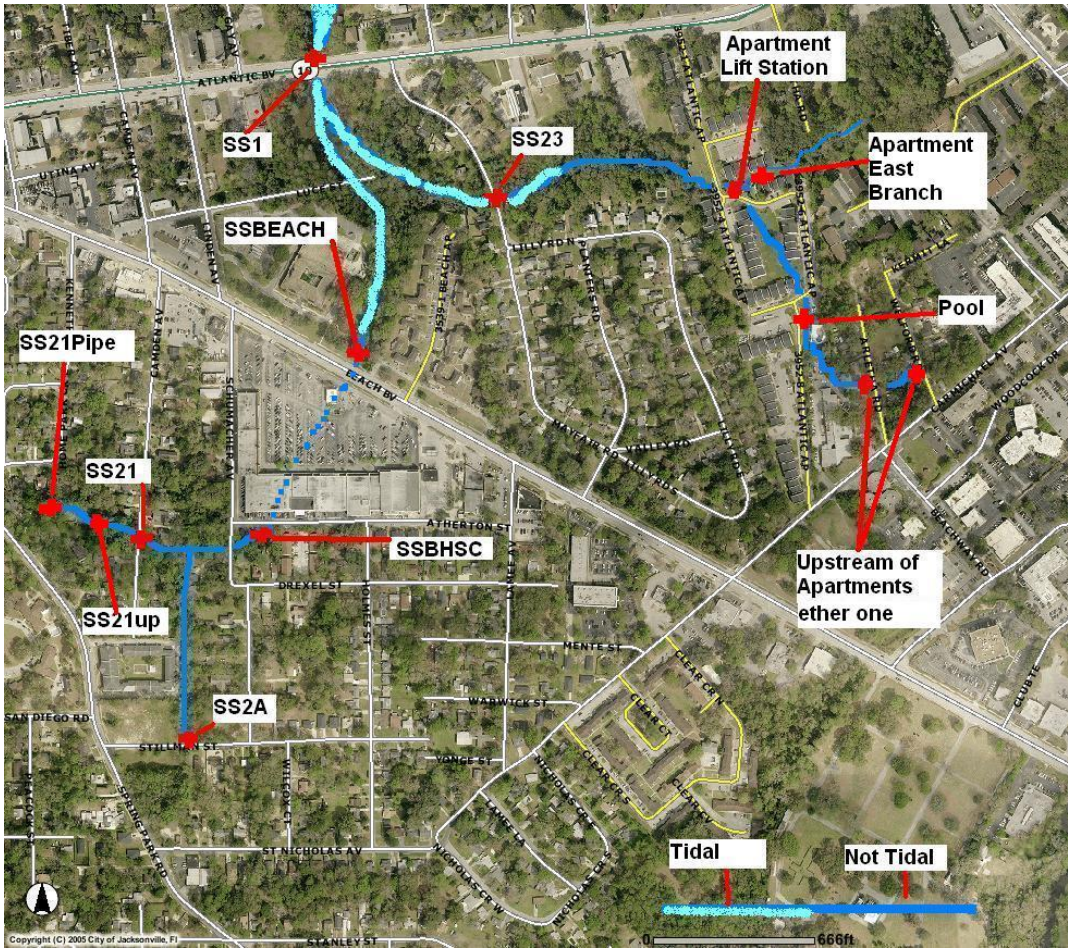


Jacksonville Area MST Studies

Miller Creek (2287)

- Levels of sucralose were elevated, even compared to Hogan Creek

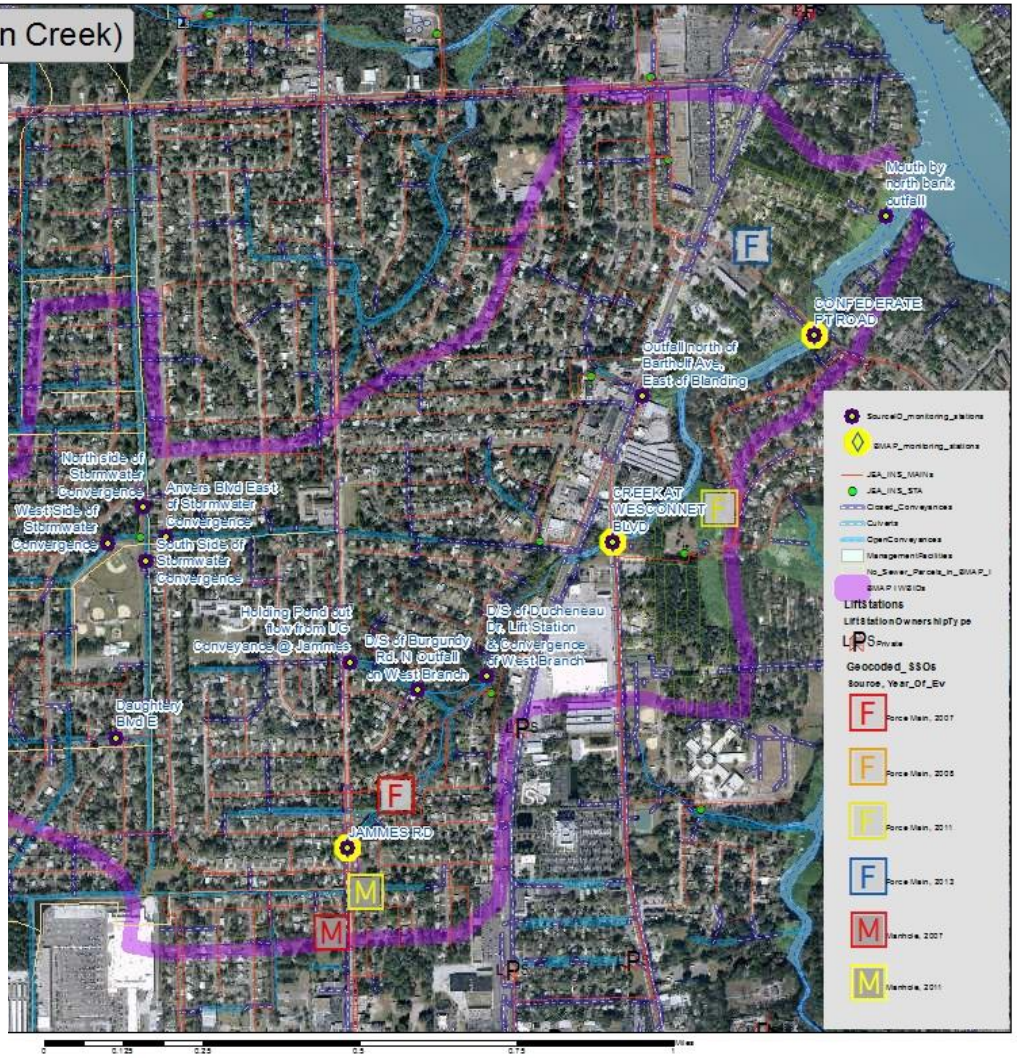
  (0.01U µg/L – 0.53 µg/L)
- Only one site (SS21) didn't have detectable sucralose.
- Pool sampling location 0.53 µg/L) sucralose
- No acetaminophen assays run.
- Despite having bacteria levels in the thousands of CFU/100 ml in water samples and tens of thousands in sediments at several of the sites, no HF183 marker was detected



Jacksonville Area MST Studies

Butcher Pen Creek (2322)

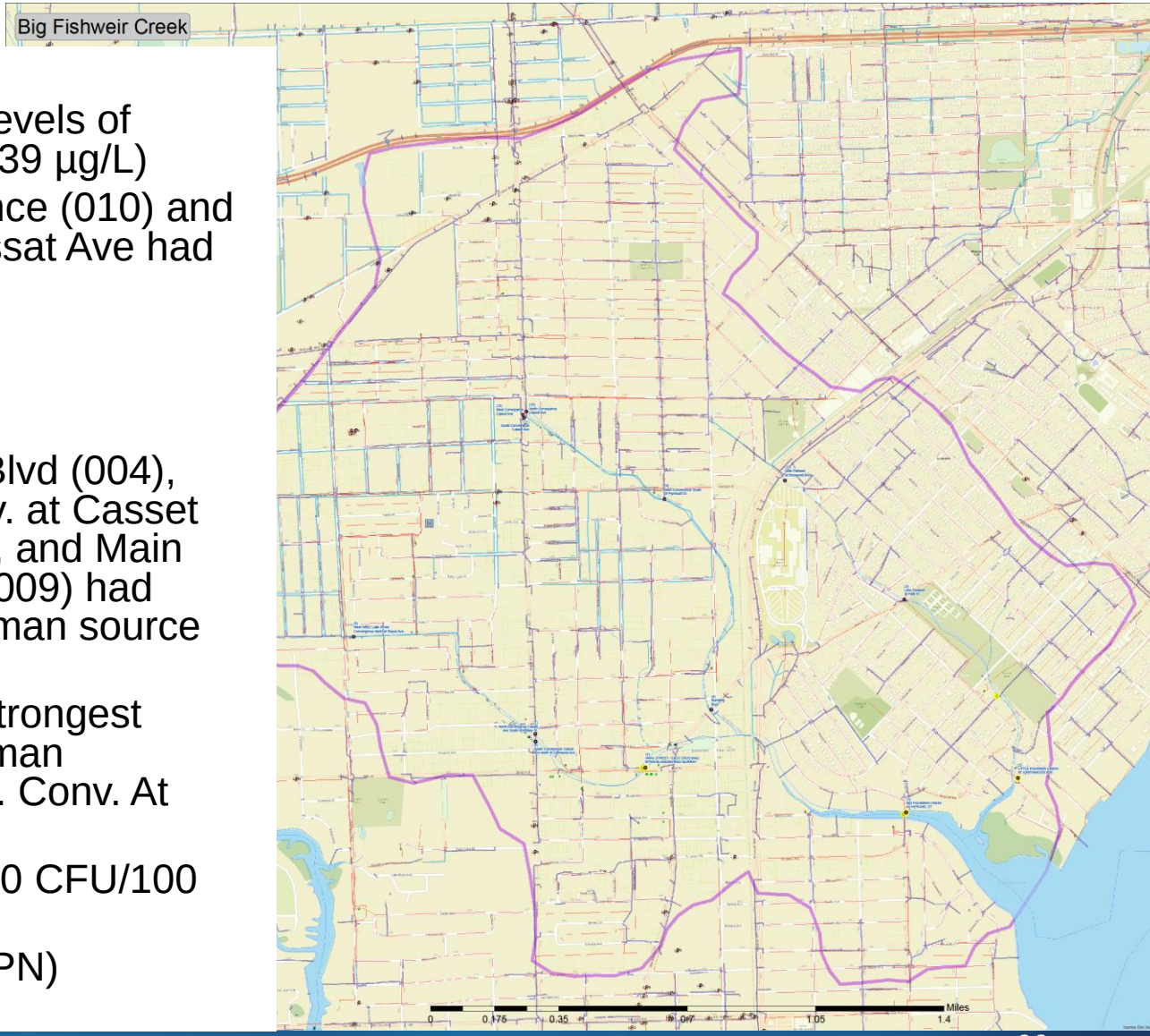
- All sites had measureable levls of sucralose
  (0.041 µg/L – 0.22 µg/L)
- Only Butcher Pen Ck @ Wesconnet Blvd (004) had more than trace levels of acetaminophen (0.0061-0.0072 µg/L)
- All sites but the Outfall N. of Bartholf Ave E. of Blanding(003) had amplification of HF183 human source marker
- Fecal bacteria
  - (520 – 5,400 CFU/100 mL)
- *E. coli* bacteria
  - (248.9 – 2,419.6 MPN)



Jacksonville Area MST Studies

Big FishWeir Creek (2280)

- All sites had measurable levels of sucralose (0.074 µg/L – 0.39 µg/L)
  - Only the North Conveyance (010) and South Conv. (011) at Cassat Ave had more than trace levels of acetaminophen
  - N. Conv. - 0.20J µg/L
  - S. Conv. - 0.036 µg/L
- All sites except Blanding Blvd (004), Park Street (005), N. Conv. at Casset Ave S. of Yerkes St. (007), and Main Conv. S. of Plymouth St. (009) had amplification of HF183 human source marker
- N. Conv. At Cassat St. – strongest indication of untreated human wastewater, followed by S. Conv. At Cassat St.
- Fecal bacteria (210 – 9,000 CFU/100 mL)
- *E. coli* (112.6 – 2,419.6 MPN)



Jacksonville Area MST Studies

Deer (2256)

All sites had at least trace levels of sucralose (0.046 µg/L – 0.093 µg/L)

All sites had measurable acetaminophen (0.0034 µg/L – 0.057 µg/L)

Aplification of HF183 human source marker in Deer Creek @ Talleyrand (001) and Culvert (002) samples

Low but similar concentrations of sucralose and acetaminophen with HF183 human source marker suggests dilute untreated human wastewater present

Fecal bacteria (2,700 CFU/100 mL – TNTC)

E. coli (All > 2,419.6 MPN)

Jacksonville Area MST Studies

Terrapin (2204)

- Sucralose was detected at both sites, at least at trace levels (0.030 µg/L – 0.24 µg/L), with Terrapin Ck @ Alta Rd. (001) having the 0.24 µg/L.
- Acetaminophen was not detected at Terrapin Ck @ Alta Rd. (001), but was detected at trace levels at Terrapin Ck @ Faye Rd (002) (0.00067µg/L – 0.00069 µg/L)
- Low levels of HF183 human marker were detected at both sites
- Fecal bacteria
  - (30 – 530 CFU/100 mL)
- *E. coli* (435.2 – 1,266.8 MPN)

Questions?

Contact Information

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