

# Who Pooped in our Water? Lessons Learned Using Microbial Biomarker Methods

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Charlotte-Mecklenburg Storm Water Services

# Charlotte-Mecklenburg



Fecal Coliform Total Maximum Daily Load for the Irwin, McAlpine, Little Sugar and Sugar Creek Watersheds, Mecklenburg County

Final  
February 2002

## Catawba River Basin



Prepared by:  
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Water Quality Section – Planning Branch  
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**Fecal Coliform Total Maximum Daily Load for the McKee and Clear Creek Watersheds, Mecklenburg and Cabarrus Counties, North Carolina**

Final Report  
June 2003  
(Approved August 2003)

## Yadkin River Basin

Prepared by:  
NC Department of Environment and  
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Division of Water Quality  
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Through assistance from:  
TMDL Modeling and Support Section  
EPA, Region 4, Atlanta, GA

Total Maximum Daily Load – Steele Creek  
(Hydrologic Unit Code 030501030108)  
Stations CW-009, CW-011, CW-203  
Fecal Coliform Bacteria

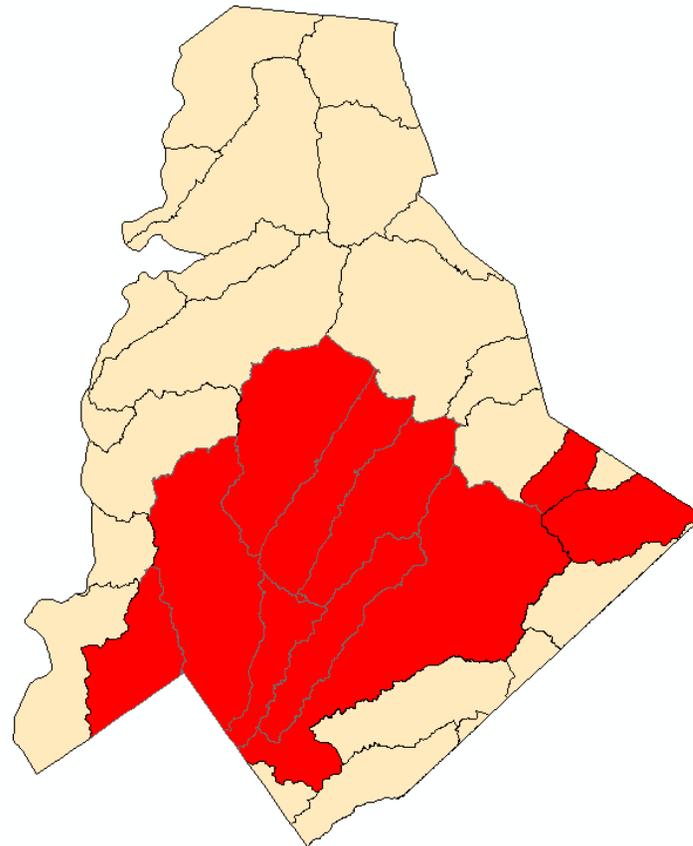


May 2007  
Bureau of Water



SCDHEC Technical Report Number: 010-07

# Fecal TMDL Watersheds



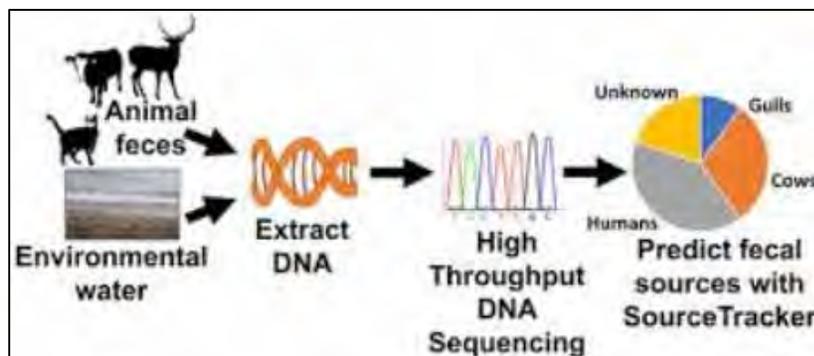
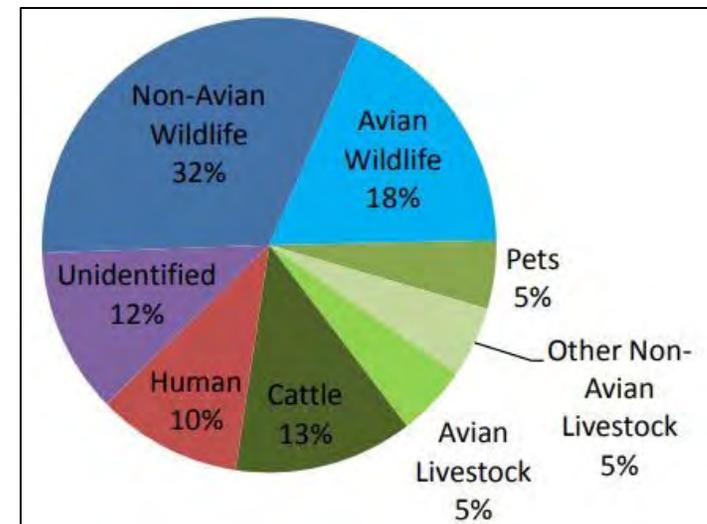
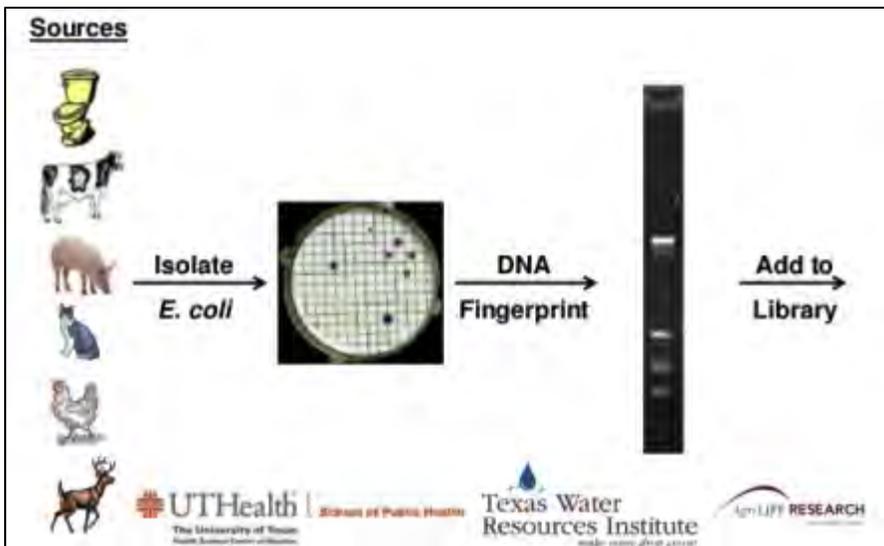
# Microbial Source Tracking



Methods used to identify and quantify the extent to which different source species are contributing to the microbial contamination in a water body.



# Library-Based Methods



# 2005 Study for TMDL



*Pathogen Source Assessment  
for  
TMDL Development and Implementation in  
North Carolina Piedmont and Coastal Plain Watersheds*

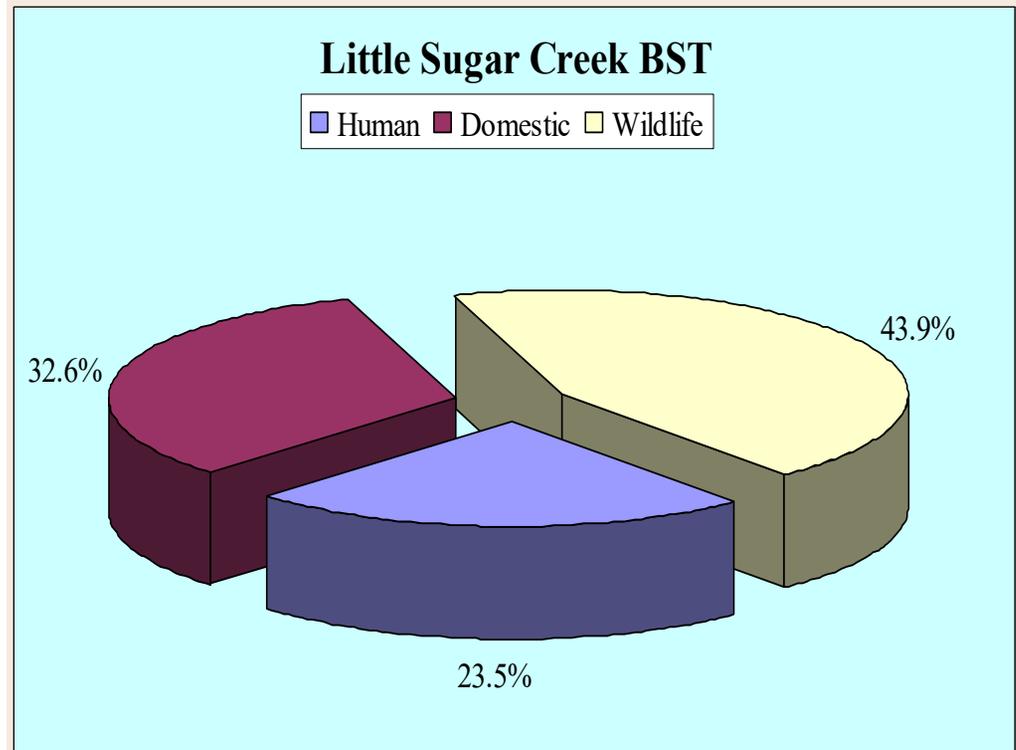
Prepared for:  
North Carolina Department of Environment and  
Natural Resources Division of Water Quality  
(RFP # 16-EW03032)



# 2005 Source Tracking Study



- Antibiotic Resistance Analysis (ARA)
- Develop library of known fecal sources
- Isolate colonies
- Subject “isolates” to antibiotics
- Identify source-related patterns
- Compare to patterns in water samples



# Library-Dependent Methods



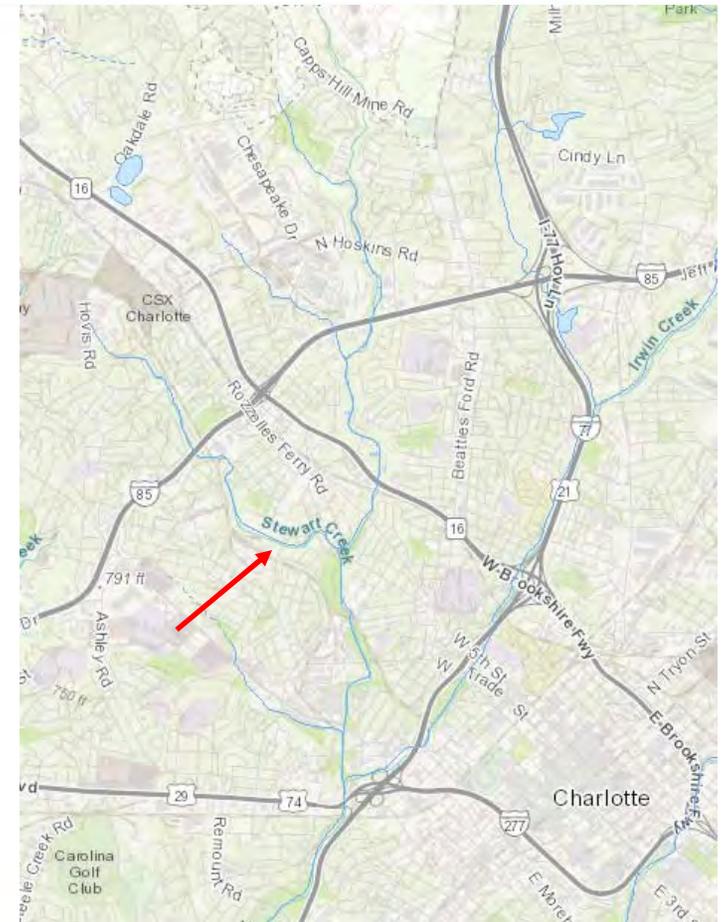
## Challenges & Limitations

- Time & investment required for library development
- Geographic variability
- Traits that are assayed show considerable overlap among species
- High rate of false positives
- Pie charts inaccurate

# Stewart Creek, 2013-2014



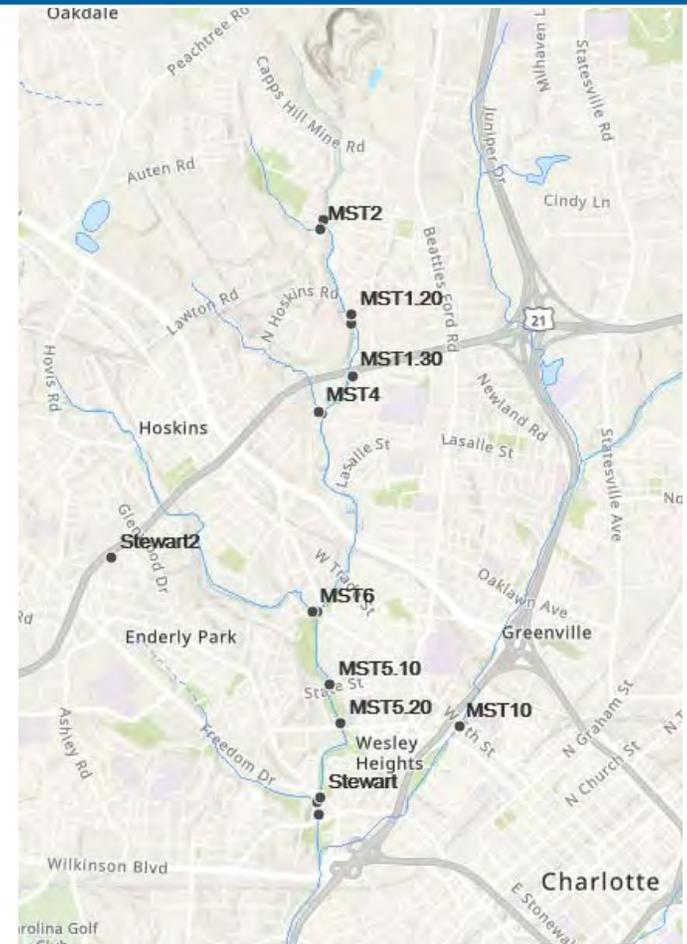
- Flooding
- High Fecal
- Equity concerns
- Stewart Creek Environmental Association
- Does the City or County care?
- What are they going to do about this?



# Where is it coming from?



- Is it human or just pet waste?
- New Molecular Source Tracking Methods
  - Species specific molecular biomarkers
  - Biomarker quantification
  - No library needed
  - Less geographic variability



# MST monitoring 2014-2017

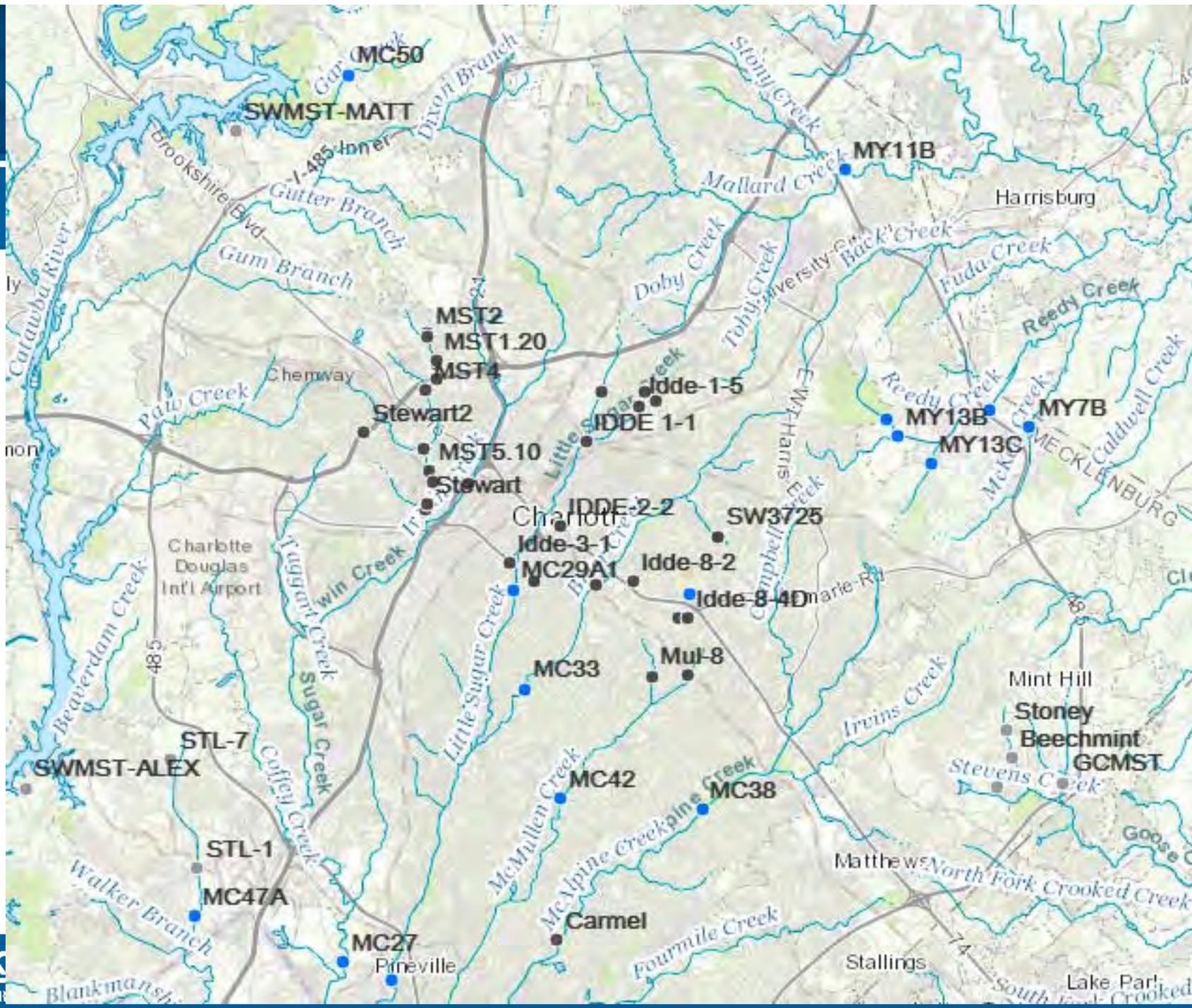


- Fecal, E. coli, and Enterococcus collected alongside MST samples
- Stewart Creek
- Stream walks (samples >1,000 CFU/100mL)
- Fixed interval sites with high fecal numbers
- IDDE sites with high fecal
- Watersheds with fecal TMDL

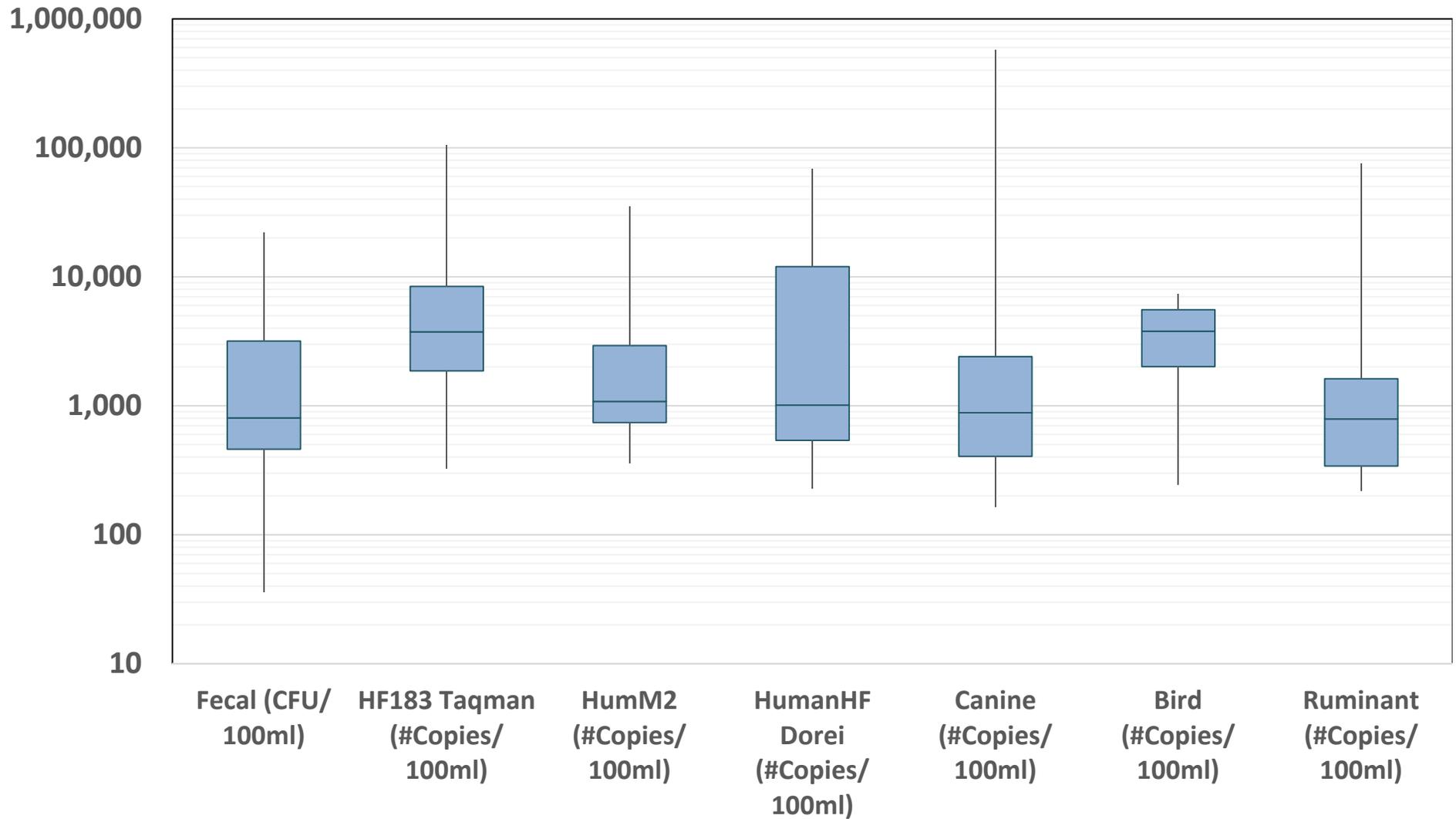
# MST monitoring

- 57 sites sampled
- Storm & ambient
  - Stewart Creek
  - Reedy Creek
- Ambient only
  - TMDL
  - FIM
  - IDDE
- Most sites sampled only once/condition (screening)
- Biomarkers - Human, canine, bird, ruminant, beaver





# Fecal Coliform & Biomarker Ranges All Data



# Human Biomarkers



- 2014 – HF183 & HumM2
- 2015 – HF183 only
- 2016 & 2017 – HF Dorei



	Site	Date	Fecal (CFU/100ml)	HF183 Taqman (#Copies/100ml)	HumM2 (#Copies/100ml)
A m b i e n t	MST3	1/2/2014	450	10,900	4,060
	MST5	1/2/2014	160	2,160	740
	MST6	1/2/2014	200	665	780
	MST7	1/2/2014	160	10,500	2,150
	MST8	1/2/2014	1,500	95,300	35,300
	MST9	1/2/2014	150	11,300	2,930
	MST10	1/2/2014	520	673	-
S t o r m	MST3	1/10/2014	3,000	6,220	2,810
	MST5	1/10/2014	1,200	5,180	728
	MST6	1/10/2014	2,700	trace	618
	MST7	1/10/2014	2,000	2,570	1,040
	MST8	1/10/2014	3,800	56,500	12,500
	MST9	1/10/2014	940	4,410	1,080
	MST10	1/10/2014	3,700	1,020	357

HF183 routinely  
3 times HumM2



	Site	Date	Fecal (CFU/100ml)	HF183 Taqman (#Copies/100ml)	HumM2 (#Copies/100ml)
A m b i e n t	MST3	1/2/2014	450	10,900	4,060
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HF183 routinely  
3 times HumM2



	Site	Fecal (CFU/100ml)	HF183 Taqman (#Copies/100ml)	Canine (#Copies/100ml)	Bird (#Copies/100ml)
A m b i e n t	MST3	450	10,900	ND	ND
	MST5	160	2,160	ND	ND
	MST6	200	665	359	ND
	MST7	160	10,500	ND	ND
	MST8	1,500	95,300	ND	ND
	MST9	150	11,300	ND	ND
	MST10	520	673	ND	ND
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	MST5	1,200	5,180	ND	ND
	MST6	2,700	trace	trace	ND
	MST7	2,000	2,570	ND	ND
	MST8	3,800	56,500	ND	trace
	MST9	940	4,410	ND	ND
	MST10	3,700	1,020	ND	ND

Fecal Coliform increases during wet weather but human biomarker decreases and no other biomarkers are detected.



	Site	Fecal (CFU/100ml)	HF183 Taqman (#Copies/100ml)	Canine (#Copies/100ml)	Bird (#Copies/100ml)
A m b i e n t	MST3	450	10,900	ND	ND
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	MST6	200	665	359	ND
	MST7	160	10,500	ND	ND
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	MST6	2,700	trace	trace	ND
	MST7	2,000	2,570	ND	ND
	MST8	3,800	56,500	ND	trace
	MST9	940	4,410	ND	ND
MST10	3,700	1,020	ND	ND	

Makes sense



			HF183		
	Site	Fecal (CFU/100ml)	Taqman (#Copies/100ml)	Canine (#Copies/100ml)	Bird (#Copies/100ml)
A m b i e n t	MST3	450	10,900	ND	ND
	MST5	160	2,160	ND	ND
	MST6	200	665	359	ND
	MST7	160	10,500	ND	ND
	MST8	1,500	95,300	ND	ND
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	MST6	2,700	trace	trace	ND
	MST7	2,000	2,570	ND	ND
	MST8	3,800	56,500	ND	trace
	MST9	940	4,410	ND	ND
	MST10	3,700	1,020	ND	ND

Low fecal during dry but several biomarkers detected. During wet, fecal increases but no biomarkers were detected.



	Site	Fecal (CFU/100ml)	HF183 Taqman (#Copies/100ml)	Canine (#Copies/100ml)	Bird (#Copies/100ml)
A m b i e n t	MST3	450	10,900	ND	ND
	MST5	160	2,160	ND	ND
	MST6	200	665	359	ND
	MST7	160	10,500	ND	ND
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	MST8	3,800	56,500	ND	trace
	MST9	940	4,410	ND	ND
	MST10	3,700	1,020	ND	ND

Fecal higher in wet compared to dry, human higher in dry vs wet, no other biomarker contributions.

# Non-human data examples



Site	Date	Fecal (CFU/100ml)	HumanHF Dorei (#Copies/100ml)	Canine (#Copies/100ml)	Bird (#Copies/100ml)
Fairington	6/28/2017	10,800	228	6,010	trace

Site	Date	Fecal (CFU/100ml)	HumanHF Dorei (#Copies/100ml)	Canine (#Copies/100ml)	Ruminant (#Copies/100ml)
MC50	5/25/2016	1,000	trace	trace	1,530

# Summary of Results



	HF183 Taqman	HumM2	HumanHF Dorei	Canine	Bird	Ruminant	Beaver
Total samples	67	22	29	82	84	59	29
% not detected	18%	41%	10%	32%	55%	63%	62%
% trace	6%	0%	34%	27%	43%	15%	28%
<b>Total % quantified</b>	<b>76%</b>	<b>59%</b>	<b>55%</b>	<b>41%</b>	<b>2%</b>	<b>22%</b>	<b>10%</b>
Max (#Copies/100mL)	106,000	35,300	68,300	573,000	7,320	75,100	3,180
Avg (#Copies/100mL)	8,840	2,959	11,175	11,301	158	1,802	295

# Summary of Results



	HF183 Taqman	HumM2	HumanHF Dorei	Canine	Bird	Ruminant	Beaver
Total samples	67	22	29	82	84	59	29
% not detected	18%	41%	10%	32%	55%	63%	62%
% trace	6%	0%	34%	27%	43%	15%	28%
Total % quantified	76%	59%	55%	41%	2%	22%	10%
Max (#Copies/100mL)	106,000	35,300	68,300	573,000	7,320	75,100	3,180
Mean (#Copies/100mL)	8,840	2,959	11,175	11,301	158	1,802	295

# Actions taken



- Stewart Creek
  - Human influence was demonstrated
  - Fecal and HF183 used to prioritize sewer investigations
  - Pet waste information targeted to areas with high canine biomarkers

# Questions



- Significance of different human biomarkers?
- Trace?
- Relationship between biomarkers & FIB #?
- What can we conclude so far?
- How should we proceed with future monitoring?

# Review of data by expert



- All MST data
- FIB
- pH
- Turbidity
- Rainfall
- Temperature
- Monitoring location descriptions
- Map

MST RESULTS												
	Sit	Dat	Time	Flow [CFD/1000]	Flow [MGD]							
A	MC204	8/27/2017	11:00	4,400	607	78						
	MC206	8/27/2017	12:30	525	462	23						
	MC45	8/27/2017	11:20	405	483	45						
D	Brookdale	8/28/2017	8:20	4,400	4,283	unloaded						
	Patricialee	8/28/2017	8:27	48,800	42,835	unloaded						
	Shaw	8/28/2017	8:40	4,800	4,515	unloaded						
E	GCMSY	8/28/2017	10:30	5,200	5,427	264						
	STL-2	8/28/2017	10:00	637	4,300	10						
	STL-4	8/28/2017	10:05	510	420	455						
T	DUPMST-ALEX	8/28/2017	18:45	3,400	2,385	3,240						
	DUPMST-PMY	8/28/2017	3:15	510	883	410						
A	D09	5/18/2016	8:58	300	800	540				7,300	404	leaver
	MC204	5/18/2016	2:48	8,400	3,400	300				59,300	leaver	leaver
	MC306	5/18/2016	8:05	370	470	300				leaver	leaver	leaver
D	MC308	5/18/2016	8:05	300	4,800	250				4,300	284	leaver
	MST8	5/18/2016	3:25	2,400	2,800	500				12,300	leaver	leaver
	MUL-10	5/18/2016	8:55	6,500	3,100	840				leaver	leaver	leaver
E	MUL-8	5/18/2016	8:55	5,400	2,700	2,700				825	leaver	leaver
	MY15	5/17/2016	3:45	540	850	520				leaver	leaver	leaver
	MY15B	5/17/2016	18:35	4,500	4,400	2,800				leaver	leaver	leaver
T	MY15D	5/17/2016	18:10	5,400	850	4,300				leaver	leaver	leaver
	MY15C	5/17/2016	18:20	300	4,500	850				leaver	leaver	leaver
	MC58	5/25/2016	3:15	4,800	630	540				leaver	leaver	leaver
T	DUP152	5/25/2016	18:35	4,200	4,100	500				865	leaver	leaver
	DUP224	5/25/2016	18:25	16,800	16,800	20,000				leaver	leaver	leaver
T	MY15	8/8/2016	8:10	15,800	14,800	3,200				leaver	5,720	leaver
	MY15B	8/8/2016	8:30	12,800	16,800	3,800				leaver	24,500	leaver
	MY15D	8/8/2016	8:25	28,800	16,800	5,400				leaver	4,900	leaver
H	MC15C	8/8/2016	2:45	8,200	16,800	4,800				leaver	18,500	leaver
D	Stewart	8/15/2015	11:00	840	4,100	4,300				20,300	5,100	unloaded
	IDDE-1-7	8/15/2015	18:20	3,200	2,300	2,800				874	25,300	leaver
	IDDE-1-8	8/15/2015	18:30	22,400	3,200	24,000				2,270	570,400	unloaded
E	IDDE-B-4C	8/15/2015	18:45	630	4,200	4,100						unloaded
	IDDE-B-4D	8/15/2015	18:48	6,200	6,800	7,000				leaver	300	leaver
	MC204	8/15/2015	7:55	200	200	100				10,500	leaver	leaver
H	MC35	8/17/2015	8:10	700	450	450				100,000		
	MC42	8/17/2015	8:30	500	740	2,400				4,350	844	845
	MC38	8/17/2015	8:50	850	700	850				leaver		
H	MC45B	8/17/2015	18:20	800	650	200				5,300	leaver	leaver
	MC27	8/17/2015	18:30	320	4,400	3,100				1,820	282	200
	MC17M	8/17/2015	18:50	5,100	500	350				leaver		5,100
H	MY17	8/17/2015	18:10	200	900	600				4,800	4,550	
	MY15	8/17/2015	18:25	630	400	560				5,000	2,200	leaver
	MY15B	8/17/2015	18:30	600	4,100	4,300				5,740	323	leaver
H	Stewart	8/26/2015	11:00	3,100	2,300	2,800					424	leaver
	MC306	8/26/2015	3:20	500	520	2,800					2,400	leaver
	IDDE-B-2	8/26/2015	3:30	630	500	4,100						

# Sensitivity & Specificity



- Sensitivity - likelihood of biomarker X being detected in fecal samples from species X
- Specificity/Cross reactivity - likelihood of detecting biomarker X in fecal samples from species Y or Z

# Sensitivity & Specificity



- HF183 – very sensitive and specific
- DogBac – low sensitivity and specificity
  - Detected in deer, human, and goose feces
- Rum2Bac – low sensitivity and specificity
  - Detected in bird, human, and dog feces

# Main observation

- HF183 results are dependable and indicate significant human contributions at multiple locations



# Limitations



- Inadequate information from the lab
  - Couldn't interpret "trace"
  - Unclear why different human biomarkers were used
  - Insufficient assessment of sensitivity and specificity
- Low number of samples per location and condition

# Recommendations



- Focus on human sources – most closely related to human illness
  - HF183
  - Other methods – viral
- Collect at least 10 wet & 10 dry samples per location
- Non-human biomarkers – avoid or test for sensitivity and specificity using 10 fecal samples each for human, dog, ruminant, bird

# Lessons Learned



- New and experimental monitoring – involve experts who can help develop monitoring plan and interpret results
- Up front - try to understand meaning of results and how to interpret
  - What is high?
  - What is trace?
  - What is good vs bad?
- Understand strengths and weaknesses

# Fecal Coliform Next Steps?



It



s!

# Next Steps

- Future use of MST TBD
- Charlotte Water lab biomarker development
- Follow development of the science as biomarkers improve and new methods are developed



# Questions?

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