Use of Host Specific Fecal Indicator Bacteria Microbial Source Tracking to Identify Contributions to Bacterial Impairments in Tidal Creeks along the Grand Strand, SC

J. Michael Trapp, Susan Libes,

Amanda Sturgeon, and Erin J. Burge,

The Times-Union, Jacksonville, Thursday, August 9, 2001

Beach closings nearly double due to bacteria, group says

Associated Press

WASHINGTON — Swimmers faced sewage-polluted waters that closed beaches across the nation nearly twice as often last year as the prior year, an environmental group said yesterday.

A survey released by the Natural Resources Defense Council cites 11,270 beach closings and advisories in 2000, with 85 percent due to elevated bacteria counts that exceeded federal swimmer safety standards.

The group wants the Bush administration to implement new federal water quality standards, announced just before President Clinton left office, aimed at cleaning up coastal pollution and reducing storm water and agriculture runoff polluting about 21,000 lakes, ponds, streams and rivers across the country.

"They've been put on hold indefinitely," Nancy Stoner, director of the NRDC's clean water program, said. "It's time to clean up our impaired waters."

HITTING HOME The following Northeast Florida beaches are monitored every two weeks: Hanna Park, Neptune, Atlantic, Jacksonville, Seminole, Huguenot and Little Talbot, according to the Natural Resources Defense Council.

Although the high bacteria levels were mainly due to increased rain and more frequent municipal and state monitoring, the council's 11th annual report also points to a 40 percent jump in the number of beaches reporting pollution problems from an …

bums" for a second year in a row for failing to regularly monito… their coastlines. Texas an… Washington state had been i… that category last year but wer… removed for having limite… monitoring.

In the past year, 11 states — Alabama, California, Florid… Georgia, Maine, Massachusett… Mississippi, North Carolin… Ohio, South Carolina and Texa… — initiated or expanded mon… toring programs. Californi… Massachusetts, and Florida als… passed legislation requiring be… ter beach monitoring and publ… notification.

More than a third of the beac… closures and advisories — i… … lar spots … ix in the … Beach in … t and Mi-

MORE DIRTY BEACHES

Michigan is spending millions on tourism campaigns to draw visitors to the state's impressive shoreline -- a shoreline that is suffering more and more from environmental degradation.

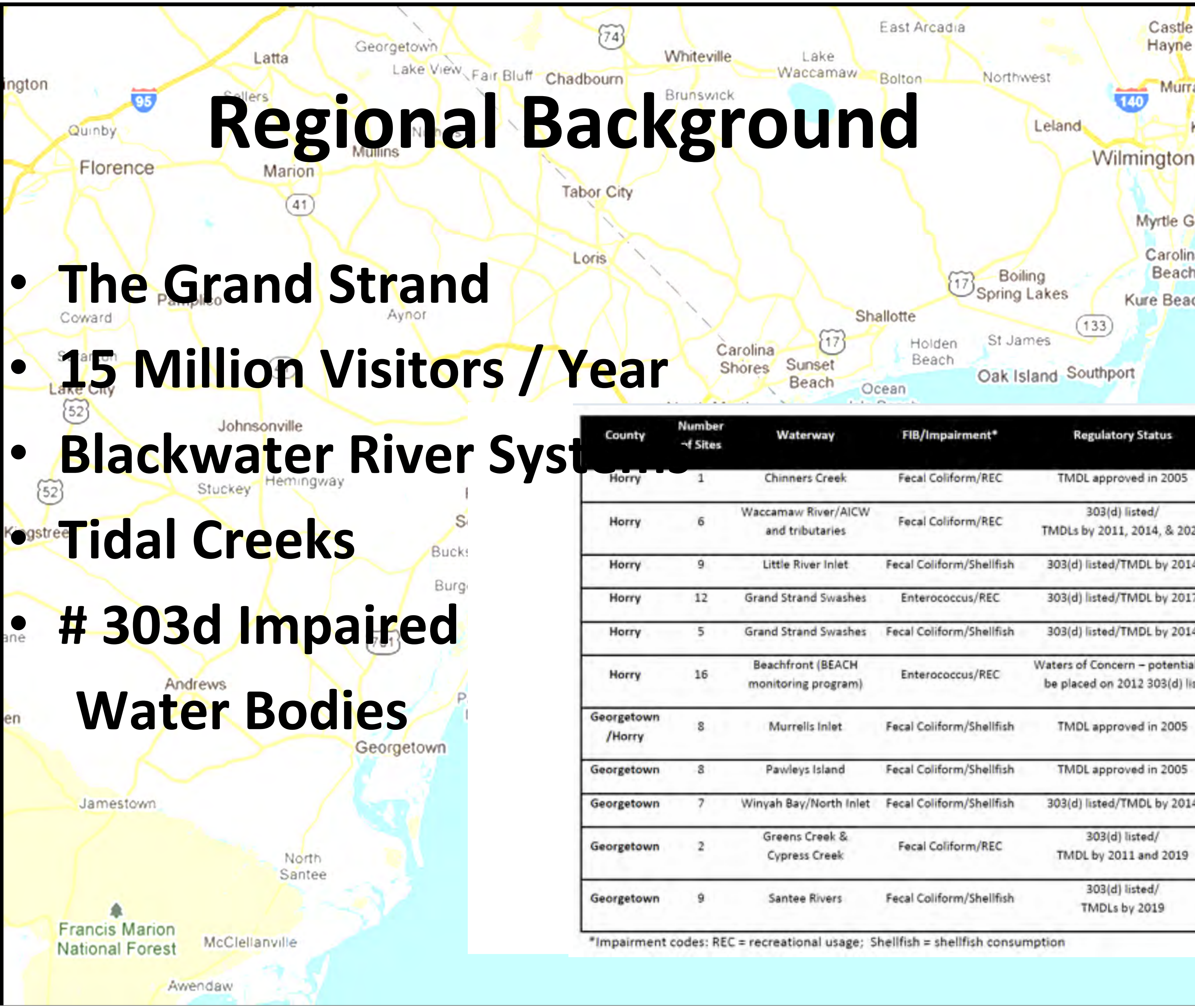
BEACH CLOSURES AT MICHIGAN BEACHES

YEAR	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
DAYS CLOSED	122	211	>140	578	474	886	1,568	697	1,596	1,003	536*
% OF MONITORED BEACHES W/CLOSURES	NA	NA	10.2	13.5	19	18.4	2.9	14.8	19.8	24.3	24*

* 2011 DATA WAS THROUGH SEPT. 1

SOURCE: MICHIGAN DEPARTMENT OF ENVIRONMENTAL QUALITY

Regional Background



- The Grand Strand
- 15 Million Visitors / Year
- Blackwater River Systems
- Tidal Creeks
- # 303d Impaired Water Bodies

County	Number of Sites	Waterway	FIB/Impairment*	Regulatory Status
Horry	1	Chinners Creek	Fecal Coliform/REC	TMDL approved in 2005
Horry	6	Waccamaw River/AICW and tributaries	Fecal Coliform/REC	303(d) listed/ TMDLs by 2011, 2014, & 2023
Horry	9	Little River Inlet	Fecal Coliform/Shellfish	303(d) listed/TMDL by 2014
Horry	12	Grand Strand Swashes	Enterococcus/REC	303(d) listed/TMDL by 2017
Horry	5	Grand Strand Swashes	Fecal Coliform/Shellfish	303(d) listed/TMDL by 2014
Horry	16	Beachfront (BEACH monitoring program)	Enterococcus/REC	Waters of Concern – potential to be placed on 2012 303(d) list
Georgetown /Horry	8	Murrells Inlet	Fecal Coliform/Shellfish	TMDL approved in 2005
Georgetown	8	Pawleys Island	Fecal Coliform/Shellfish	TMDL approved in 2005
Georgetown	7	Winyah Bay/North Inlet	Fecal Coliform/Shellfish	303(d) listed/TMDL by 2014
Georgetown	2	Greens Creek & Cypress Creek	Fecal Coliform/REC	303(d) listed/ TMDL by 2011 and 2019
Georgetown	9	Santee Rivers	Fecal Coliform/Shellfish	303(d) listed/ TMDLs by 2019

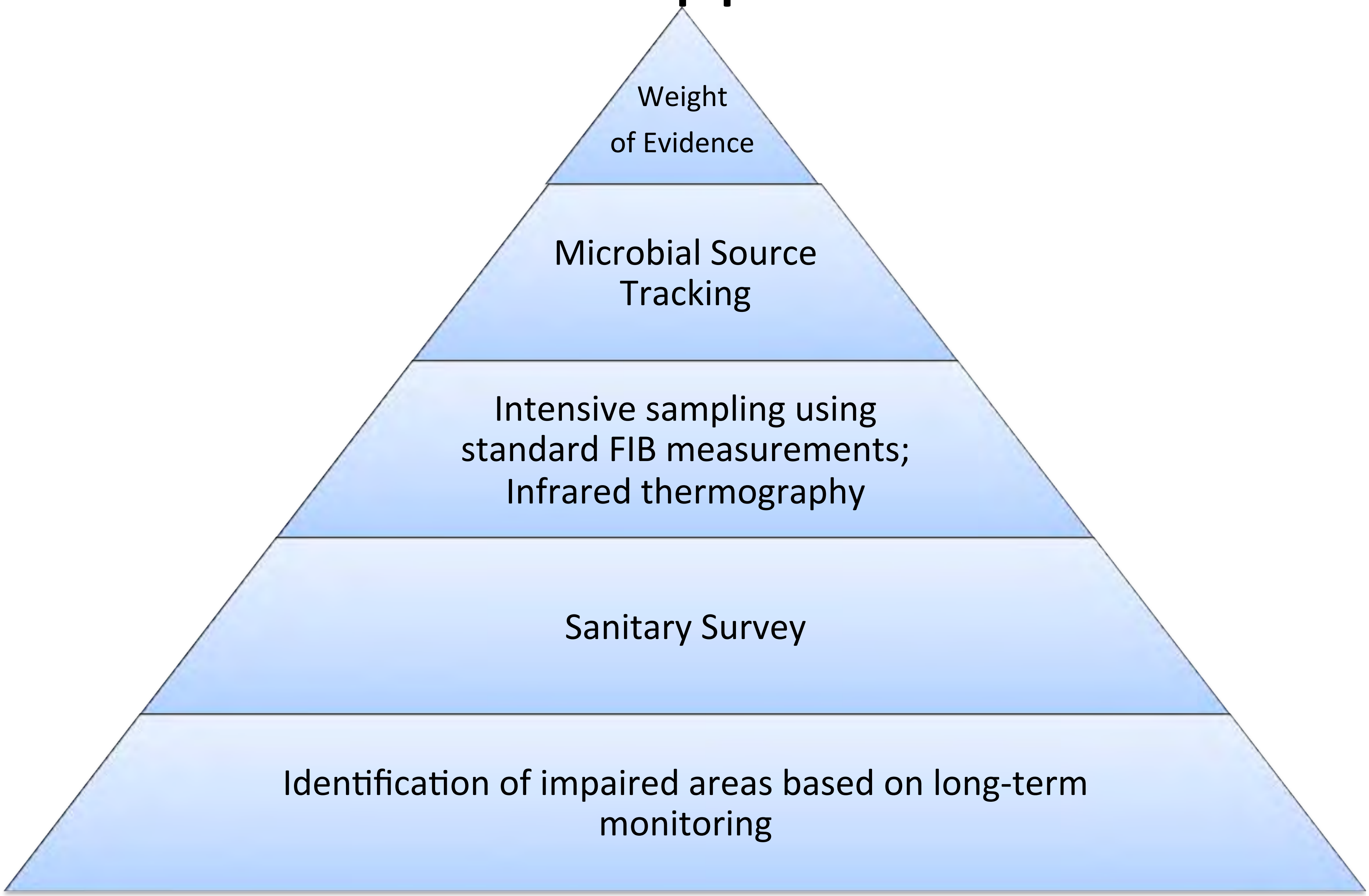
*Impairment codes: REC = recreational usage; Shellfish = shellfish consumption


12:56 JAN/24/2012

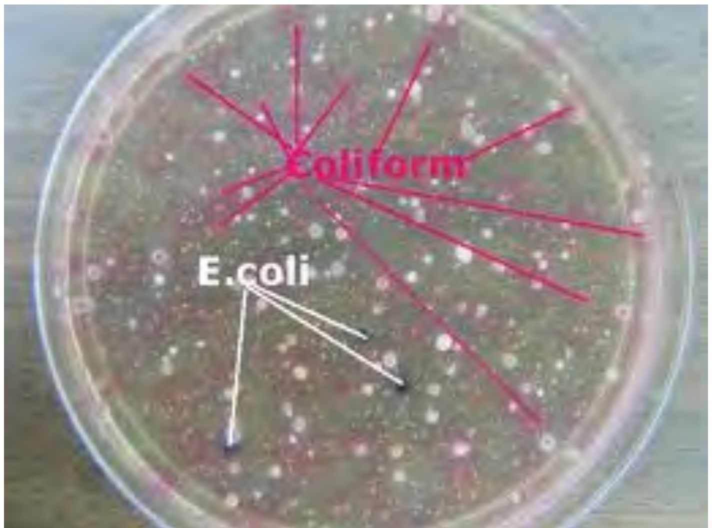
Microbial Source Tracking

- Pathogen Contamination
  - What is it?
  - Where is it coming from?
  - Who is making it?
  - When is it occurring
  - How can we address it?

Tiered Approach



Traditional Culturing Methods

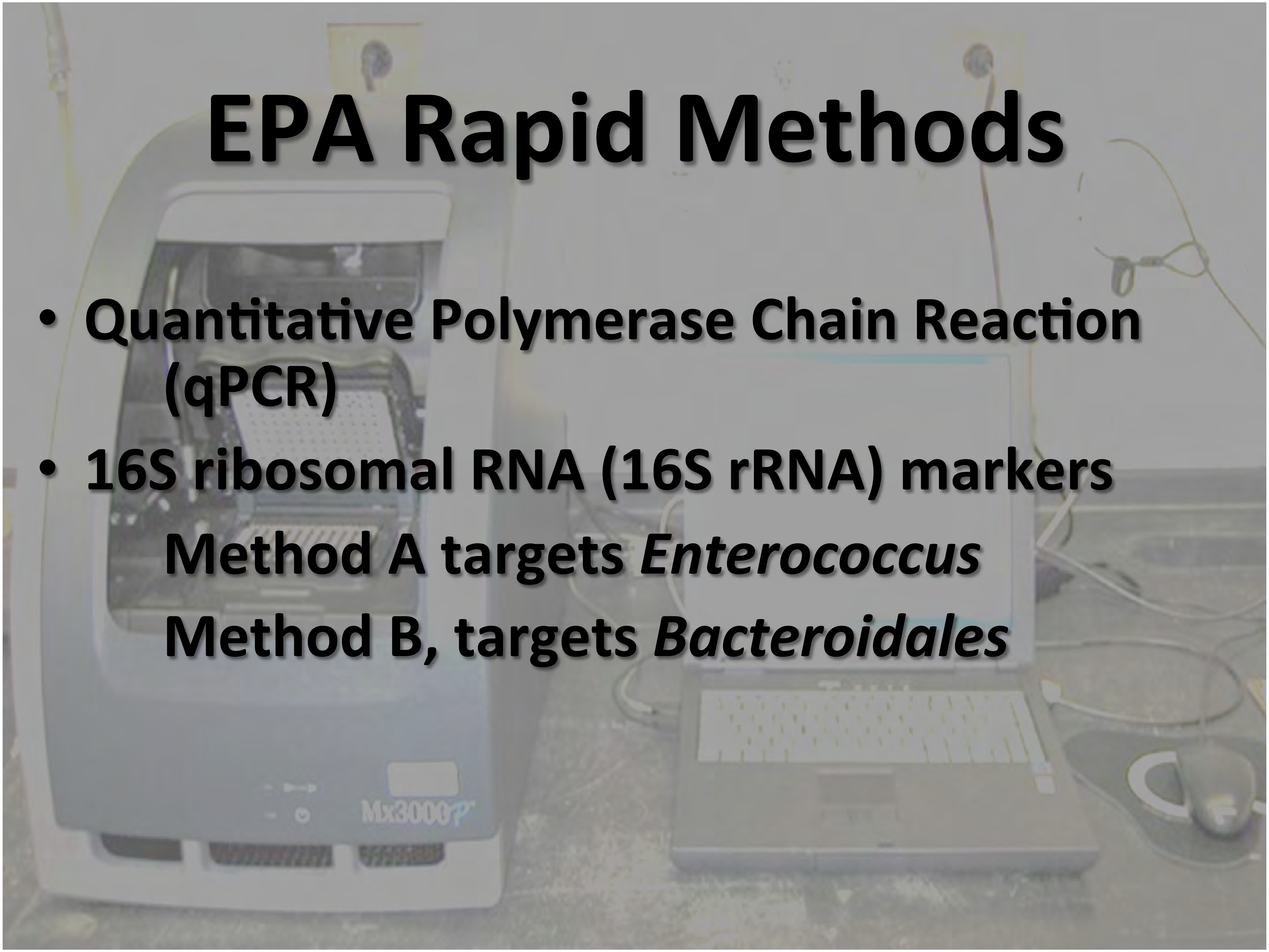


EPA Rapid Methods

- **Quantitative Polymerase Chain Reaction (qPCR)**
- **16S ribosomal RNA (16S rRNA) markers**

  Method A targets *Enterococcus*

  Method B, targets *Bacteroidales*



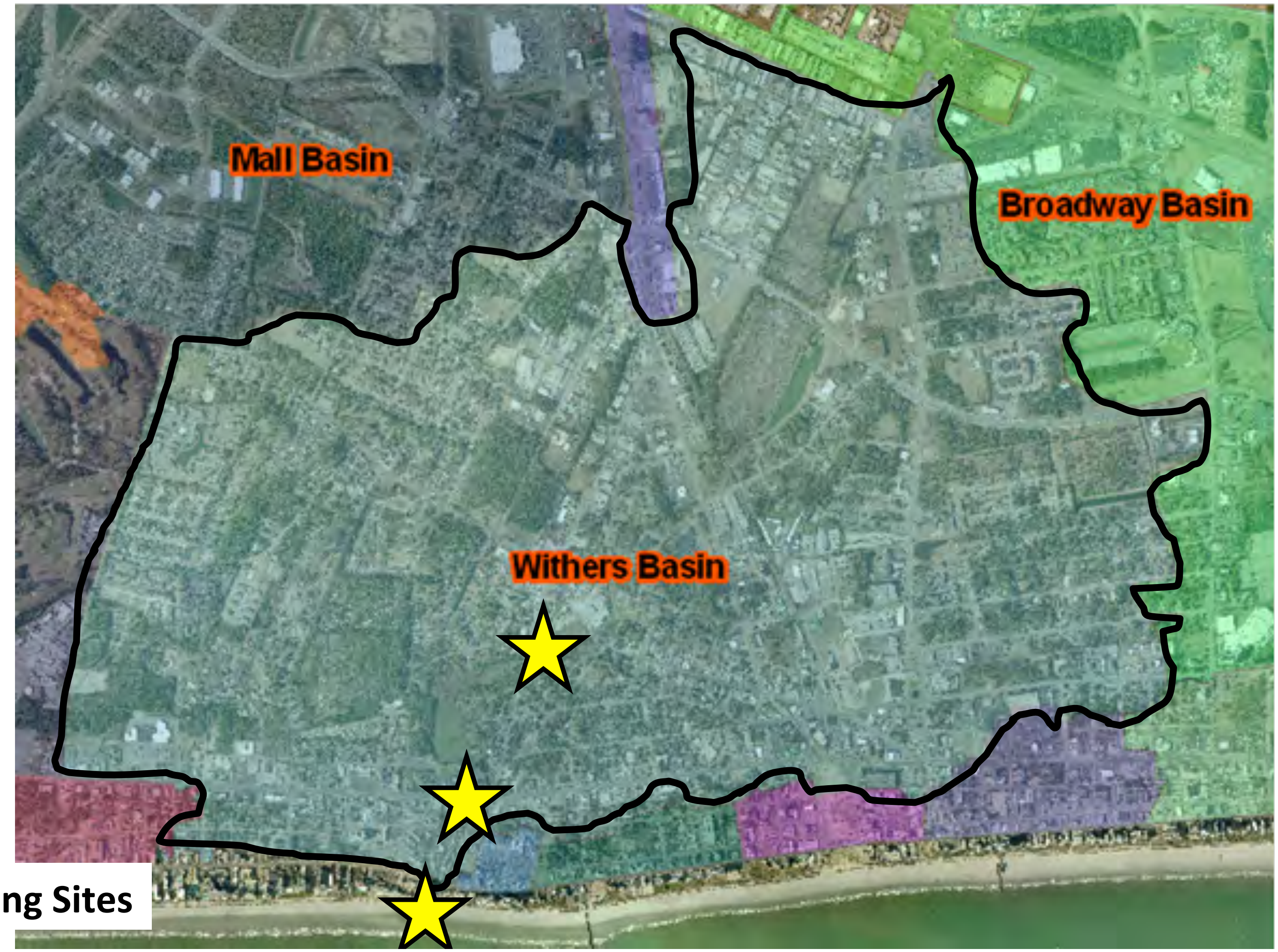
qPCR in Microbial Source Tracking

- 16S rRNA gene found in nearly all bacteria and Achaea
- Small changes in genes allow for identification of hosts
- qPCR allows for quantification of specific host inputs

PLANNING ASSISTANCE TO STATES PROJECT

Stormwater Management Planning: Development of a Pilot Investigative Approach to Remediate Bacterial Source Impairments along the Grand Strand

Identification of impaired areas based on long-term monitoring



SC-DHEC Sampling Sites

Statistics f

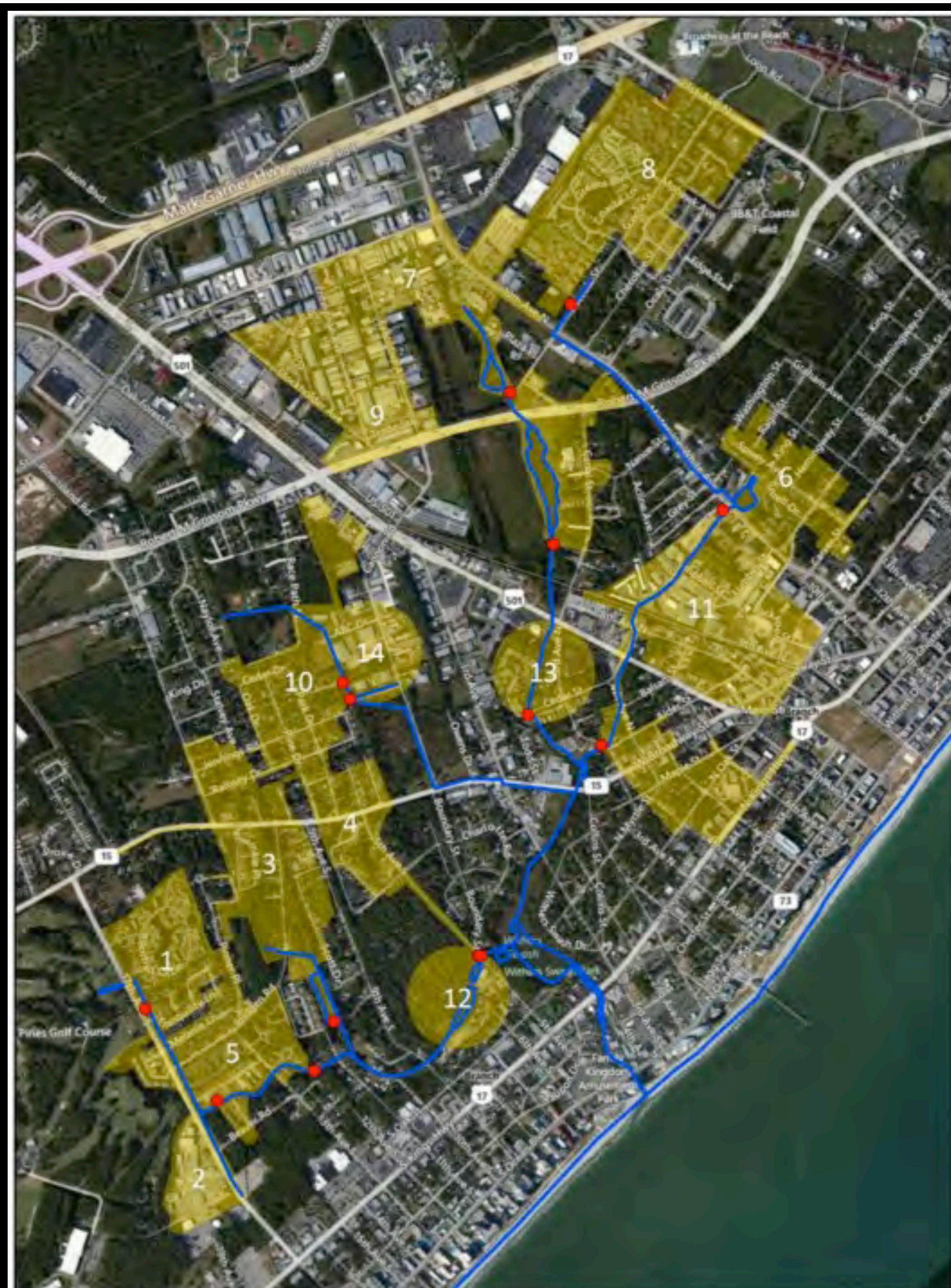
Sit							Min
WAC-22A							9
WAC-22AS1	641	87%	58%	5,762.6	776	24,197	5
WAC-22AS2	564	86%	70%	3,854.6	1,153	24,197	9

http://bccmws.coastal.edu/enteroview/

Murrells Inlet

- Several of the tidal creeks have chronically high FIBs.
- Targeted monitoring being conducted by volunteers.
- High FIBs seem to be everwhere.
- High fecal coliform concentrations are leading to shellfish closures.

Sanitary Survey


Mark Garner Hwy
8
7
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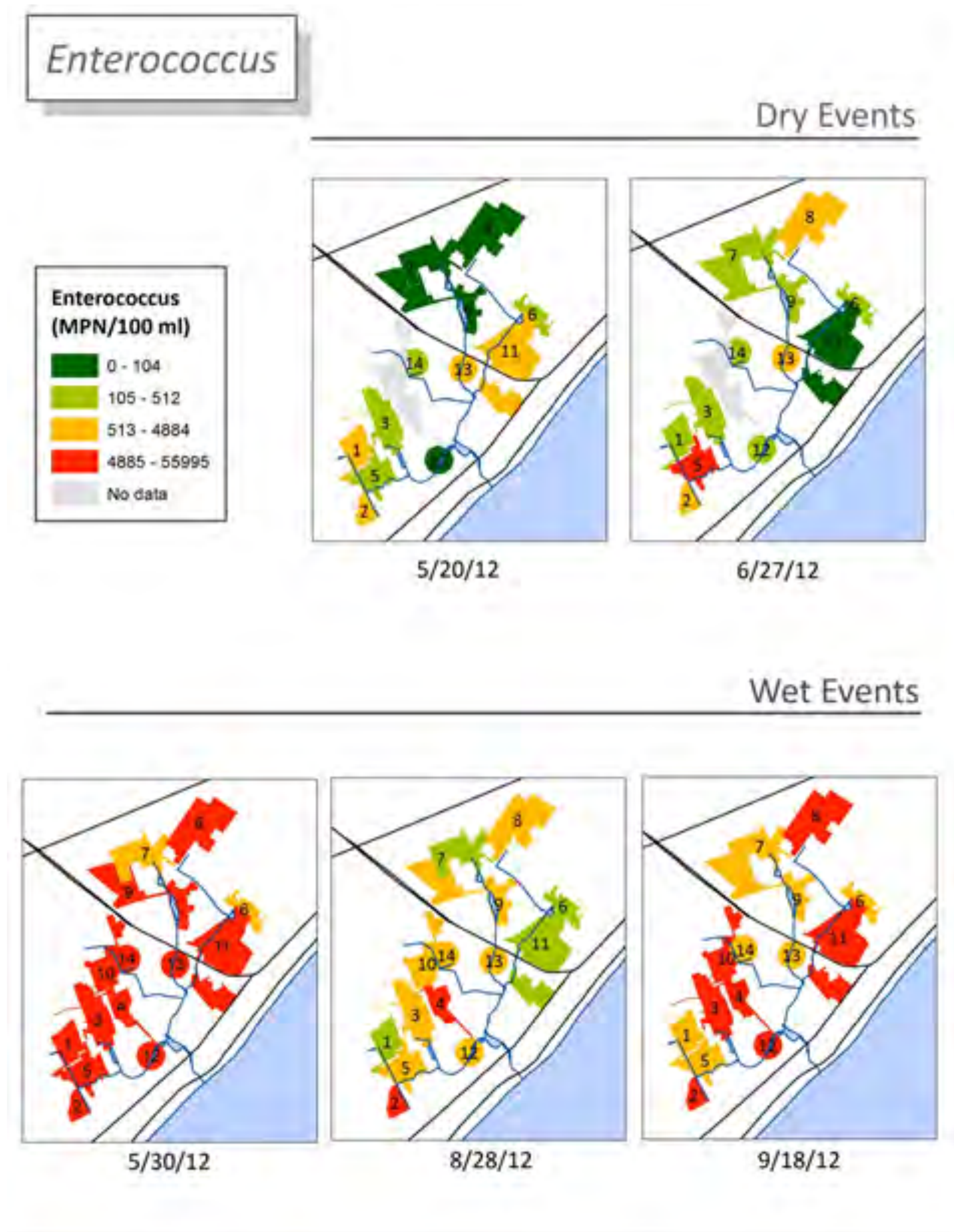
2
Pines Golf Course

ArcGIS Data Visualization Tools



Weight of Evidence (FIBs)

Site	DRY 5/20/2012	DRY 6/27/2012	WET 5/30/2012	WET 8/28/2012	WET 9/18/2012	Average Wet	Average Dry	Wet + Dry Average
1	5	3	8	3	5	5.3	4.0	4.8
2	6	7	8	7	7	7.3	6.5	7.0
3	4	3	8	7	8	7.7	3.5	6.0
4	NS	NS	8	8	8	8.0	NS	8.0
5	4	7	8	6	6	6.7	5.5	6.2
6	6	3	6	4	5	5.0	4.5	4.8
7	2	4	4	3	5	4.0	3.0	3.6
8	2	4	7	4	7	6.0	3.0	4.8
9	2	4	7	5	4	5.3	3.0	4.4
10	NS	NS	7	5	7	6.3	NS	6.3
11	5	2	8	4	8	6.7	3.5	5.4
12	4	5	8	7	8	7.7	4.5	6.4
13	5	5	8	6	6	6.7	5.0	6.0
14	4	3	7	5	5	5.7	3.5	4.8

FIB Rank Order Summary

Weight of Evidence

site	NH4-N (mg/L)	BOD5 (mg O2/L)	TSS (mg/L)	VSS (mg/L)	Percent VSS (%)	Turbidity (NTU)	Toxicity (+/-)	Optical Brighteners (+/-)	Caffeine (ng/mL)	DO (% saturation)	pH	Conductivity (uS/cm)
1	3.0	2.6	1.8	1.8	3.3	1.4	2.2	1.0	2.0	1.6	2.0	2.8
2	2.8	2.6	3.2	3.0	1.4	2.2	2.2	2.2	1.5	1.6	2.0	2.8
3	3.0	4.0	2.8	3.2	2.8	2.0	2.2	1.0	4.0	1.6	2.8	1.8
4	3.7	3.7	2.0	2.0	2.7	2.0	1.0	4.0	4.0	2.0	3.0	1.7
5	1.4	2.4	2.0	2.0	3.2	1.2	2.2	3.4	2.0	2.4	2.6	2.6
6	3.6	2.8	2.6	3.0	3.4	1.8	2.8	2.8	2.5	2.2	2.8	2.4
7	1.6	2.2	2.2	1.8	2.8	1.6	2.8	1.6	2.5	1.8	2.6	2.4
8	2.4	3.4	2.8	3.0	2.2	2.8	3.4	1.6	4.0	2.0	2.6	1.4
9	2.2	2.4	2.2	1.8	2.4	1.0	1.6	1.0	2.0	2.4	3.0	3.2
10	2.3	3.7	3.7	3.7	1.0	4.0	3.0	1.5	2.5	2.0	2.0	2.0
11	1.8	3.0	3.2	3.2	3.2	2.2	2.2	1.6	3.0	2.4	3.2	2.4
12	2.2	2.4	1.8	1.8	2.8	1.0	3.4	2.8	1.0	1.8	3.0	4.0
13	2.4	2.2	2.0	1.8	1.8	1.4	2.2	2.8	2.0	1.8	2.6	2.8
14	3.0	2.6	3.0	2.8	1.8	2.6	2.8	1.6	2.0	1.8	2.0	2.2

Rank	NH4-N (mg/L)	BOD5 (mg O2/L)	TSS (mg/L)	VSS (mg/L)	Percent VSS (%)	Turbidity (NTU)	Toxicity (+/-)	Optical Brighteners (+/-)	Caffeine (ng/mL)	DO (% saturation)	pH	Conductivity (uS/cm)
1	0.001715 - 0.012714 (0% - 25%)	0 - 2.0 (0 - WQS)	0 - 13.2 (0% - 25%)	0 - 4.7 (0% -25%)	0 - 28 (0%-25%)	0 - 25	-	-	0 - 0.192 (0% - 25%)	0 - 60 (0 - WQS)	0 - 6 (<WQS)	0 - 149 (0 - 25%)
2	0.012714 - 0.044842 (25% - 50%)	2.0 - 3.7 (WQS - 33%)	13.2 - 37.8 (25% - 50%)	4.7 - 12.0 (25% -50%)	28 - 33 (25% -50%)	25 - 50 (<WQS)			0.192 - 0.325 (25% - 50%)	60 - 76 (WQS - 33%)	6 - 7.25	150 - 243 (25% - 50%)
3	0.044842 - 0.1027815 (50% - 75%)	3.7 - 6.5 (33% - 67%)	37.8 - 113.0 (50% - 75%)	12.0 - 28.3 (50% -75%)	33 - 44 (50% - 75%)	50 - 100 (>WQS)			0.325 - 0.388 (50% - 75%)	76 - 90.9 (33% - 67%)	7.25 - 8.5	244 - 355 (50% - 75%)
4	0.1027815 - 0.46579 (75% - 100%)	6.5 - 31.2 (67% - 100%)	113.0 - 997.5 (75% - 100%)	28.3 - 232.5 (75% -100%)	44 - 77 (75% - 100%)	100 - 710	+	+	0.388 - 4.32 (75% - 100%)	90.9 - 236.5 (67% - 100%)	8.5 - 9.55 (>WQS)	356 - 42994 (75% - 100%)

WQ Parameter Rank Order Summary

Weight of Evidence

- **Distillation of data by site to qualitative terms for management adaption**

Site	Regulatory Indicators	Warm Blooded	Canine	Human
1	Strong	Strong	Strong	Significant
2	Very Strong	Very Strong	Strong	Significant
3	Very Strong	Strong	Significant	Very Strong
4	Very Strong	Strong	Very Strong	Strong
5	Very Strong	Strong	Strong	Minor
6	Strong	Strong	Significant	Minor
7	Significant	Strong	Significant	Significant
8	Strong	Significant	Strong	Minor
9	Strong	Significant	Strong	Significant
10	Very Strong	Very Strong	Very Strong	Minor
11	Strong	Very Strong	Significant	Strong
12	Very Strong	Significant	Very Strong	Very Strong
13	Very Strong	Strong	Strong	Very Strong
14	Strong	Significant	Strong	Significant

Summary Findings

Sources have been identified as likely to be contributing to the high and widespread levels of FIB,

- Pets – especially dogs
- Sewer line breaks and private sanitary sewer overflows
- Homeless
- Other: birds (geese, ducks and gulls), horses, raccoon, and deer.

Science to Management

- **Recommended Remediation Strategies (Action Plans)**

-Science and Regulatory efforts

-Outreach Education

-Land Use Planning

Dog Waste Outreach Campaign

Coastal Waccamaw
Stormwater Education Consortium

It's your Doo-ty... Pick it up!

FACT: Dog waste left on the ground travels to coastal waters after rain events through ditches, storm drains, streams, swashes, and rivers.

- Because of stormwater runoff, it is important to pick-up pet waste even when you are not near water!

FACT: Local scientists have found high levels of bacteria from dog waste in our coastal South Carolina waters.

- In some local areas, shellfish harvesting has been prohibited due to the presence of this bacteria.

FACT: Bacteria from dog waste can cause local beach advisories and unhealthy swimming conditions.

- An average dog poops 3/4 pounds a day, containing 6 billion fecal coliform bacteria.

Protect Our Waters!

Pick Up Pet Waste To Help Reduce Bacterial Pollution!

Visit **www.cwsec-sc.org** for more information.

What You Can Do To Help

- Be sure to pick up after your pets and encourage your neighbors to do so, even if you don't live close to water.
- Carry disposable, biodegradable bags when you walk your dog in your neighborhood or at a park.
- Flush pet waste down the toilet if possible. This way, pet waste is treated at a sewage treatment plant.
- NEVER put pet waste in a storm drain!
- Make sure that your community areas have at least one pet waste bag dispenser and that the bags are refilled as needed.
- Spread the word that dog waste is harmful in our waterways.

You can help Protect our Waters by picking up pet waste!

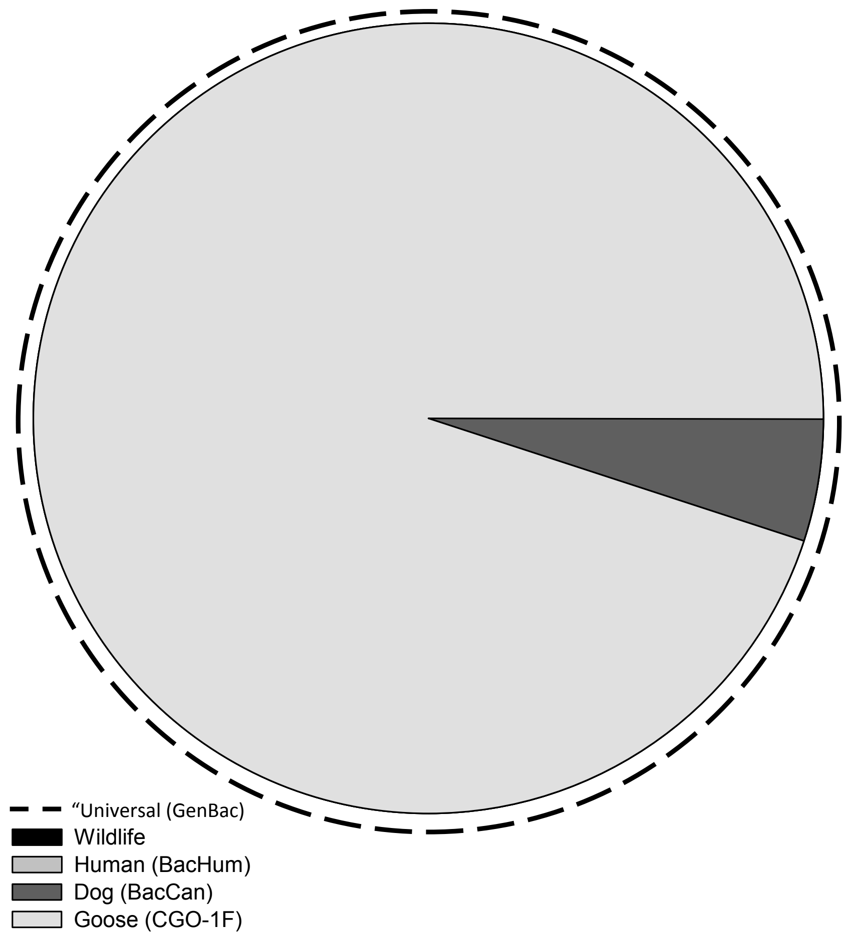
Visit **www.cwsec-sc.org** for more information.

Coastal Waccamaw
Stormwater Education Consortium

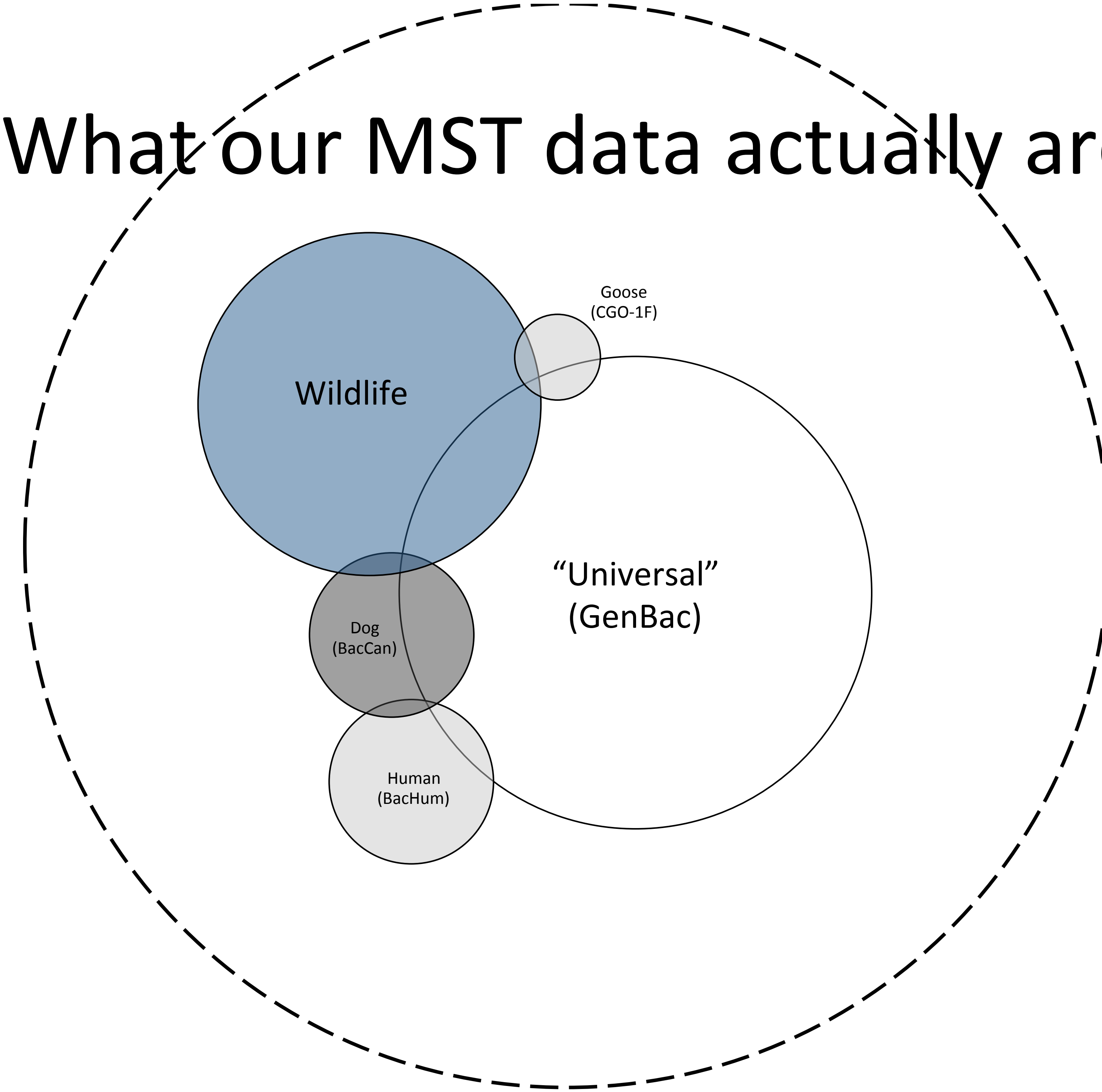
Where is the need for research?

- Missing host (need for more host assays)
- Reliable sources of standards
- More data needed to interpret results
- False negatives or positives (greater validation of assays and individual to individual variability)
- Temporal variability in the host source signal
- Other sources of FIB (sediments)

What we want our MST data to be



What our MST data actually are



Examining the Colonization and Survival of *E. coli* from Various Host Sources in Drainage Basin Sediments and Stormwater

Kyle Curtis[1], J. Michael Trapp[2]

[1]Department of Marine and Wetland Studies, Coastal Carolina University, P.O. Box 261954, Conway, South Carolina 29528-6054
[2]Burroughs & Chapin Center for Marine and Wetland Studies, Coastal Carolina University, P.O. Box 261954, Conway, South Carolina 29528-6054

Savannah Presbytery M.K. Pentecost Ecology Fund

Abstract

Stormwater drainage has a significant impact on the health of tidal creek systems via regular inputs of runoff from the surrounding watershed. Due to this hydrologic connection, contamination of the upstream drainage basin will have a direct effect on estuaries and tidal creeks that often act as receiving waters. This study builds on the growing body of research emphasizing the importance of drainage basin sediments as they enhance the persistence and transport of the fecal indicator bacteria (FIB) *E. coli* within a watershed. Experiments presented here use microcosm environments with drainage basin sediments and stormwater to investigate *E. coli* colonization of stagnant waters and the importance of host sources to bacterial survival. The colonization of sterile sediment environments is also examined using two common host sources (human and avian). Each experiment uses sediments of varying grain size and organic content to examine the influence of physical characteristics on bacterial prevalence. Results indicate an extended persistence of *E. coli* in sediments influenced significantly by grain size and host source of bacteria.

Introduction

Objectives:

1. Investigate the ability of sediment-borne FIB to colonize overlying waters in the absence of flow/agitation
2. Examine the effects of physical sediment characteristics on FIB colonization and persistence
3. Examine the effects of host organism on the survival and persistence of FIB in sediment and stormwater matrices

Figure 1. Sites of sediment sample collection in the Withers Swash drainage basin.

Hypotheses

Experiment 1

1. *E. coli* from ambient sediments will enter the water column without agitation or flow
2. Microcosms with smaller grain size will sustain higher concentrations of FIB which persist longer

Experiment 2

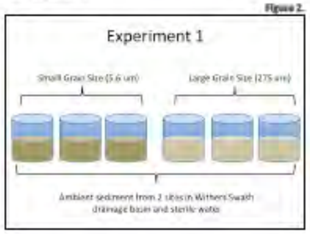
1. *E. coli* from the water column will colonize sterile sediments
2. Host source of FIB will significantly influence FIB concentration and survival

Experimental Design

Microcosms were established to mimic drainage basin conditions. Local watershed sediments and stormwater were used to incorporate the effects of microbial predation and competition on FIB. The influence of host source was investigated using spikes of human (mixed liquor from wastewater treatment facility) and avian (seagull feces) origin.

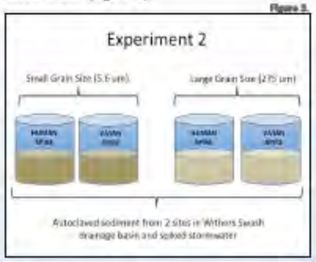
Experiment 1 tested the ability of *E. coli* in sediments to enter the water column in the absence of flow. Grain size effects on *E. coli* concentration were examined using two particle size treatments (Figure 2).

Figure 2.



Experiment 2 examined the ability of *E. coli* from the water column to colonize sterile sediments. Concentration and persistence were examined based on host source of FIB and grain size treatments (Figure 3).

Figure 3.



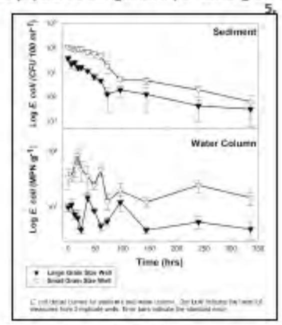
Throughout each experiment microcosm water and sediment were sampled successively. Each were analyzed for bacterial concentration using Colilert (IDEXX).

Figure 4. Sediment extraction

Results

Experiment 1 Microcosms with smaller grain size sediments had significantly higher *E. coli* concentrations and lower decay rates (5.). Multivariate analysis of variance (MANOVA) found a significant grain size effect on bacterial concentration for sediment ($p<0.001$) and overlying waters ($p<0.001$)(6.). Decay rates (7.) were also significantly affected grain size in sediment ($p=0.016$) and overlying waters ($p=0.005$).

5.



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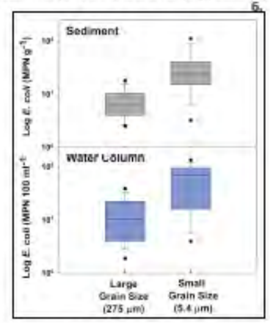

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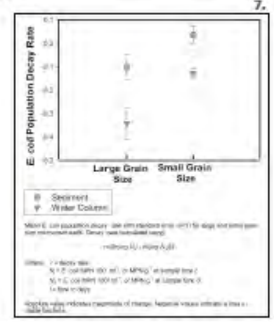
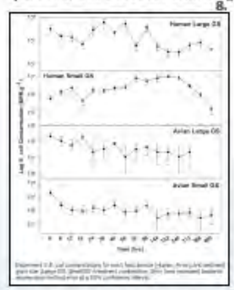


Experiment 2 *E. coli* from human source spikes exhibited higher concentrations and lower decay rates (8., 9.). MANOVA results (10.) indicated a significant host effect on bacterial concentration for sediment ($p<0.001$) but not overlying waters ($p=0.138$). Host source significantly influenced *E. coli* persistence in both the sediment ($p=0.01$) and overlying water ($p=0.001$) matrices (11.).

8.



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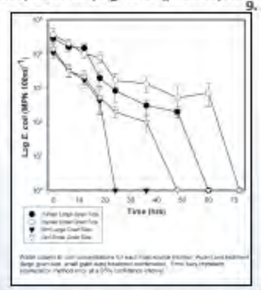

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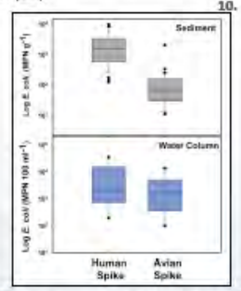

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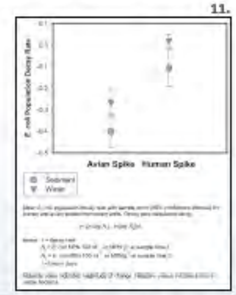

Figures 5-11. *E. coli* population decay throughout each experiment are shown in figures 5.,8., and 9. MANOVA results for *E. coli* concentration (6.,10.) are shown using boxplots with boxes representing 1st and 3rd quartiles, lines indicating median, whiskers indicating 10th and 90th percentiles, and dots indicating outliers. Figures 7. and 11. show mean population decay rate with associated error.

Conclusions

The findings of this study emphasize the importance of sediments as a source/sink for FIB within a watershed.

Experiment 1

- ***E. coli* from sediment rapidly colonized overlying sterile water**
- **Sediment particle size significantly influenced *E. coli* concentration and persistence**

Experiment 2

- ***E. coli* in the water column rapidly colonized sterile sediments**
- **Host source significantly influenced *E. coli* concentrations in sediments and decay rates in the sediment and water column**

Our results suggest *E. coli* may not be an effective indicator of microbial impairment within a watershed. The efficacy of using FIB as a proxy measure for other pathogenic species is being increasingly called into question as they are known to persist in sediments and return to the water column during times of increased flow. Studies also suggest that their survival in the environment is not well correlated with the survival of pathogenic species for which they are an indicator (Lemarchand & Lebaron, 2003; Harwood et al., 2005; Noble & Fuhrman, 2001). As typical bacterial analyses cannot distinguish between recent and long-survived (or even indigenous) FIB, their use as an indicator of microbial water quality is likely problematic. Our results suggest interpretation of these measurements may be further confounded by local conditions such as physical drainage basin characteristics and host source of bacterial input.

Acknowledgements

We would like to thank the M.K. Pentecost Ecology Fund, the Coastal Carolina University Research Council, and the CCU College of Science, for their support of this research. We also thank the Coastal Carolina Environmental Quality Lab for their input and assistance in developing and conducting these studies.

References

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Contact information

Kyle Curtis → mkcurtis@g.coastal.edu
Dr. J. Michael Trapp → jtrapp@coastal.edu

Comparison of Regulatory Fecal Indicator Bacteria and Host Specific Genetic qPCR markers in Fecal Matter of Common Sources to Tidal Creeks

Aleksandar Dimkovikj and J. Michael Trapp
Coastal Carolina University, P.O. Box 261954, Conway, SC 29528-6054 USA

Abstract

Fecal indicator bacteria (FIB) are an important form of pollution and are of particular concern in tidal creeks, which often serve as conduits of stormwater, transporting high concentrations of bacteria to the aquatic environment. In this study, we conduct a comparison of regulatory FIB (*E. coli*) to host-specific qPCR assays on direct canine and sea bird fecal grab samples from the Grand Strand of South Carolina. Results suggest that inter-specimen variability makes interpretation of qPCR results difficult to attribute a percentage of the FIB load to a particular host. Temporal variability of the addition of waste to the system further complicates interpretation.

Introduction

Water quality impairments are commonly associated with elevated concentrations of fecal indicator bacteria (FIB). Microbial source tracking (MST) aims to identify the sources of FIB pollution so targeted remediation strategies can be used to improve water quality. Bacterial culture-based methods dependant on active substrate metabolism are typically used to quantify FIB and can offer some geographic source information. Results from these methods, however, do not provide information on the source of the pollution. Molecular techniques, such as polymerase chain reaction (PCR), offer a quick and sensitive approach for quantifying FIB concentrations and host specific quantification by targeting genetic markers in the bacteria unique to the host organism.

In this study, dog and bird fecal grab samples were examined for regulatory *E. coli* (Colilert-18), a general FIB qPCR assay (GenBac) and host specific qPCR assays for dog and avian sources (BacCan and Bird GFD) assays. In a second experiment dog and bird fecal matter was aged and sampled over time to compare how the ratio between *E. coli* and genetic markers change temporally.

Methodology

- ❑ Fecal samples collected from selected canines were diluted to 10^{-4} mL final dilution factor, while selected sea bird (Laridae) samples were combined and diluted to 10^{-4} mL final dilution factor.
- ❑ Collected samples from an Italian Greyhound and bird mass-fecal sample were incubated (22C for ~ 14 days) and dilution and filtering protocols were repeated.
- ❑ Bacterial DNA was extracted from filters by mechanical and chemical cell lysis, and quantitative PCR was used to quantify the presence of FIB with the GenBac Assay, the BacCan Assay and the Bird GFD Assay. Additionally culturable *E. Coli* was measured by Colilert-18.

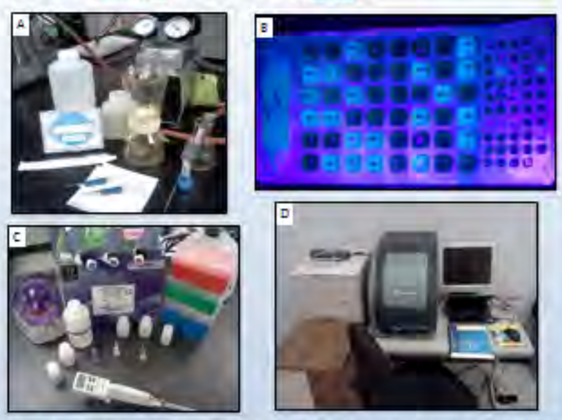

Figure 1. A: Homogenized canine (n = 12) and sea bird (n = 25) fecal samples were diluted (1X PBS) and vacuum filtered in clean environment. B: Subsamples were analyzed with Colilert-18 for *E. coli*. C: DNA was extracted from filters using the UltraClean Soil DNA Isolation Kit Method. D: Quantitative Polymerase Chain Reaction (qPCR) was conducted by TaqMan with Strategene

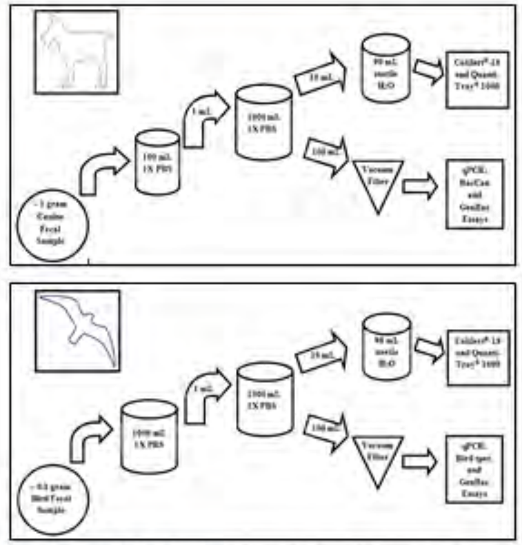

Figure 2. Flow chart indicating how canine (top) and sea bird (bottom) fecal samples were diluted with Phosphate Buffered Saline (1X PBS) and analyzed for qPCR and FIB concentrations.

Results

- ❑ Concentrations of FIB and species specific genetic markers varied greatly between individuals. Surprisingly some individuals showed no E. coli in fresh feces but had relatively strong genetic marker results.
- ❑ A weak correlation existed across individually sampled canines between *E. coli* and BacCan (R^2 = 0.0366; p = 0.0187), and *E. coli* and GenBac (R^2 = 0.0366; p = 1.97 X 10^{-5}) determined FIB concentrations.
- ❑ Genetic marker decay rates for aged canine fecal sample was rapid and logarithmic in slope. Corresponding *E. coli* concentrations progressively increased with time to maximum quantifiable level.
- ❑ Decay rate for aged sea bird fecal sample was very rapid while *E. coli* persisted longer with high concentrations.

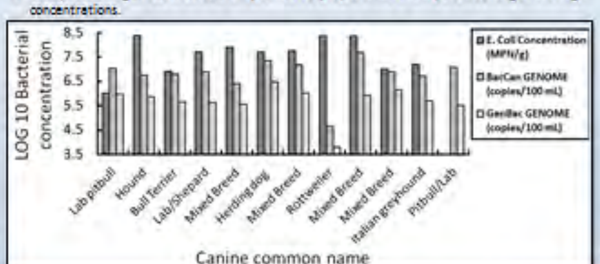

Figure 4. Individual canine samples exhibit high interspecies and applied method variability for FIB.

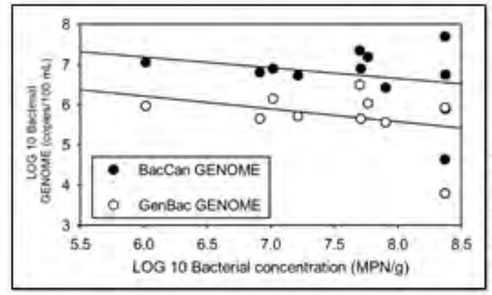

Figure 5. Weak correlation between culture based and qPCR determined bacterial concentrations for canine fecal samples.

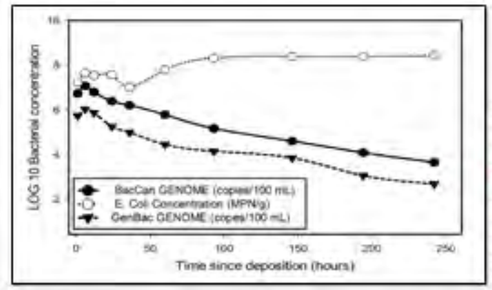

Figure 6. Genetic marker decay rates determined with qPCR were accelerated and exponential in slope (R^2 = 0.991) as compared to *E. Coli* for canine fecal matter.

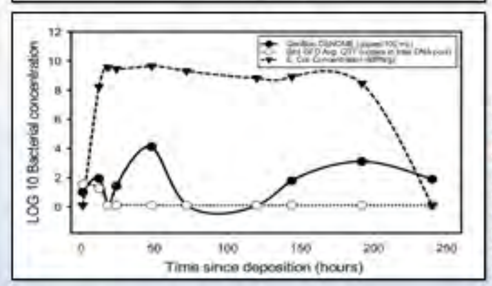

Figure 7. Genetic marker decay rates (qPCR) for sea bird fecal matter were highly accelerated as compared to *E. Coli* which persisted longer with high concentrations.

Conclusion

- ❑ There is a great deal of variability in the concentration of FIBs and genetic markers between individuals.
- ❑ The source specific signal seems to disappear quickly while FIB concentrations appear to continue to rise after leaving the organism. Thus any detection of a source specific signal should be considered significant .
- ❑ This variability between bacterial concentrations in fecal samples limits interpretation of qPCR and Colilert results which complicates the assignment of FIB percent load to a particular host.
- ❑ These results suggest that a multiple tracer weight of evidence approach including traditional WQ measurements and qPCR methods are necessary for meaningful data interpretation.

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Contact Information: J. Michael Trapp (trapp@coastal.edu); Aleksandar Dimkovikj (adimkov@coastal.edu)


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