MICROBIAL SOURCE TRACKING OF *E. COLI* AND TOTAL COLIFORMS IN MURRELLS INLET, SOUTH CAROLINA

Justin A. Anderson and Lauren M. Greoski

Faculty Advisor: S. Libes

December 10, 2010

Prepared for Georgetown County, SC

Tracy Jones, Stormwater Department

BACKGROUND:

Bacteria source tracking is a way to discover the source of pollution to a certain location. Indicator bacteria such as Fecal coliform and *E. coli* are commonly used to assess the safety of drinking water, recreational water, and shellfish waters (Anderson et al. 2005). However certain studies have indicated that the number of bacteria in coastal sediments can be 10-10,000 times greater than that of the water column (Craig et al. 2002) and that *E. coli* in sediments have shown persistence over time (Craig et al. 2004). Re-suspension of particles from the sediment can cause the number of coliform bacteria to increase in the water column at certain locations. This increase of bacteria can lead to greater health risks from swimming, drinking, and the consumption of shellfish. Therefore it is important to evaluate the potential of sediments as a legacy source of bacteria to waters that are experiencing chronic bacterial contamination.

Fecal coliform, *Enterococci* and *E. coli* are indicator bacteria used to assess the risk of acquiring enteric disease from swimming in marine waters (US EPA 1986)(Figure 1).

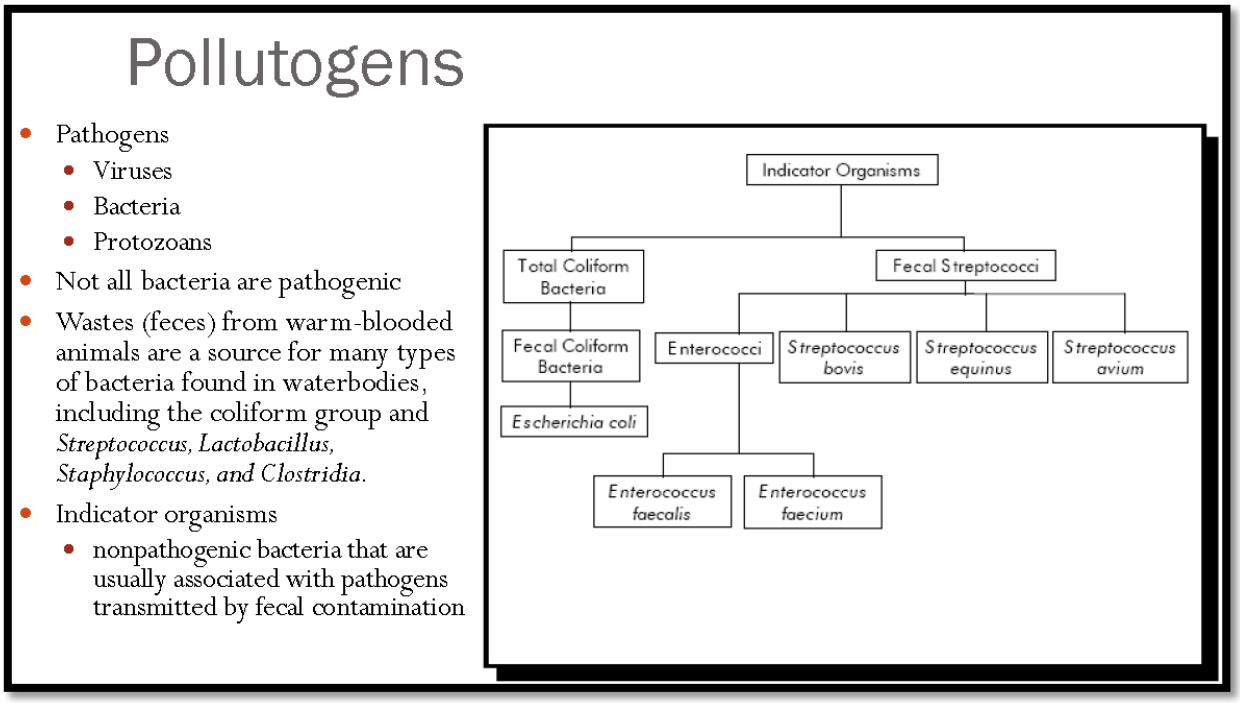


Figure 1. Fecal Indicator Bacteria Relationships

US EPA's Water Quality Standards (WQS) for safe use of recreational water for each of these fecal indicator bacteria (FIB) is shown in Table 1. In 1986, the US EPA recommended the use of *E. coli* as a FIB for recreational usage in freshwaters and that fecal coliforms no longer be used.

Table 1: US EPA Recreational Water Quality Standards for fecal indicator bacteria. (US EPA 1986)

			Single Sample Maximum Allowable Density			
	Acceptable Swimming Associated Gastro-enteritis Rate per 1000 swimmers	Steady State Geometric Mean Indicator Density	Designated Beach Area (upper 75% C.L.)	Moderate Full Body Contact Recreation upper 82% C.L.)	Lightly Used Full Body Contact Recreation upper 90% C.L.)	Infrequently Used Full Body Contact Recreation (upper 95% C.L.)
Freshwater						
enterococci	8	33 (1)	61	78	107	151
E. coli	8	126 (2)	235	298	409	575
Marine Water						
enterococci	19	35 (3)	104	158	276	501

GEOGRAPHIC SETTING

The Murrells Inlet estuarine system drains roughly 10,000 acres of land comprised of forest, open water/beach, urban buildup, wetlands, and urban/recreational grasses (Figure 2).

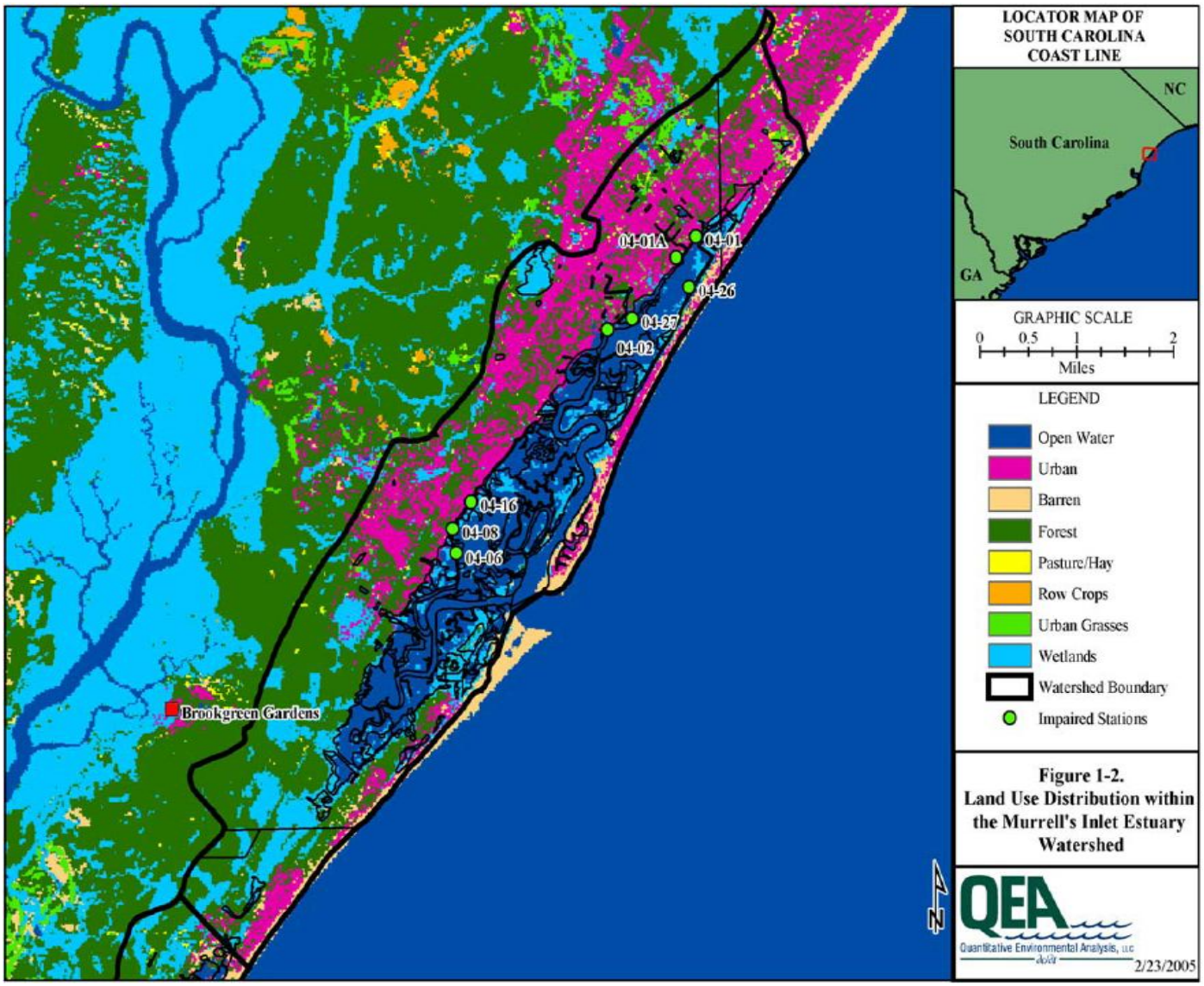


Figure 1-2.
Land Use Distribution within the Murrell's Inlet Estuary Watershed

Figure 2: Murrells Inlet land use. SCDHEC (2005)

The Inlet is a tidally-dominated waterbody situated in the Pee Dee Coastal Frontage Basin. This watershed is made up of small tidal creeks. In April 2005, six sites in Murrells Inlet were deemed restricted from shellfish harvesting for direct marketing in these locations (SCDHEC 2005). In order to establish water-quality based controls to reduce pollution and maintain and restore water quality resources, "The state of South Carolina is required to develop total maximum daily loads (TMDLs) for water bodies not meeting water quality standards (WQS) in accordance with Section 303(d) of the Clean Water Act and the United States Environmental Protection Agency's

Water Quality Planning and Management Regulations" (SCDHEC 2005). The TMDL process establishes the amount of loadings of pollutants or other quantifiable parameters allowed for a specific waterbody in relation to the relationship the source of pollution and the water quality in the stream (SCDHEC 2005).

Due to the history of chronic bacteria contamination in Murrells Inlet, a volunteer water quality monitoring program was initiated in 2008. The volunteers monitor eight land-based sites along the inlet (http://bccmws.coastal.edu/volunteermonitoring/ and Figure 3) and have continually found high concentrations of *E. coli* and *total coliform* at certain sites (Figures 4 and 5). The sites with the highest, most persistent FIB concentrations, Boat House Run (BHR) and Harrelson's Seafood (HS), were chosen as starting points for sediment-based source tracking.

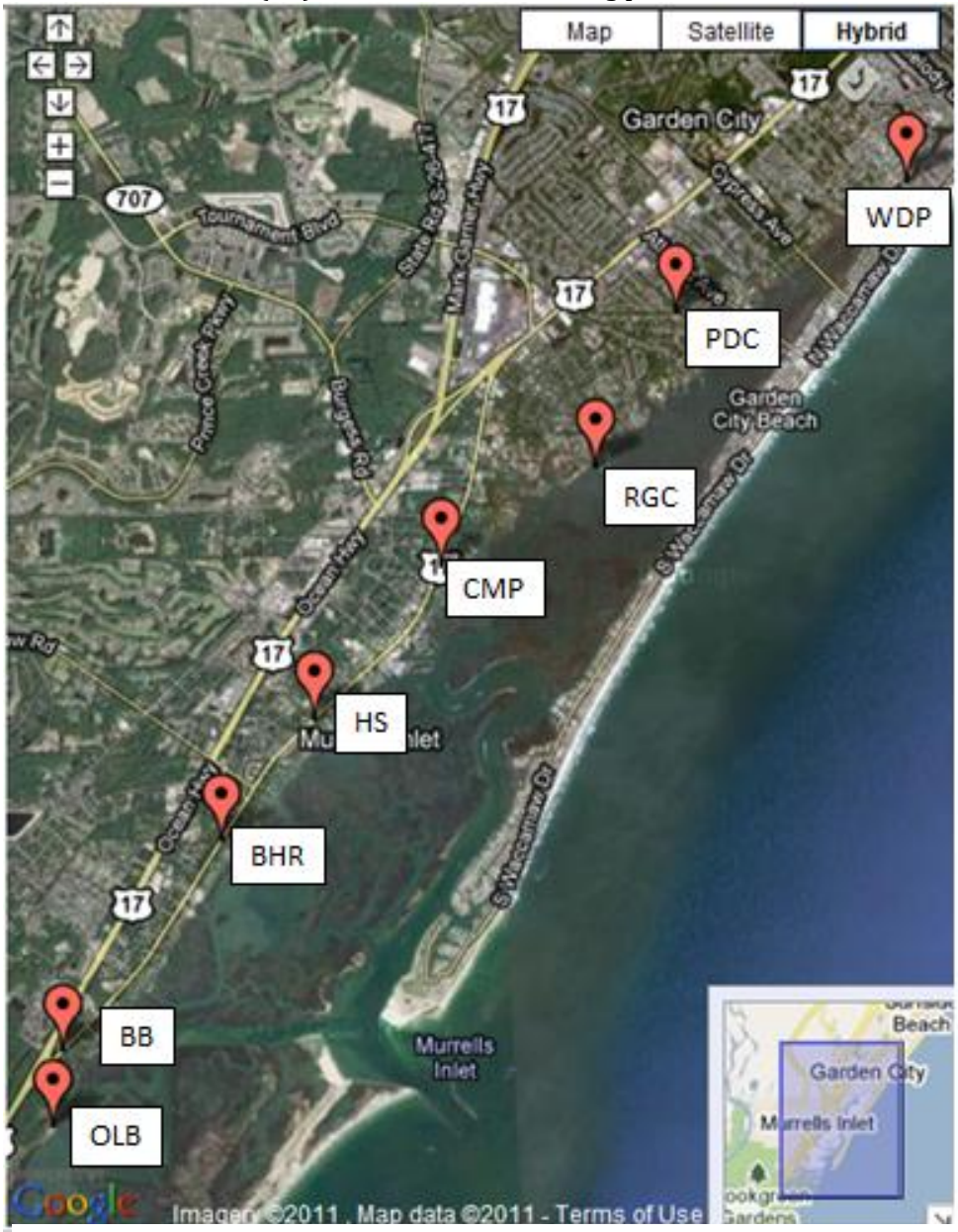


Figure 3: Murrells Inlet Volunteer Monitoring Sites.

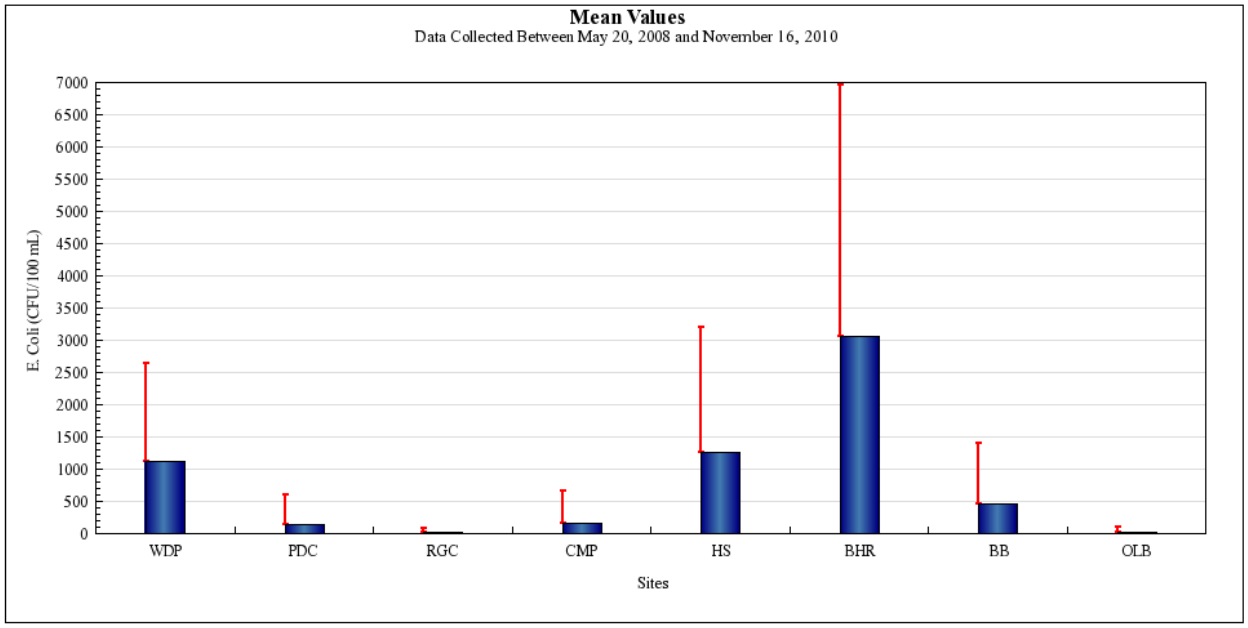


Figure 4: Mean *E. coli* concentrations (CFU/ 100 mL) (± 1 SD) measured biweekly from May 20, 2008 to November 16, 2010 by the Waccamaw Watershed Academy's Volunteer Water Quality Monitoring program at the sampling sites shown in Figure 3.

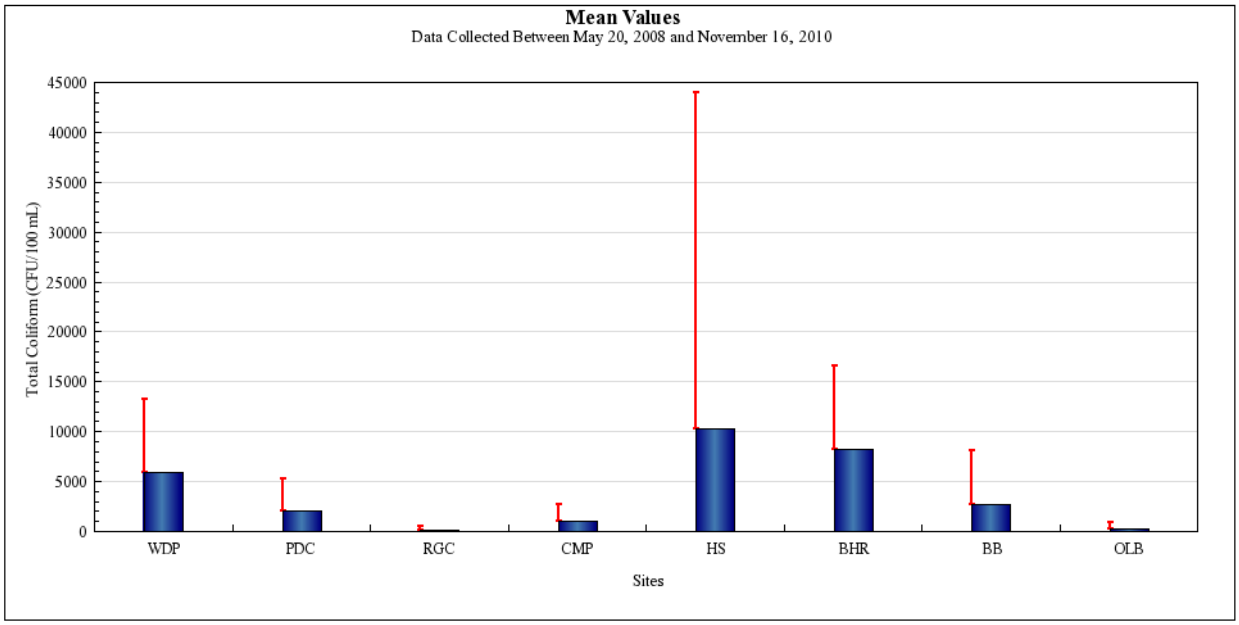


Figure 5: Mean total coliform concentrations (CFU/ 100 mL) (± 1 SD) measured biweekly from May 20, 2008 to November 16, 2010 by the Waccamaw Watershed Academy's Volunteer Water Quality Monitoring program at the sampling sites shown in Figure 3.

STATEMENT OF HYPOTHESIS

The volunteer water quality monitoring project in Murrells Inlet was continuously finding high bacteria levels in water samples. The persistently high concentrations observed at the terminus of two tributaries (BHR and HS) are attributable to re-suspension of contaminated sediments located upstream. Identification of sedimentary contamination patterns will provide insight into sources of the contamination.

EXPERIMENTAL DESIGN

To identify sources of FIB pollution to BHR and HS, several water quality parameters were measured along with microbial concentrations to provide a weight-of-evidence approach. These parameters included: conductivity, turbidity, and optical brighteners. Their source tracking potential is described below.

Conductivity is a measure of the total dissolved solids in a water sample. Knowing the conductivity range of potential end member water masses can help quantify their contributions to a sample (Pitt 2004). For example, septage, groundwater, and ocean water have relatively high conductivities.

Turbidity is measured to quantify suspended particle concentrations. Because bacteria tend to adhere to particles, FIB concentrations can be highly correlated with particle concentrations. The higher the particle load, the greater the chance that high levels of FIB will be present.

Optical brighteners (OBs) are fluorescent whitening agents that emit light in the blue range (415-445 nm) and are found in most laundry detergents. They are used to cover up unwanted yellowing of clothes (Cao et al. 2009). The detection of OBs is an indication that human wastewaters are present when there is no known non-anthropogenic source, such as color dissolved organic matter (CDOM) present. The main route of entry of human wastewaters is assumed to be from septic tank discharges and failing community sewage lines (Dickerson et al. 2007). Fluorometry is used to measure OB levels. Various methods have been published to detect OB's in the presence of CDOM (Hartel et al. 2007 and Cao et al. 2009). These methods rely on brief exposure of the sample to ultraviolet light, causing the OB's to rapidly degrade. Since CDOM does not rapidly degrade, this irradiation technique eliminates any non anthropogenic interferences. The irradiated and unirradiated fluorescence measurements are compared with the difference providing and semi-quantitative estimate of the OB concentration.

ANALYTICAL METHODS

BACTERIAL ENUMERATION

E. coli and total coliform concentrations were measured using Micrology, Inc.'s Easygel Plus media following the Waccamaw Watershed Academy's SOP (WWA 2010a). This method provides a double confirmation of *E. coli* under visible and UV light. This is the same technique used by the

Waccamaw Watershed Academy's volunteer water quality monitoring program. The technique was used to measure water column concentrations and concentrations in extracts as described in the next section.

SEDIMENT EXTRACTION OF BACTERIA

An extraction method was used that was optimized for silt and clay (Craig et al. 2002). Since a goal of this project was to evaluate the degree to which natural re-suspension of sediments could be a significant source of water column FIB concentration elevations, an extraction technique was adopted that most closely mimicked a natural re-suspension process. This technique involved shaking the sediment samples to extract bacteria as opposed to sonicating the samples or other harsh treatments.

The full SOP is provided in Appendix A. In brief, *E. coli* and total coliforms concentrations in the sediments were measured as follows: (1) approximately 50 g of sediment was collected in a sterile plastic snap-top vial. (2) In the lab, 99mL of sterile buffered water was added to the vial, which was snapped shut and shaken vigorously for 1 min. (3) After settling for 10 min, 1 to 3 mL of supernatant was pipetted into Micrology media using a sterile pipette. (4) The media was then treated as per WWA (2010a). (5) The sediment remaining in the sterile cup was placed in a drying oven at 60C, until completely dry. (6) A dry weight of the sediment was then measured using an average weight of 24.8g for the empty clean cup.

The details of how bacteria concentrations in the supernatant were used to infer concentrations per 100 g dry sediment can be found in appendix A. The reported sediment concentrations (CFU/100 g sediment) do not include contributes from the pore waters which were assumed to have FIB levels equivalent to that of the over-lying waters.

OPTICAL BRIGHTENERS

Water samples were collected in 100mL acid-washed amber glass bottles. They were stored in the dark at 4˚C until analysis. Immediately prior to analysis, the samples were brought up to a temperature of 20˚C using an incubator. OB concentrations were measured using the method of Cao et al. (2009) as modified in CCU EQL (2010). This also enables production of concentration estimates as Tide detergent equivalent units (Dickerson et al. 2007) and comparison with the % degradation protocol of Hartel et al. (2007). Briefly, the raw fluorescence (RFU) of each sample was measured in a plastic cuvette. The cuvette was exposed to UV light for 5 minutes in a temperature controlled incubator. Its raw fluorescence was then remeasured. The irradiation process was repeated and the fluorescence measured a third time. A significant level of OB's was reported if the percent reduction after 10 minutes of exposure to the percent reduction after 5 minutes of exposure was less than 1.5%. The process was performed in triplicate.

CONDUCTIVITY

Conductivity was analyzed in the laboratory after the water samples were warmed to room temperature. A Hach Sension 5 conductivity meter was then used to measure for conductivity using the SOP in WWA (2010b).

TURBIDITY

Turbidity was analyzed in the laboratory as per ***SM 2130 B. (21st ed.)*** (CCU EQL 2007)***.*** Briefly, analysis was performed on the water samples after they reached room temperature using a Hach 2100N turbidimeter.

SAMPLING METHODS AND LOCATIONS

On April 4, 2010, several sediment and water samples were collected at Oyster Landing, Murrells Inlet, SC to serve as a control sites. The volunteer water quality monitoring program had demonstrated low E. coli concentrations were present at that site (Figure 4)

The remainder of the sampling sites was selected in collaboration with Chris Allen and Tracey Jones (Georgetown County, Stormwater) based on their knowledge of suspected septic tank locations. The most downstream sites where BHR and HS, i.e. the volunteer water quality monitoring sites. The remainder of the sampling was conducted upstream (Figures 6 and 7).

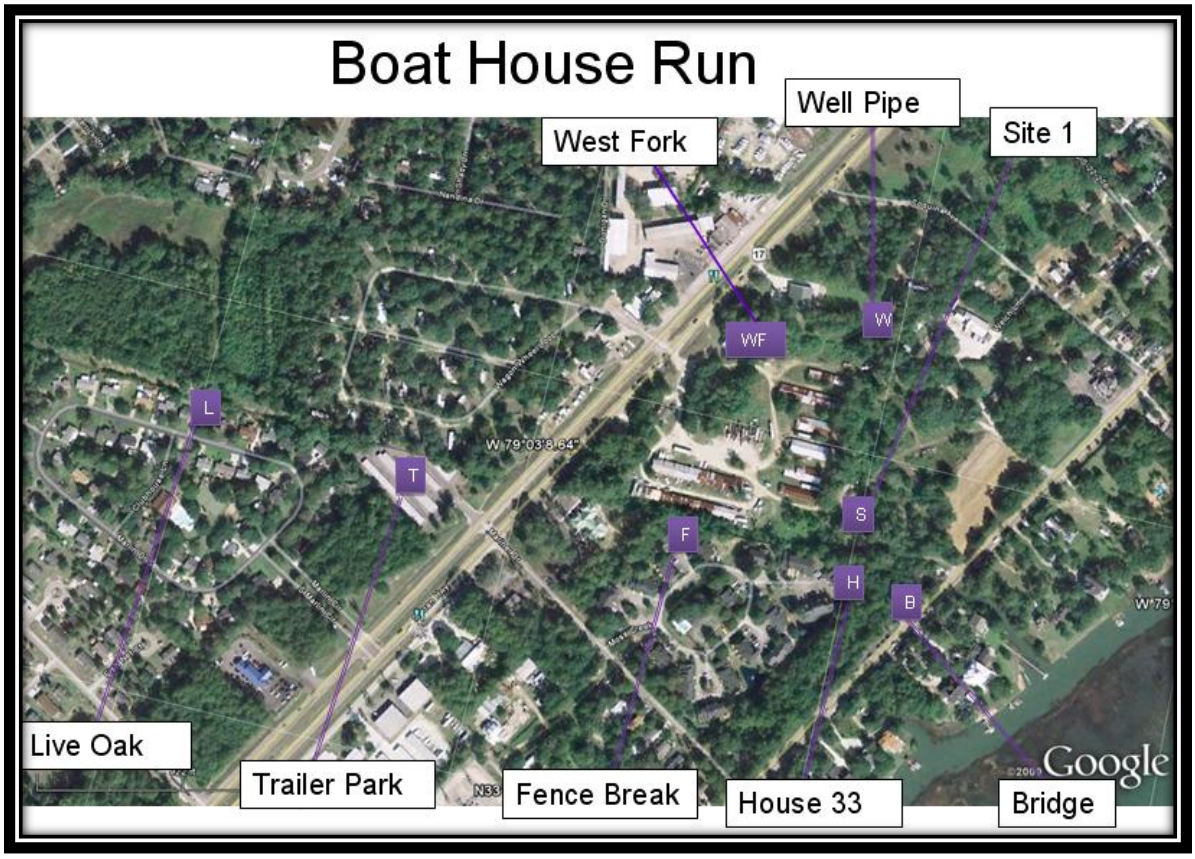


Figure 6: Boat House Run tidal creeks sampling sites.

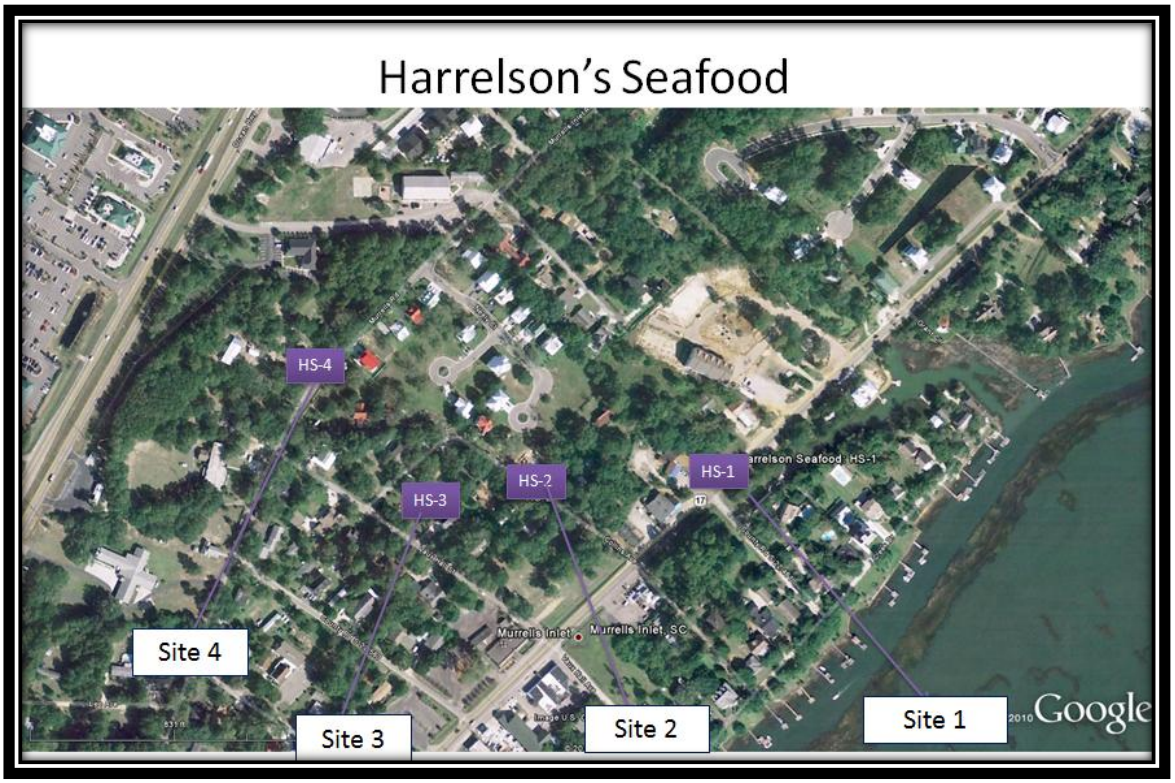


Figure 7: Harrelson's Seafood tidal creek sampling sites.

Once the sampling sites were chosen, a 1-in diameter clear plexiglass coring liner was inserted about 2 inches into the sediment. Approximately 50g of sediment, with minimal overlying water entrainment, was carefully placed into a sterile 240-mL snap top polypropylene vial (Fisher 03-341-76A) and labeled. Next, a water sample from the over-lying water column was collected in a 120-mL sterile snap top polypropylene vial (Fisher 03-341-75E) and labeled. Another water sample was collected in a 500-mL PE screw top bottle for laboratory measurement of conductivity and turbidity. Finally, a 100-mL acid-washed amber glass bottle was filled with over-lying water for OB analysis. All samples were placed into a cooler at 4˚C for transportation.

Sediment masses of 25g, 50g, and 100g, extract volumes of 1mL, 2mL, and 3mL, and incubation times of 18hr, 21hr, and 24hr were evaluated to see which yielded the optimal bacterial counts specified by Micrology, Inc. Due to high concentrations of bacteria in the sediments, extract volumes of 1 mL were generally used to prevent over growth of *E. coli* in the Micrology media.

SAMPLING DATES AND RAINFALL

On April 4, 2010, Boat House Run was sampled 3 times in the same location (*Bridge*) to determine the best method for future samplings. Fine grain and sands were sampled to assess the influence of sediment composition on extraction efficiency. Significant prior rain included 0.18" on Apr 8-9.

On May 5, 2010, Boat House Run sites: *Bridge, House 33, Fence Break, Trailer Park*, and *Live Oak* (Figure 6), were sampled to explore sources of FIB pollution to the Boat House Run tidal creek. This sampling was conducted 24 hours after a rain event during which 0.60 inches fell on May 4, 2010.

On May 11, 2010, Boat House Run sites: *Site 1, West Fork*, and *Well Pipe* (Figure 6), were sampled to explore sources of FIB pollution on the west branch of Boat House Run's tributary. This sampling occurred during a dry event, the previous rain event having occurring on May 4, 2010.

On October 30, 2010, Boat House Run sites: *Bridge, House 33, Fence Break, Trailer Park*, and *Live Oak* (Figure 6), were sampled to further explore FIB pollution sources and to assess temporal variability via comparison with the previous sampling which had taken place on May 5, 2010. Significant prior rain included 0.98" on Oct 25, 2010 and 0.13" on October 28, 2010.

On November 6, 2010, Harrelson's Seafood sites: *HS-1, HS-2, HS-3, HS-4*, and the Boat House Run site: *Live Oak* (Figures 6, 7) were sampled. These sites were used to explore the sources of FIB pollution in the Harrelson Seafood tidal creek and to further assess temporal variability of at the Live Oak site, the latter having been previously sampled on May 5 and October 30, 2010. Significant prior rain included 0.66" on Nov. 5-6, 2010.

RESULTS

Table 2 lists the *E. coli* and total coliform concentrations in the sediment, sediment plus pore water, and the overlying water column for each sampling site on each sampling date. It was assumed that pore waters had a concentration similar to that in the overlying water, which was frequently considerable. To remove the influence of pore water on the sediment concentrations, a correction was made by subtracting off the pore water bacteria to generate a sediment concentration. The samples in which the *E. coli* comprised more than 50% of the total coliforms are labeled in red.

Field duplicates were collected at the *Bridge* site on October 30, 2010 and at HS-3 on November 30, 2010 to assess reproducibility. The % difference in the sediment sample duplicates at the Bridge site was 0% and at HS-3, 20%. Varying volumes of supernatant (2 mL and 1 mL) were analyzed using sediment from the *Live Oak* site on October 30, 2010, with both extracts yielding zero *E. coli*.

The turbidity samples were overall low, except for those collected from *Live Oak, West Fork,* and *HS-1*. During each sampling, the *Live Oak* site had the highest turbidities. Conductivity values were relatively low, except at *Well Pipe*, where a groundwater influence is suspected. Optical brightener results are shown in three formats: (1) Tide detergent equivalent units where concentrations greater than 33 µl/L are deemed significant (Dickerson et al. 2007), (2) a presence/absence determination based on the % degradation after five minutes exposure to UV light (Hartel et al. 2007), and (3) a presence/absence determination based on a dual UV degradation protocol (Cao et al. 2009). The samples taken on 10/30/10 were all positive while the samples on 11/6/10 were all negative except at Live Oak. After an additional 5 minutes of UV exposure, the samples taken on 11/6/10 all received values less than 1.5 and were concluded to be positive.

Table 2: All results for all sampling events listing sediment and water *E. coli* and total coliform concentrations, turbidity, conductivity, and optical brightener results listed by date.

Bacteria in Sediment of Murrells Inlet streams and ditches

Site	Date	Sedimentary *E. coli* (CFU/100g)	Sedimentary + Pore Water *E. coli* (CFU/100g)	Water Column *E. coli* (CFU/100ml)	Sedimentary Total coliforms (CFU/100g)	Sedimentary + Pore Water Total Coliforms (CFU/100g)	Water Column Total Coliforms (CFU/100ml)	Turbidity (NTU)	Specific Conductivity (µS/cm)	% sedimentary +PW *E. coli* from Porewater	% sedimentary bacteria that are *E. coli*	% pore water bacteria that are *E.coli*	OB Tide Equivalent Units based on Dickerson et. al. (2007)	OB results based on Hartel et. al. (2007)	OB results based on Cao et. al. (2009)
Boat House Run -fine grain	4/11/2010	5,456	6,552		7,410	9,100				17%	74%				
Boat House Run -fine grain	4/11/2010	10,071	11,293	2,400	11,919	13,802	3,700	1.7	659	11%	84%	65%			
Boat House Run - sands	4/11/2010	8,766	9,272		12,145	12,925				5%	72%				
	Ave±SD =	8098 + 2379	9039 ± 2379		10,491 + 2671										
Bridge	5/5/2010	2,470	3,723	4,800	3,287	4,800	5,800	5.61	436	34%	75%	83%			
House 33	5/5/2010	68,057	69,464	3,300	78,716	80,421	4,000	1.41	414	2%	86%	83%			
Fence Brk	5/5/2010	1,038	1,280	800	1,554	2,067	1,700	7.78	388	19%	67%	47%			
Trailer Park	5/5/2010	15,652	16,123	200	22,806	26,103	1,400	4.41	306	3%	69%	14%			
Live Oak	5/5/2010	207,011	207,011	0	206,620	207,011	1,300	8.79	287	0%	100%	0%			
Site 1	5/11/2010	8,027	62,237	70,400	17,772	85,534	88,000	3.05	871	87%	45%	80%			
West Fork	5/11/2010	6,034	6,034	0	10,360	10,434	100	12.4	568	0%	58%	0%			
Well Pipe	5/11/2010	965	1,030	100	2,055	2,318	400	2.9	1058	6%	47%	25%			
Bridge	10/30/2010	11,504	11,672	700	15,900	16,275	1,567	2.08	402	1%	72%	45%	50	Positive	Positive
Bridge Dup	10/30/2010	4,850	5,018	633	6,579	6,961	1,433	2.04	403	3%	74%	44%	50	Positive	Positive
House 33	10/30/2010	14,528	14,864	733	32,554	33,517	2,100	1.44	299	2%	45%	35%	53	Positive	Positive
Live Oak (2mL)	10/30/2010	0	90	367	44	630	2,167	14.5	393	NA	0%	17%	46	Positive	Positive
Trailer Park	10/30/2010	49,138	49,253	67	65,282	66,200	533	2.23	249	0%	75%	13%	50	Positive	Positive
Live Oak (1mL)	10/30/2010	0	0	NA	494	1,080	NA	NA	NA	NA	0%	NA	NA	NA	NA
HS-1	11/6/2010	19,040	22,240	2,800	28,315	32,963	4,067	13.8	661	14%	67%	69%	33	Negative	Positive
HS-2	11/6/2010	12,429	12,641	700	22,237	22,792	1,833	1.49	372	2%	56%	38%	36	Negative	Positive
HS-3	11/6/2010	9,710	10,017	833	20,322	20,913	1,600	1.34	368	3%	48%	52%	37	Negative	Positive
HS-3 Dup	11/6/2010	16,139	16,408	800	49,211	49,895	2,033	1.96	370	2%	33%	39%	37	Negative	Positive
HS-4	11/6/2010	58,432	59,565	1,500	116,344	118,006	2,200	2.18	368	2%	50%	68%	39	Negative	Positive
Live Oak	11/6/2010	0	0	33	0	0	867	7.76	247	0%	NA	36%	38	Positive	Positive

NOTES: Cases where *E. coli* comprise more than 50% of the total coliforms are highlighted in red. Limitations in the resolution of the FIB measurements lead to some cases where the *E. coli* concentrations are numerically a bit higher than the total coliform concentrations. These are not significant differences.

Summary statistics for the results in Table 2 are presented in Table 3. These include the mean, median, standard deviation, maximum and minimum values for each parameter. Some sites were resampled to provide insight into the time scales over which bacteria sediment concentrations vary. These results are summarized in Table 4 and suggest no consistent behavior. No consistent trends were observed, with some sites showing increases and others decreases over time. No sites had stable concentrations.

Table 3: Summary statistics for results in Table 2.

	Sedimentary E. coli (CFU/100g)	Sedimentary + Pore Water E. coli (CFU/100g)	Water Column E. coli (CFU/100ml)	Sedimentary Total coliforms (CFU/100g)	Sedimentary + Pore Water Total Coliforms (CFU/100g)	Water Column Total Coliforms (CFU/100ml)	Turbidity (NTU)	Specific Conductivity (µS/cm)	% sedimentary bacteria that are E. coli	% pore water bacteria that are E.coli	OB Tide Equivalent Units based on Dickerson et. al. (2007)
Mean	23,014	25,904	4,362	31,823	35,815	6,340	4.9	456	58%	45%	43
Median	9,710	11,293	767	15,900	16,275	1,767	2.6	391	67%	44%	39
Std. Dev.	44,176	44,756	14,805	47,762	48,933	19,271	4.4	210	25%	26%	7.0
Max	207,011	207,011	70,400	206,620	207,011	88,000	14.5	1058	100%	83%	53
Min	0	0	0	0	0	100	1.3	247	0%	0%	33

Table 4: Temporal variations in sedimentary *E. coli* (CFU/100g) in the Boat House Run tidal creek.

	Sampling Date				
Sites	***5/5/2010***	***10/30/2010***	***11/6/2010***	***% RPD***	***Time Trend***
Bridge	2,470	11,504	NA	129%	Increase
(Field Dup)		4,850		38%	
House 33	68,057	14,528	NA	-130%	Decrease
Trailer Park	15,652	49,138	NA	103%	Increase
Live Oak	207,011	0	0	Huge	Decrease

The frequencies of high (> 10^4), medium, (10^3 to 10^4) and low (<10^3) *E. coli* and total coliform concentrations in sediment (CFU/100 g) and overlying water (CFU/ 100mL).are shown in Figure 8. In both cases, the most frequently observed values in the sediments exceed 10^4 CFU/100g and the most frequently observed values in the sediments are less than 10^3. This suggests a strong partitioning of FIB from the water column into the sediments.

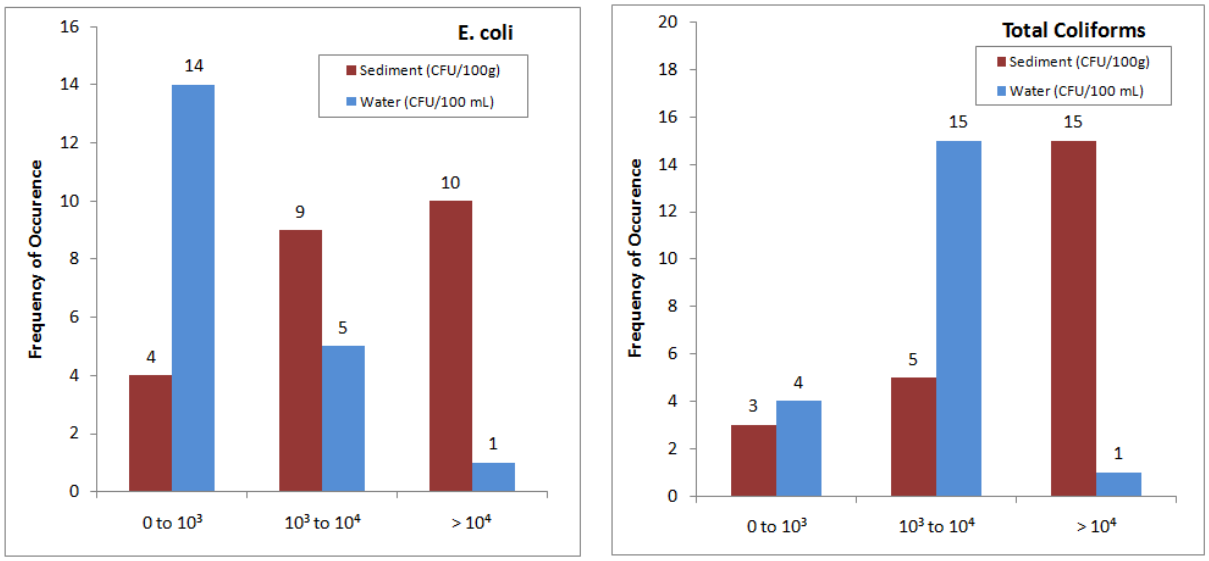


Figure 8: Frequency of high (> 1 x 10^4), medium, (1 x 10^3 to 1 x 10^4) and low (<1 x 10^3) *E. coli* and total coliform concentrations in samples of sediment (CFU/100 g) and overlying water (CFU/100mL).

Variations in the percentage that *E. coli* comprises of the total coliform concentrations in the sediment and water column are shown in Figure 9 and 10 for the two tributaries of the Boat House Run tidal creek, and the Harrelson Seafood tidal creek, respectively. Eleven out of the 20 samples had a higher % *E. coli* in the sediment as compared with the overlying water column. The median % *E. coli* in the sediments was 67% as compared with 45% in the water column, suggesting at *E. coli* are more strongly partitioned into the sediments than are total coliforms or that their survival is in the sediments exceeds that for total coliforms. At the *Bridge* site, values remained constant over time in the sediment, but decreased in the overlying water column, a trend seen at the other sites. The latter suggests a longer survival rate for E. coli in the sediments as compared with the water column. The relative constancy in the % E. coli in the sediments at Trailer Park (69 to 75%) and Bridge (72 to 77%) suggests a constancy in the nature of the source of FIB to these sites.

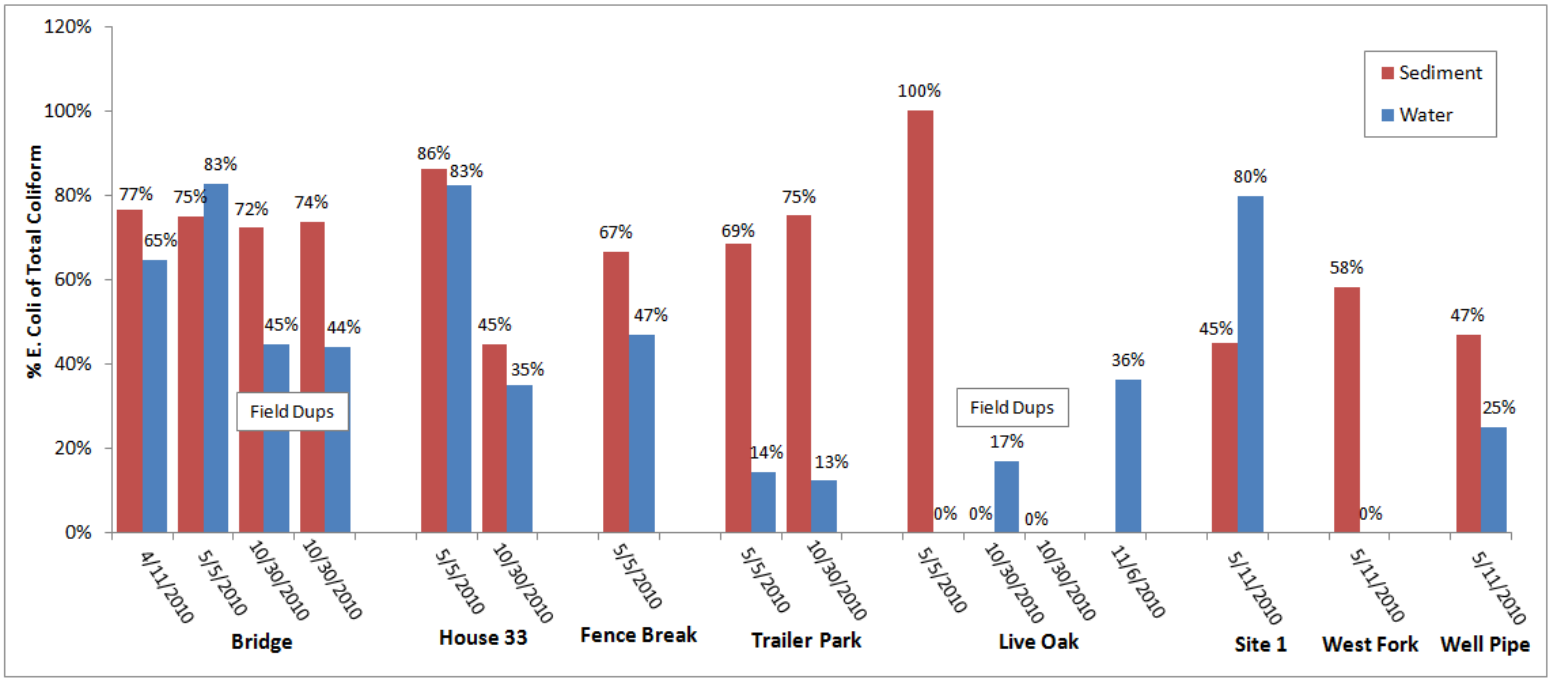


Figure 9: Percentage of total coliforms comprised by *E. coli* in sediment and water samples in the Boat House Run tidal creek.

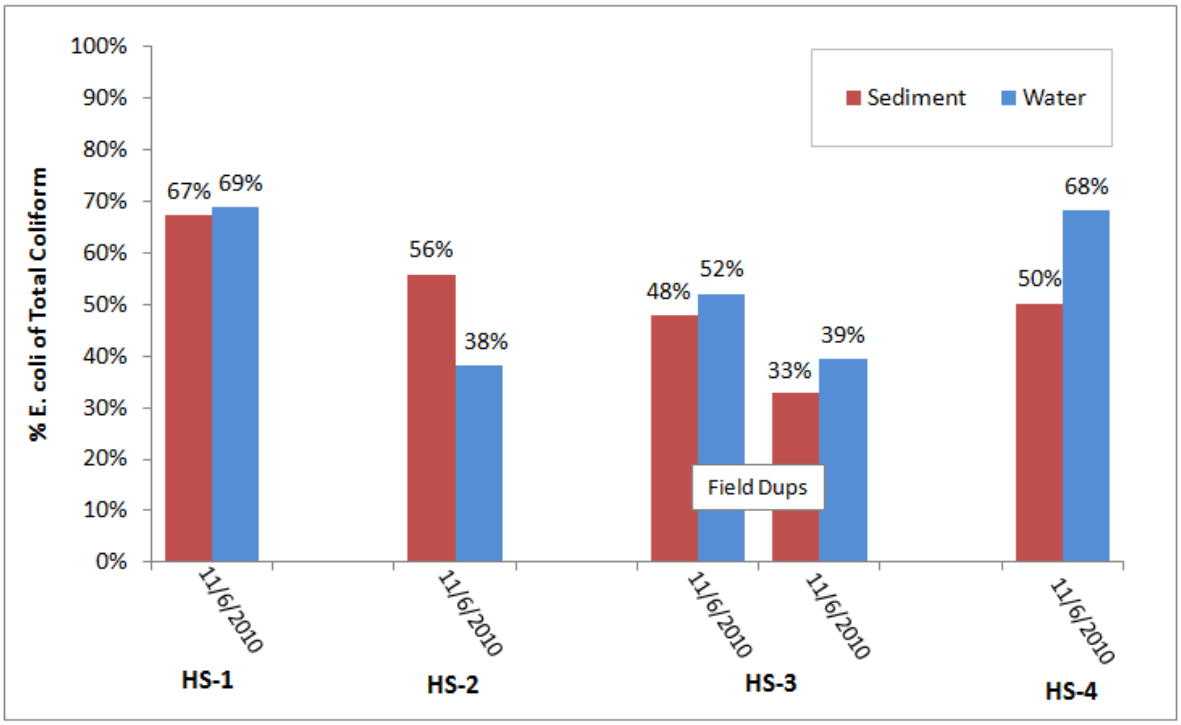


Figure 10: Percentage of total coliforms comprised by *E. coli* in sediment and water samples in the Harrelson's Seafood tidal creek sampling sites.

E. coli and total coliform concentrations are shown in their geographic settings for the two tributaries of the Boat House Run tidal creek in Figures 11 and 12, and for the Harrelson Seafood tidal creek in Figure 13. These representations are used to identify likely upstream locations of FIB sources. The sediment and water concentrations are included for all sampling dates.

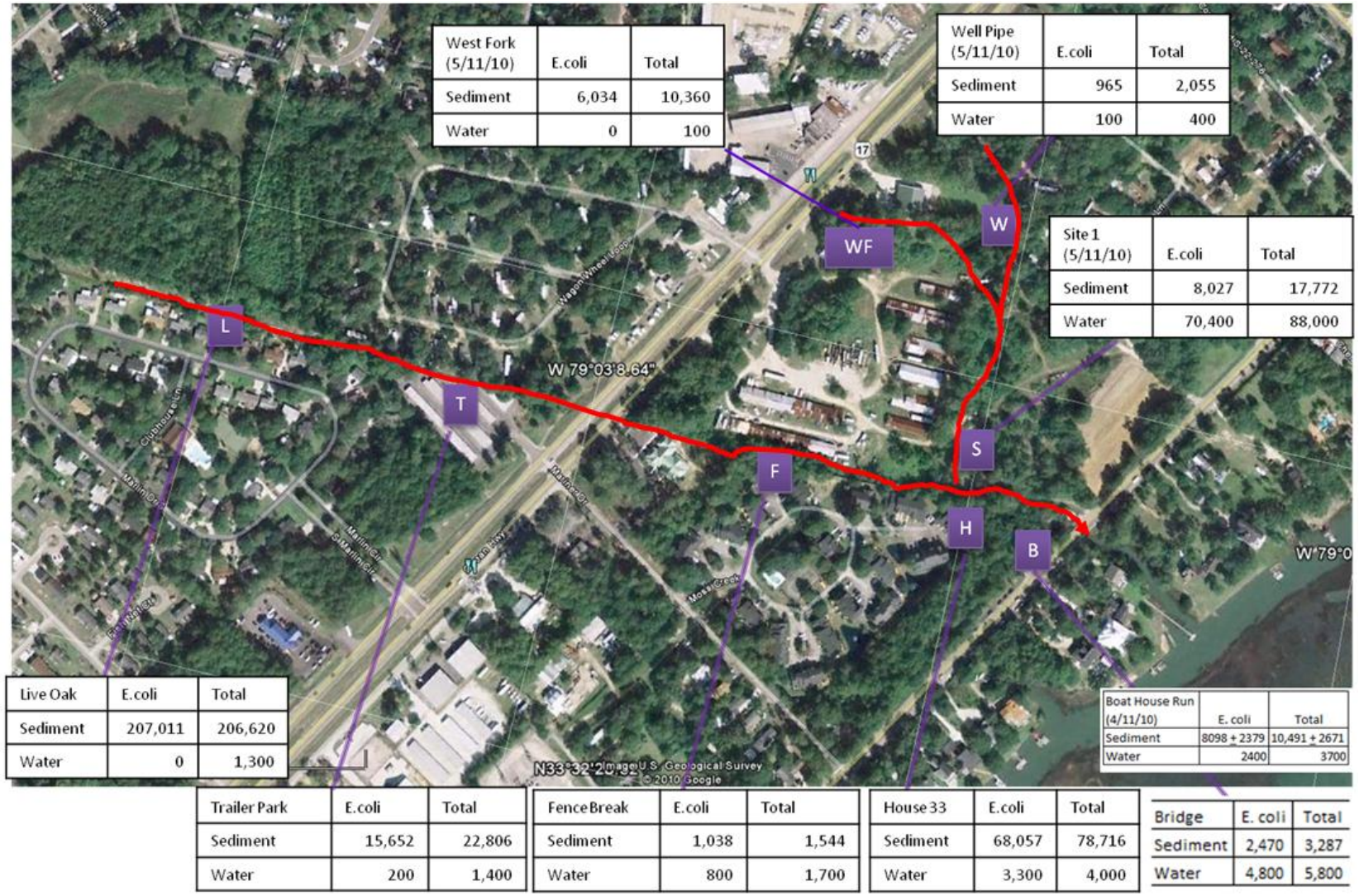
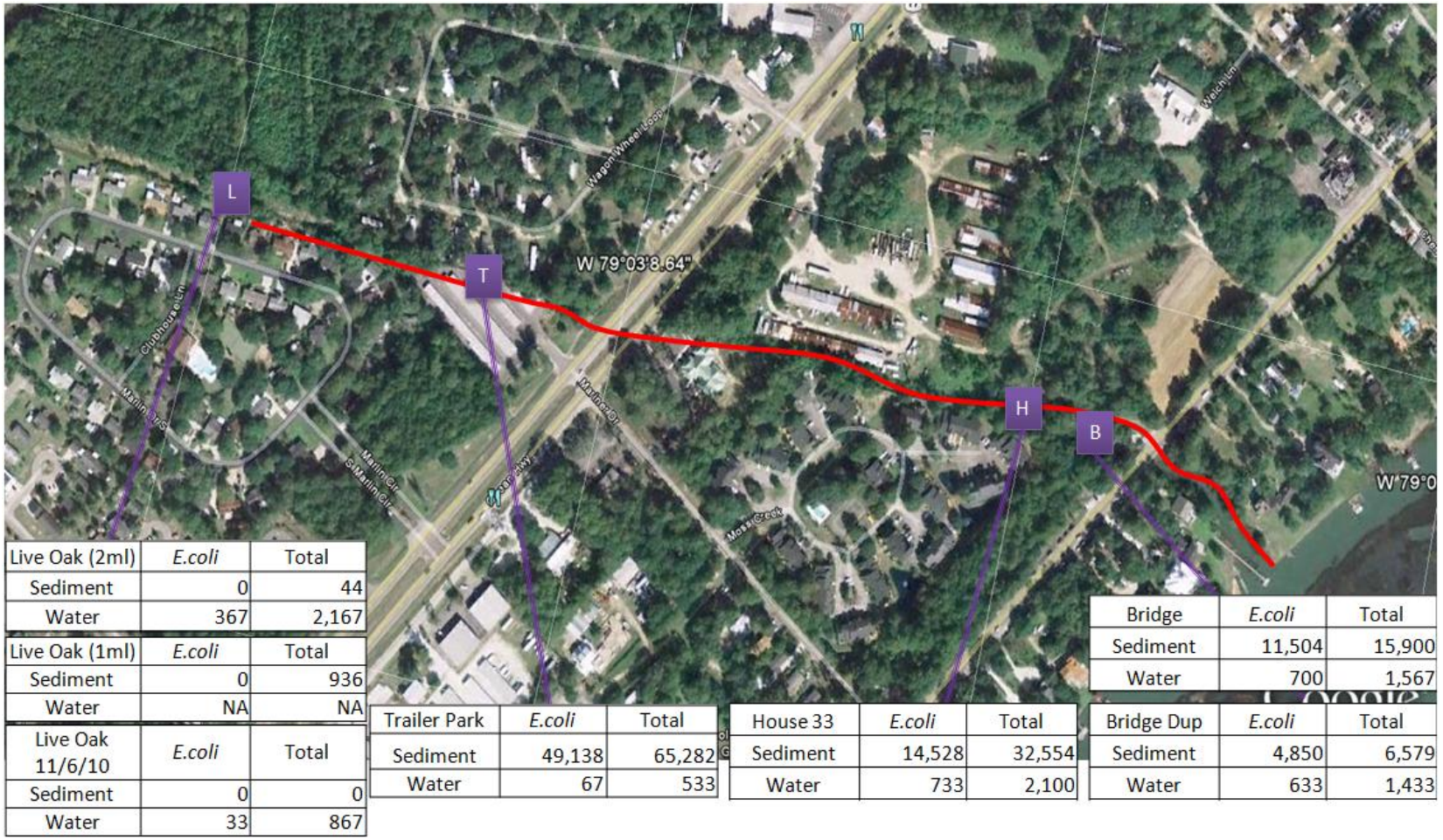


Figure 11: Boat House Run tidal creek: *E. coli* and total coliform concentrations in water and sediment on 5/5/11 unless otherwise indicated.



Live Oak (2ml)	*E.coli*	Total
Sediment	0	44
Water	367	2,167

Live Oak (1ml)	*E.coli*	Total
Sediment	0	936
Water	NA	NA

Live Oak 11/6/10	*E.coli*	Total
Sediment	0	0
Water	33	867

Trailer Park	*E.coli*	Total
Sediment	49,138	65,282
Water	67	533

House 33	*E.coli*	Total
Sediment	14,528	32,554
Water	733	2,100

Bridge	*E.coli*	Total
Sediment	11,504	15,900
Water	700	1,567

Bridge Dup	*E.coli*	Total
Sediment	4,850	6,579
Water	633	1,433

Figure 12: Boat House Run tidal creek: *E. coli* and total coliform concentrations in water and sediment on 10/30/2010 unless otherwise indicated.

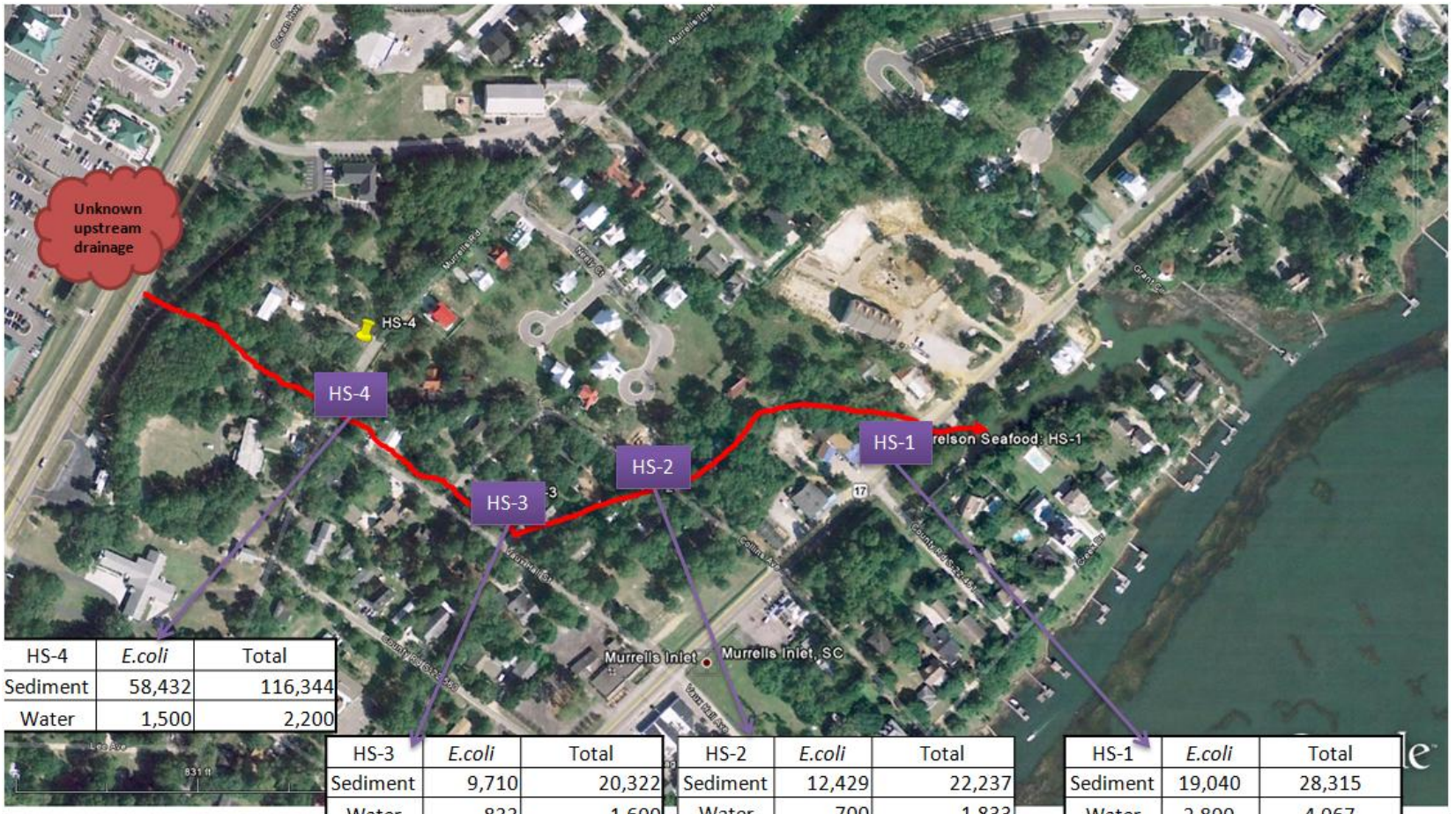


Figure 13: Harrelson's Seafood tidal creek: *E. coli* and total coliform concentrations in water and sediment on 11/6/2010.

CONCLUSIONS

Evidence for contamination in the sediments

As shown in Table 2, the sedimentary *E. coli* concentrations ranged from 0 to 207, 011 with a mean value of 23,014 ± 44,176 CFU/100 g. The median value was 9710 CFU/100g. The highest value (207,011 CFU/100g) was observed at the *Live Oak* site in the Boat House Run tidal creek on 5/5/2010 following 0.6" rain on 5/4/2011. In comparison, the water column concentrations ranged from 0 to 70,400 with a mean value of 4,362 CFU/100 mL and a median of 767 CFU/100 mL. Fourteen of the twenty water column measurements exceeded the recreational WQS. The highest value (70, 400 CFU/100 mL) was observed at *Site 1* in the Boat House Run tidal creek on 5/11/2011 (prior rain was on 5/4/11).

As shown in Figure 8, most of the sediment concentrations were greater than 10^3 g/100g. This is similar to the range reported by Craig et al. (2002) for fecal coliform concentrations in sediments located near sewage treatment plant discharges. Since E. coli are a subset of the fecal coliform population, which is a subset of the total coliform community (Figure 1), the total coliform concentrations provide an upper limit for comparison with the values reported by Craig et al. (2002). In Murrells Inlet, the sedimentary total coliform concentrations ranged from 0 to 206,620 with a mean value of 31,823 ± 47,762 CFU/100 g. The median value was 15,900 CFU/100g. The highest value (206,620 CFU/100g) was observed at the *Live Oak* site in the Boat House Run tidal creek on 5/5/2010 following 0.6" rain on 5/4/2011. ***In summary, the FIB concentrations in the sediments of Murrells Inlet appear to be at least equal to or somewhat higher than those reported in Craig et al. (2002) for sediments known to be contaminated by treated municipal sewage effluent.***

The observation that the lowest and highest sedimentary values were observed at the Live Oak site is discussed further in the section labeled "**Temporal Trends and Legacy Effects**". To evaluate the precision of the sedimentary measurements, field duplicates were measured at the Boat House Run *Bridge* site on 4/11/2010 (5456 and 10,071 CFU/100g) and 10/30/2010 (11,504 and 4,850 CFU/100 g), and HS-3 on 11/6/2010 (9710 and 16,139 CFU/100 g), yielding relative percent differences of 60, 81, and 50%, respectively. This is less than the %RPD limits for FIB as measured by standard methods, reflecting the notably high degree of uncertainty inherent in standard microbial test methods.

Source Tracking

Overall conductivity, turbidity and optical brightener trends

Conductivities decreased with increasing distance from the Inlet. Levels in western tributary of the Boat House Run tidal creek were somewhat lower on 10/30/2010 as compared with 4/11/10 and 5/5/2010, with the exception of the *Live Oak* site, which was 31% higher, coinciding with a high turbidity (14.5 NTU) and water column *E. coli* concentration (367 CFU/100 mL). The conductivity of this site had declined to former values by 11/6/2010. The northern tributary, sampled on 5/11/2010, had much higher conductivities than the western tributary, with a "hot spot" located at *Well Pipe* (1058 μS/cm), suggesting groundwater discharge. The Harrelson's

Seafood tidal creek, sampled on 11/6/2010, had conductivities similar to that of the western tributary of the Boat House Run tidal creek, with levels decreasing with increasing distance upstream from the Inlet.

Turbidity plays a role in the transfer of FIB to the sediments. Re-suspension of particles, which can occur from scouring during a time of increased water velocities, can lead to higher concentrations of FIB in the water column. Since FIB have a tendency to adhere to fine grain particles, they can be transported to where ever the particles eventually settle out. Evidence for this transport mechanism is seen in the elevated turbidity (14.5 NTU) and *E. coli* (367 CFU/100 mL) concentrations in the water column at the *Live Oak* site on 10/30/2010. In contrast, no *E. coli* were observed in the pore-water corrected sediments. Other samples with notably high turbidities were *HS-1* (13.8 NTU) and *West Fork* (12.4 NTU). The former had an elevated *E. coli* (2800 CFU/100 mL) and the latter had no *E. coli*.

Using the method of Cao et al. (2009), all of the samples measured (10/30/2010 and 11/6/2010) appeared to have been contaminated with optical brighteners. The levels were somewhat higher on 10/30/2010 in the Boat House Run tidal creek sites than in the Harrelson seafood tidal creek on 11/6/2010.

Boat House Run Tidal Creek (Figure 12 and 13): The Boat House Run tidal creek receives drainage through a western and a northern tributary. **The highest sedimentary *E. coli* levels were observed in the western tributary, particularly *Live Oak, House 33* and *Trailer Park*. The latter two had the highest concentrations on both 5/5/2010 and 10/30/2010.** High levels at the most upstream site, *Live Oak*, were only observed on 5/5/2010. This site is located in a retirement community, west of Hwy 17. Sampling was conducted in the area where the yards sloped into the tributary and the grass was very lush, even in October. Intermediate sedimentary *E. coli* levels were observed at the *Bridge, West Fork*, and *Site 1*. Very low levels were seen at *Well Pipe* and *Fence Break*. The highest water column *E. coli* concentrations were observed at *House 33* and *Bridge* on 4/11/, 2010, 5/5/2010 and 1/30/2010. ***In summary, a likely source appears to be present in the western tributary, with most persistent influence in the water and sediments at the two most downstream sites, House 33 and Bridge.*** Note that the only samples collected in the northern tributary were during relatively dry weather (7 days of no rainfall).

Harrelson Seafood Tidal Creek (Figure 14): Conductivity is highest at HS-1 reflecting occasional intrusions of sea water to this most downstream site. On the sampling date, Nov 6, the day after a 0.66" rain, upstream conductivities were uniformly low, ranging from 368 to 372 μS/cm. Likewise, no gradient was observed in turbidity, except for a high value (14 NTU) at HS-1. At HS-1, a PVC pipe was observed to be discharging directly into the tidal creek at the site where the highest water column FIB concentrations were observed. This site had the second highest sedimentary FIB concentrations. The most upstream site sampled, HS-4, had the highest sedimentary FIB concentrations and the second highest water column FIB concentrations in this tidal creek. This site had the densest aquatic vegetation in the ditched streambed as compared with the other sites, suggesting nutrient loading. Because sampling was not conducted upstream (west) of Murrells Inlet Road, it is not possible to further isolate potential sources emanating from the extensive upstream drainage area. The latter appears to include ditches bordering Hwy 17, and possibly a watershed extending west of Hwy 17. ***In summary, a source upstream of HS-4 is indicated as***

well as at HS-1, where a PVC pipe was discharging. Also notable were the relatively high water and sediment concentrations at the other sites in this tidal creek.

Temporal Trends and Legacy Effects

As shown in Table 4, considerable variability was observed over time at sites that were resampled. Although decay rates for bacteria in sediment are slower than in the overlying water, Craig et al. (2004) reports that 90% removal is achieved within 1 to 7 d with rates decreasing with increasing temperature. At 20 C, 90% removal was observed within 2 to 5 d. With regards to FIB, the term "removal" means that the bacteria could no longer be cultured from the water samples. Causes for removal include: (1) grazing, or (2) inactivation (kill) by exposure to heat, solar radiation, salt, or lack of nutrients (Sinton et al. 2002; Whitman et al. 2004).

These results suggest that the sources contaminating the sediments are not persistent over periods of months. For example, at the *Live Oak* site, the highest sedimentary concentrations were observed on 5/5/2010 and then no *E. coli* was observed at this site on 10/30/2010 or 11/6/2010 although *E. coli* was observed in the overlying waters. The highest water column *E. coli* concentrations were observed on 10/30/2010 in concert with the highest turbidity (14.5 NTU) following 1" rain on 10/25/10 and 0.13" rain on 10/28/10. In other words, an episodic source appears to be present at the *Live Oak* site. This is also likely the case at the other three sites where resampling showed significant changes over time, i.e., *Bridge, House 33*, and *Trailer Park* (Table 4).

Decay rates for FIB are greater in saltwaters as compared to freshwater (Hood et al. 2002; Sinton et al. 2002). Therefore episodic incursions of saltwater upstream could contribute to kills of sedimentary bacteria, leading to the variability observed over time at the *Bridge* site. The volunteer water quality monitoring program has documented that periodic incursions of seawater do occur at the *Bridge* site in the Boat House Run creek and the *HS-1* in the Harrelson Seafood tidal creek.

In summary, temporal variability in the sedimentary E. coli concentrations suggest that the sediments do not serve as a long-term legacy source to the water column. In some cases, sedimentary FIB concentrations increased, indicating that a current source is present. This is further supported by the widespread presence of optical brighteners, which should degrade rapidly upon exposure to sunlight. Because of the widespread use of septic tanks in Murrells Inlet, leakage from these units is a likely source.

RECOMMENDATIONS FOR FUTURE WORK

Sampling should be repeated at the same sites to better assess the temporal variability of sedimentary bacterial contamination. Grain size and composition of sediment should be analyzed to see if these characteristics influence bacteria levels, especially since Craig et al. (2002) notes the influence of grain size on extraction efficiency. GPS coordinates should be taken to better locate the sampling sites and septic tank maps should be created to assist in associating FIB contamination with proximity to septic tank systems.

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APPENDIX A

Standard Operating Procedure:
E. coli in fine-grained sediments

Description

The measurement of *E. coli* in sediments involves the extraction of bacteria adsorbed to particles by shaking sediment samples briefly with sterile buffered water. This method works will for fine-grained sediments and evaluated by Craig et al., 2002. An aliquot of supernatant is used to enumerate extracted bacteria. At sites where overlying waters have high concentrations of E. coli, corrections for pore water contributions are made by subtracting the E. coli concentration in water samples collected just above the sediments.

Equipment

1. Boots
2. Hand Sanitizer/Latex Gloves
3. PE Core liner
4. Rubber/Foam Ball
5. "Model Sample"
6. Permanent marker
7. 240 mL sterile polypropylene containers (Fisher 03-341-76A) and 120 mL sterile containers (Fisher 03-341-75E)
8. 99 ml vials of sterile buffered water (Fisher 02-686-202B DILU-LOK PHOS BFR WM99ML 50/CS)
9. Cleaning supplies for PE core liner – including spray bottle of ethanol.
10. Micrology supplies – sterile pipette, 30 mL sterile vial, media, and plate

PROCEDURE

1. Check that incubator is at 35C.
2. Take media out of freezer and lay out micrrology supplies and run log.
3. Put COC sheet in clipboard for field use.
4. Clean PE core liner with antiseptic soap and water. Rinse with deionized water. Finish with Ethyl alcohol from squirt bottle.
5. At site, fill out COC sheet. Collect sediment samples as follows:
6. Push core liner into sediment about 0.5 to 1 in deep.

$$3.14 \times 1 \times 1^2 = 3.14 \text{ in}^3 \times 2.54^3 \text{cm/in} \times 2.6 \text{ g/cm}^3 = 133 \text{ g sediment}$$

7. Use rubber ball (or palm of hand) as one way valve to keep sediment in place. Minimize the amount of water entrained in the sample.
8. Deliver into sterile snap top 240 mL vial (or 120 mL vial). Let settle 1 minute. Pour off water. Pour off excess sediment. Determine excess sediment by comparing volume collected to Model Sample (240 mL vial with level marked in permanent ink at 50 and 100g.)
9. Collect a water sample.
10. Put both samples on ice.
11. Wash core liner with sterile water between samples and rinse with EtOH.
12. Return samples to lab and within 6 hours of collection, process as follows.
13. At lab, analyze the water sample as per SOP.
14. To analyze the sediment sample, first weigh the sediment sample in its cup.

15. Pour premeasured 99 mL buffered sterile water into sediment.
16. Snap the cap back on the cup and mix vigorously for 1 minute.
17. Let settle 10 minutes.
18. Pipette 1 to 3 mL of supernatant into Micrology media using a sterile pipette.
19. Let sediment settle for 3 days. Pour off supernatant. Dry sediment in drying oven. Measure dry weight of sample in the cup. Weight of the cup is 24.8 g.
20. Compute bacteria concentration (CFU/100 g sediment) corrected for pore water contributions by assuming the water concentrations is equal to that in the pore water.
    a. Compute the volume of pore water in the sample = (Wet wt of cup + sample) – (Dry wt of cup + sample)
    b. CFU in the combined sediment + PW as

$$CFU(sediment + PW) = \frac{CFU\ counts\ from\ sediment\ sample\ plate \times (99\ mL + ml\ PW)}{mL\ supernatant}$$

    c. CFU in the PW as

$$CFU(PW) = \frac{CFU\ counts\ from\ water\ sample\ plate \times ml\ PW}{mL\ supernatant}$$

    d. CFU in the sediment as :

    CFU (sediment) = CFU(Sediment + PW) – CFU (PW)

    e. CFU in the sediment per 100 g dry weight sediment as :

$$\frac{CFU}{100\ g} = \frac{CFU(sediment)\ x100}{measured\ g\ dry\ weight}$$

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CCU EQL Bench Sheet

Bacteria in Sediment

Page _____

Analyst: _____

QA Reviewer: _____ Date: _____

Sample ID	Sample Site	Date/ Time Collected	Sediment Wt		Extraction		Incubation								
							E. coli			Fecal Coliform			Enterococcus		
			Wet (g)	Dry (g)	Sterile Buffer (mL)	Shaking/Settling time (min)	mL subsampled	Start/End	CFU	mL subsampled	Start/End	MPN	mL subsampled	Start/End	MPN