

Assessing Fecal Bacteria Sources in the Wrightsville Beach Area, 2007-2008: Report to the Town of Wrightsville Beach

By

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April, 2009

Sampling period:

The waters surrounding Wrightsville Beach were sampled in August, November and December 2007 and February, March, April, June, August, October, November and December 2008, and January 2009 for fecal coliform bacteria, and all months except August 2007 were also analyzed for *Enterococcus*. Nine stations were sampled:

Stations:

WB-CM: Causeway Marina. This is sampled from the end of the docks at Seapath Marina on the causeway. GPS: N 34 12.716 W 77 48.279

WB-WP: Wynn Plaza. This station is at the docks at Wynn Plaza (the gazebo at the corner of South Lumina across from Wings). GPS: N 34 12.502 W 77 47.819

WB-CYC: Carolina Yacht Club. Sampled from the end of a dock at Carolina Yacht Club. GPS: N 34 12.105 W 77 48.093

WB-JPB: Jack Parker Boulevard. This is sampled from the Coast Guard dock at the end of Jack Parker. GPS: N 34 11.357 W 77 48.765

WB-JM: Johnny Mercer's. We sampled here in the surf at Johnny Mercer's Pier. GPS: N 34 12.849 W 77 47.302 (*note this site was dropped after several months of data showed no water quality contact violations and little human source influence*)

WB-LB: Lollipop Bay. Samples are taken from a dock at a private residence at 2201 N Lumina Ave. GPS: N 34 13.486 W 77 47.128

WB-SB: Salisbury Bridge. This station is sampled from a private dock on Salisbury Street at Salisbury Bridge, where the old Pizza Hut was located. GPS: N 34 13.000 W 77 47.702

WB-WR: Wildlife Ramp. These samples are taken from the end of a dock at the Wildlife Ramp. GPS: N 34 13.114 W 77 48.687

WB-JM: Marina Street. These samples were taken from the dock of the Wrightsville Yacht Club. GPS N 34 13.017 W 77 48.770 (this station was initiated after we ceased sampling at Johnny Mercer's Pier).

Methods:

Fecal coliform bacteria and *Enterococcus*: Samples were collected for fecal coliform bacteria and *Enterococcus* bacteria at the above locations. In the laboratory fecal

coliform bacteria and enterococcus were analyzed by membrane filtration using Standard Methods (APHA 1998), with fecal coliform bacteria results reported as colony-forming units (CFU)/ 100 mL of water. While on-site additional samples were taken for water temperature, salinity, turbidity and dissolved oxygen using YSI instrumentation.

Optical brighteners, compounds added to laundry detergents, adsorb to clothing and form a light reflective layer creating the appearance of whiter whites and brighter colors. These compounds are excited by light in the near UV range (360-365nm) and emit light in the blue range (400-440nm). After light absorption, fluorescence is given off during the second excited state and can be measured by a fluorometer. In the United States, 97% of all laundry detergents contain one or both of two types of fluorescent whitening agents. Since household plumbing systems combine wastewater from toilets and washing machines, the presence of optical brighteners and fecal coliform bacteria in a waterway may indicate an input of human origin. Optical brightener samples were collected by filling Nalgene 125mL opaque collection bottles 10 cm below the surface facing into the stream. Collection bottles were acid washed and triple rinsed before sampling. Samples were refrigerated in the dark at 8° C and read within 8 days.

Fluorometry was performed with a laboratory fluorometer (Model 10-AU-000, Turner Designs, Sunnyvale, California). A kit was added to the fluorometer that included a lamp (10-049) emitting near UV light at 310-390 nm, a filter (10-069R) for the 300-400nm light range, and finally a 436 nm filter was added to greater decrease background fluorescence (Hartel 2007a). A standard curve was created using serial dilutions from 100 mg of Tide (Procter and Gamble, Cincinnati, Ohio) in one liter of deionized water. Tide is a commonly used laundry detergent known to contain optical brighteners. When the fluorometer was adjusted to an 80% sensitivity scale, the fluorometric value of 100 was equal to 100mg of Tide in 1 L of deionized water. The standard curve demonstrated that there was a linear relationship between the fluorometric response and detergent-sourced optical brighteners up to a reading of 100. Following field collections, each field sample was read on the fluorometer in triplicate at room temperature after 10 seconds to minimize degradation of optical brighteners by UV light (Hartel 2007b; Tavares et al. 2008). For optical brightener determination, the samples were allowed to warm to room temperature for approximately 30 minutes. Each sample was shaken and poured into a cuvette (about 1/3 full). The cuvette was then placed in the fluorometer modified as above for optical brightener measurement.

PCR Methods: The DNA extraction process on the first two trips worth of samples was begun on Tuesday January 22nd, and a second set was run beginning April 14. The extraction process takes approximately 6-8 hours for those samples (16 total). Once the DNA is extracted, the PCR (polymerase chain reaction) is set up. This process takes about 8 hours. When the PCR is complete, the results are run on an agarose gel to check for positive samples, identified by fluorescent bands of DNA. Once the positives have been identified, a second round of gels is run in order to separate these bands and a razor blade is used to cut them from the gel. This gel process takes about 3-4 hours. Once the bands have been cut, a GeneClean procedure is run in order to purify the DNA. The GeneClean process takes about 2 hours for 16 samples. The

DNA results of the GeneClean are then run through a Qubit Fluorometer to determine the concentration of DNA present in the samples. This process takes about 1 hour. Once the results are known, the appropriate amount of a digestion enzyme is added to each sample. The samples are then incubated overnight at 37C to allow for enzyme digestion. Once the samples have been digested overnight, they are loaded into a well and run through a DNA sequencer. For 16 samples, it takes about 1-2 hours for the samples to run. The result of this is a profile for each sample, with peaks that represent the bacterial groups present. We match these peaks to a database of known species to determine what is present in each sample.

Statistical analyses were performed on the fecal coliform bacteria and *Enterococcus* data sets. Bacteria concentrations were correlated against various physical and hydrological parameters including temperature, salinity, and rainfall (for the day of sample + the day before). Rainfall data were obtained from the following website: <http://www.wunderground.com/history/d>.

Preliminary Results:

Field results of fecal coliform and *Enterococcus* sampling are presented in Table 1. The North Carolina standard for recreational contact waters (fresh) is 200 CFU/100 mL. Fecal coliform samples were all below this standard in the twelve months we sampled except for a large April 2008 peak of 1,000 CFU/100 mL at WB-JPB, off the Coast Guard dock (Table 1).

The US EPA guide for *Enterococcus* for human contact waters is 104 CFU/100 mL. This standard was exceeded on four occasions at Wynn Plaza, three times at the Lollipop Bay location and Jack Parker Boulevard, twice at Carolina yacht club and once at the Salisbury Bridge location. The highest counts occurred in November 2008 during a rain event where we obtained *Enterococcus* counts of 1,000 at Carolina Yacht Club and 1,025 at Lollipop Bay. Additionally, in April 2008 we had a count of 925 CFU/100 mL at WB-JPB, off the Coast Guard dock (Table 1). **The four stations with the highest geometric mean *Enterococcus* counts for this sampling period were WB-LB (65 CFU/100 mL), WB-JPB (50 CFU/100 mL), WB-WP (43 CFU/100 mL) and WB-CYC (42 CFU/100 mL).** We suspect that the reason *Enterococcus* is much higher in polluted areas than fecal coliform bacteria is that fecal coliforms tend to die much more quickly in the high salinity marine waters of this area than do *Enterococci*; thus *Enterococci* are recommended for saltwater by EPA and used by NC Shellfish Sanitation as a beachwater standard.

Optical brightener samples were generally low at all sites and in all months, ranging from 1.7 to 4.7. These data indicate the lack of sewage pipe leaks in this area (there are no operating septic systems in Wrightsville Beach, according to Town staff). However, the April 2008 sample at WB-JPB was 8.2, which leads us to believe a sewage spill of some sort was responsible for the high fecal coliform bacteria and enterococcus counts at that site in April (Table 1).

Table 1. Fecal coliform and *Enterococcus* counts and human source signals in Wrightsville Beach area waters, August 2007-January 2009.

| Wrightsville Beach fecal bacteria project | | | | | | | | | | Fecal coliform bacteria CFU/100 mL |
|---|-------|-------|--------|--------------|-------|-------|-------|-------|-------|------------------------------------|
| Station | WB-CM | WB-WP | WB-CYC | WB-JPB | WB-JM | WB-LB | WB-SB | WB-WR | WB-MS | geomean |
| Aug-07 | 9 | 34 | 9 | 5 | 9 | 2 | 5 | 1 | | 6 |
| Nov-07 | 2 | 1 | 1 | 1 | 1 | 3 | 8 | 8 | | 2 |
| Dec-07 | 3 | 10 | 1 | 4 | 107 | 3 | 2 | 11 | | 6 |
| Feb-08 | 2 | 7 | 6 | 4 | 5 | 1 | 3 | 4 | | 3 |
| Mar-08 | 1 | 1 | 2 | 4 | 1 | 1 | 5 | 1 | | 2 |
| Apr-08 | 3 | 47 | 5 | 1,000 | 1 | 2 | 12 | 7 | | 10 |
| Jun-08 | 53 | 19 | 4 | 3 | 12 | 3 | 76 | 36 | | 14 |
| Aug-08 | 1 | 6 | 1 | 6 | | 1 | 5 | 10 | | |
| Oct-08 | 3 | 10 | 11 | 10 | | 4 | 5 | 8 | 9 | 7 |
| Nov-08 | 5 | 35 | 5 | 2 | | 64 | 8 | 27 | 11 | 11 |
| Dec-08 | 46 | 25 | 27 | 32 | | 14 | 14 | 36 | 27 | 26 |
| Jan-09 | 20 | 23 | 5 | 16 | | 3 | 8 | 13 | 27 | 12 |
| average | 12 | 18 | 6 | 91 | 19 | 8 | 13 | 14 | 19 | 9 |
| st. dev. | 18 | 15 | 7 | 287 | 39 | 18 | 20 | 13 | 10 | 7 |
| minimum | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 9 | 2 |
| maximum | 53 | 47 | 27 | 1,000 | 107 | 64 | 76 | 36 | 27 | 26 |
| geomean | 5 | 11 | 4 | 8 | 5 | 3 | 7 | 8 | 16 | 7 |

RED MEANS THERE WAS A HUMAN DNA SIGNAL IN THE FECAL BACTERIA
BOLDED = RAIN EVENT

| Wrightsville Beach fecal bacteria project | | | | | | | | | | <i>Enterococcus</i> CFU/100 mL |
|---|-------|------------|--------------|------------|-------|--------------|------------|-------|-------|--------------------------------|
| Station | WB-CM | WB-WP | WB-CYC | WB-JPB | WB-JM | WB-LB | WB-SB | WB-WR | WB-MS | geomean |
| Aug-07 | | | | | | | | | | |
| Nov-07 | 24 | 8 | 68 | 24 | 8 | 338 | 18 | 56 | | 31 |
| Dec-07 | 28 | 400 | 48 | 300 | 13 | 149 | 94 | 22 | | 71 |
| Feb-08 | 19 | 8 | 66 | 62 | 3 | 79 | 20 | 16 | | 22 |
| Mar-08 | 33 | 78 | 75 | 19 | 11 | 24 | 14 | 48 | | 30 |
| Apr-08 | 14 | 25 | 23 | 925 | 11 | 19 | 40 | 24 | | 33 |
| Jun-08 | 32 | 12 | 2 | 4 | 4 | 46 | 63 | 18 | | 13 |
| Aug-08 | 8 | 21 | 15 | 55 | | 29 | 35 | 41 | | 25 |
| Oct-08 | 18 | 10 | 20 | 9 | | 17 | 14 | 28 | 12 | 15 |
| Nov-08 | 56 | 165 | 1,000 | 15 | | 1,025 | 41 | 96 | 35 | 109 |
| Dec-08 | 10 | 110 | 126 | 59 | | 30 | 128 | 62 | 10 | 46 |
| Jan-09 | 45 | 410 | 26 | 319 | | 68 | 23 | 23 | 35 | 61 |
| average | 26 | 113 | 134 | 163 | 8 | 166 | 45 | 39 | 23 | 41 |
| st. dev. | 15 | 153 | 290 | 277 | 4 | 300 | 37 | 25 | 14 | 29 |
| minimum | 8 | 8 | 2 | 4 | 3 | 17 | 14 | 16 | 10 | 13 |
| maximum | 56 | 410 | 1,000 | 925 | 13 | 1,025 | 128 | 96 | 35 | 109 |
| geomean | 22 | 43 | 42 | 50 | 7 | 65 | 34 | 34 | 20 | 34 |

Bolded exceeds the instantaneous EPA standard of 104 CFU/100 mL

Results of the PCR source tracking for the first eleven months of collected data (August 2007 – December 2008) showed that all months except February and October 2008 had signals of human fecal contamination (Table 1). Of the months analyzed, human fecal contamination was detected on six occasions at the Causeway Marina and Carolina Yacht Club locations, and five occasions at the Wildlife Ramp and Lollipop Bay. Human fecal contamination was found on four occasions at the Salisbury Bridge site and on three occasions at Wynn Plaza and Jack Parker Boulevard (including the suspected spill situation). There were no canine signals detected during those periods at any of the sites. However, we have on occasion (June, November and December) found avian source signals (at WB-CM, WB-WR and WB-CYC), and ruminant signals (February at WB-WR; June at WB-CM and WB-WR, October at WB-SB); and in the rainy periods of November and December 2008 ruminant signals were detected in a broad variety of locations including WB-CM, WB-WP, WB-WR, WB-LB, WB-SB, WB-CYC and WB-MS) - likely sourced from deer visiting nearby marshes, with their fecal material carried by currents to those sampling sites. A rat fecal signal was detected in June at WB-CYC as well. Some locations detected mixed human and other signals and these will be rerun for further clarification.

The locations where human signals were frequently detected lead us to suspect boat-borne fecal sources entering area waters. Certainly boat use is very frequent at the Causeway Marina (WB-CM) and Wildlife Ramp (WB-WR) sites, as well as Carolina Yacht Club (WB-CYC) and Wynn Plaza (WB-WP) areas.

Statistical relationships - Fecal coliform bacteria and *Enterococcus* counts for the period August 2007 through January 2009 were strongly statistically correlated ($R = 0.502$, $p < 0.001$). This tells us that both indicators clearly serve as a proxy for fecal bacteria, but because the correlation coefficient R is not stronger, one is obviously longer-lasting in the environment.

There was a highly significant inverse statistical relationship between salinity and fecal coliform counts ($R = -0.368$, $p < 0.001$) and also salinity and *Enterococcus* counts ($R = -0.263$, $p = 0.013$). The relationship between salinity and fecal coliforms is stronger because fecal coliforms are more sensitive to saline waters than *Enterococcus* are. However, since these relationships are both statistically significant this may point toward stormwater runoff as a second source of fecal bacteria to these waters, because the statistics indicate that fresher water is related to higher fecal bacteria counts. As such, we conducted further analyses between both fecal indicators and the amount of rainfall that fell on the day of sampling plus the previous day. Rain that fell on the day of sampling but after the crews had sampled was not included in this analysis. Additionally, one data point, the very high fecal bacteria counts from the Coast Guard dock, was excluded based on the high optical brightener readings and our assessment that this was a sewage spill.

The relationship between rain and *Enterococcus* counts was not statistically significant (Table 2). However, there was a highly significant positive correlation between rainfall on the day of sampling plus that of the previous day and fecal coliform bacteria counts (r

= 0.299, P = 0.003). Individually several stations showed significant correlations between either fecal coliform or *Enterococcus* counts and rainfall (Table 2), including WB-CYC, WB-JPB, WB-WR and WB-SB. At WB-LB there was a high correlation coefficient R (0.504) but the probability value was not quite statistically significant. **These data indicate that there remains a problem of stormwater runoff being a second important source of fecal bacteria to the waters around Wrightsville Beach.**

Table 2. Correlations between fecal coliform bacteria counts, *Enterococcus* counts, and total rainfall on day of sampling plus the previous day, August 2007 – January 2009. Stations WB-JM and WB-JM are excluded due to reduced sampling effort. Data presented as correlation coefficient R / probability (p). Bolded results are statistically significant at $p < 0.05$.

| | Fecal coliforms | <i>Enterococcus</i> |
|--------------------|--------------------------------------|--------------------------------------|
| All sites combined | R = 0.299 p = 0.003 | R = 168 p = 0.121 |
| WB-CM | R = 0.445 p = 0.147 | R = -0.083 p = 0.807 |
| WB-WP | R = 0.368 p = 0.225 | R = 0.027 p = 0.937 |
| WB-CYC | R = 0.814 p = 0.001 | R = 0.422 p = 0.187 |
| WB-JPB | R = 0.736 p = 0.010 | R = -0.169 p = 0.649 |
| WB-LB | R = 0.504 p = 0.090 | R = 0.303 p = 0.366 |
| WB-SB | R = -0.027 p = 0.933 | R = 0.665 p = 0.025 |
| WB-WR | R = 0.668 p = 0.018 | R = 0.607 p = 0.048 |

Conclusions to date: Our source tracking data have shown that human waste flushing/dumping in the area around Wrightsville Beach is and has been contributing fecal bacterial contamination to these waters. These finding have led to the Town (with

efforts spearheaded by Steve Dellies) requesting State and Federal authorities to close this section of the Intracoastal Waterway to dumping of human fecal waste. To date this effort has been supported by New Hanover County and the Town of Carolina Beach. The University is pleased to have assisted the Town in this important public health endeavor.

The data also show that approximately half of the samples that were violations of State human contact bacterial standards did not contain human-generated fecal contamination, but are likely generated by stormwater runoff. Thus, in order to address and mitigate this important issue the Town and University need to continue this collaboration to 1) further define the specific areas where fecal bacteria-containing stormwater runoff is most prevalent, and 2) to obtain needed information on the organisms (animal sources) responsible for this fecal contamination.

Based on percentage of fecal bacteria water contact violations, geometric mean bacteria counts for the individual sites, and correlations between fecal bacteria and rainfall and salinity, we suggest four of our current sites should be investigated more intensely for sources (both animal and hydrological). These sites include Lollipop Bay (WB-LB), the Coast Guard Dock area (WB-JPB), Wynn Plaza (WB-WP) and the Carolina Yacht Club area (WB-CYC). Other lesser but potential areas include the Wildlife ramp (WB-WR) and Salisbury Bridge area (WB-SB). Other sites near the known problem areas may be more useful than the latter two as well.