Progress Report to Town of Wrightsville Beach

Assessing Fecal Bacteria Sources in the Wrightsville Beach Area

By
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Sampling period:
The waters surrounding Wrightsville Beach were sampled in August, November and December 2007 and February and March 2008 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 sample here in the surf at Johnny Mercer's Pier. GPS: N 34 12.849 W 77 47.302
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

Methods:

Samples were collected for bacteria and while on-site additional samples were taken for water temperature, salinity, turbidity and dissolved oxygen. In the laboratory fecal coliform bacteria and enterococcus were analyzed using Standard Methods, with results reported as colony-forming units (CFU)/ 100 mL of water.

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.

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 three months we sampled (Table 1).

The US EPA guide for enterococcus for human contact waters is 104 CFU/100 mL. The November samples showed high counts at the Lollipop Bay location. The December samples showed high counts at this location but also at several other sites, including Wynn Plaza docks, the Coast Guard dock at the end of Jack Parker Blvd., and near the Salisbury Bridge near the old Pizza Hut location. 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. February and March enterococcus samples were within NC DWQ standards.

Results of the PCR source tracking for the first three sets of samples shows that during August, November and December there were definite human sources of fecal bacteria present in the Wrightsville Beach area waters. In August human fecal bacteria signals were detected at the Wildlife Ramp, Lollipop Bay and Carolina Yacht Club locations. In November human signals were detected at the

Causeway Marina site and the Wildlife Ramp locations. In December we again detected human signals at Lollipop Bay. Thus, the Wildlife Ramp and Lollipop Bay both have had two human hits out of three PCR runs. There were no canine signals detected during those periods at any of the sites. Some locations detected mixed human and other signals and these will be rerun for further clarification.

The locations where human signals were detected leads one to suspect boat-borne fecal sources entering area waters. The December human signal at Lollipop Bay coincided with excessive enterococcus counts as well (see Table 1). Additional sampling and DNA source tracking is planned for early May 2008.

We note that in order for the Town to make data based management decisions on controlling fecal bacterial pollution in the waters surrounding Wrightsville Beach it is critical to compile a sufficiently solid data base from samples collected over all seasons during both rain and dry periods. Rain and runoff sampling has been very limited to date due the drought of 2007. We have produced a solid beginning but need to be able to have sufficient additional resources to obtain samples from rain events in summer-fall 2008 (assuming the drought has largely broken) and we need to be able to sample during the critical tourist period in summer 2008. Based on data to date, we also with to refine our collections to low tide as well as move at least one location (at a marina on the causeway) to a more representative location. Dr. Mallin is available to speak to Town government representatives as needed.

Table 1. Results of fecal coliform bacteria and enterococcus sampling in Wrightsville beach waters during late 2007 and early 2008, as well as locations of human fecal signals detected using PCR analysis.

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	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
average	3	11	4	4	25	2	5	5	
st. dev.	3	14	4	2	46	1	2	4	
minimum	1	1	1	1	1	1	2	1	
maximum	9	34	9	5	107	3	8	11	
geomean	4	7	2	3	10	3	4	4	

RED MEANS THERE WAS A HUMAN DNA SIGNAL IN THE FECAL BACTERIA

Wrightsville Beach fecal bacteria project Enterococcus CFU/100 mL

Station	WB-CM	WB-WP	WB-CYC	WB-JPB	WB-JM	WB-LB	WB-SB	WB-WR	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
average	26	204	58	162	11	244	56	39	
st. dev.	3	277	14	195	4	134	54	24	
minimum	24	8	48	24	8	149	18	22	
maximum	28	400	68	300	13	338	94	56	
geomean	26	57	57	85	10	**224**	41	35	

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