A MULTI-DISCIPLINARY, WATERSHED-BASED APPROACH TO BACTERIA SOURCE TRACKING WITHIN THE GRAND STRAND OF SOUTH CAROLINA

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Abstract. A multi-disciplinary, watershed-based approach was used to assess the potential contribution of septic tank discharges to high fecal indicator bacteria (FIB) levels in a tributary of the White Point Swash, a tidal creek located on the Grand Strand of South Carolina. In 2008, a monitoring site in the swash was placed on the 303(d) list for recreational and shellfish impairments based on *Enterococci* and fecal coliform contraventions. It is slated for total maximum daily load (TMDL) development by 2017.

The swash lies within the Myrtle Beach Urbanized Area and hence implementation of the TMDL will be required under the Clean Water Act's National Pollution Discharge Elimination System (NPDES) Phase II stormwater program. The most useful FIB TMDL's are those that contain source assessments that enable local stormwater managers to prescribe and implement best management practices (BMPs) to reduce loads. The US Environmental Protection Agency (USEPA) recommends a watershed-based approach for tracing and remediating FIB water quality problems and, hence, was adopted as the foundation of our methodology.

The study area tributary to the White Point Swash drains a watershed that includes a portion of the Town of Briarcliffe Acres, a 416-acre residential community that includes about 250 homes and the 500-acre undeveloped Meher Spiritual Center. Both are notable for low population density, low impervious cover and extensive tree canopy. Both the Town and the Spiritual Center rely on septic tanks for sanitary sewer service.

The potential contribution of septic tank discharge to the FIB impairment was evaluated by performing a watershed analysis, which included mapping of land cover, topography, septic tank locations, and stormwater utilities on a property/parcel level and collation of extant water quality data. Information on property owners' septic tank usage and maintenance activities was obtained from an anonymous mail survey.

Targeted sampling was conducted during wet and dry conditions to identify the potential geographic source(s) and conditions under which FIB impairments emanate. Tracers unique to human sources were used as putative indicators of potential septic tank discharge, which included the detection of a human-specific DNA marker in *Bacteroidetes*, a FIB common to human fecal microflora. A second tracer was the quantification of optical brighteners, a component of most laundry detergents. Concurrent measurements of *Enteroccoci*, fecal coliform, ammonia, turbidity, total suspended solids (TSS), volatile suspended solids (VSS), biological oxygen demand (BOD), salinity, temperature, dissolved oxygen and true color were used to corroborate the FIB source identification.

Preliminary results from initial sampling events indicate a correlation between high fecal coliform and *Enteroccoci* concentrations and the presence of human-specific *Bacteroidetes* gene markers and optical brighteners during wet-weather events, indicating (at least partially) a human source of the FIB impairment during these times.

This multi-disciplined, watershed-based approach has potential use for other FIB-contaminated sites along the Grand Strand's coastline. In 2008, the South Carolina 303(d) list included 11 sites within the Grand Strand listed for FIB contamination and an additional 22 sites designated as "waters of concern".

INTRODUCTION

The Town of Briarcliffe Acres, SC being subject to the requirements of a National Pollution Discharge

Elimination System (NPDES) General Permit for Discharges from a Regulated Small Municipal Separate Storm Sewer System (MS4), has entered into an interlocal agreement with Horry County to assist in compliance. The two entities are proactively addressing known water quality issues within its jurisdiction.

In 2008, a SC Department of Health and Environmental Control (SCDHEC) monitoring site in the White Point Swash, a tidal creek located within the Grand Strand of South Carolina, was placed on the 303(d) list for recreational and shellfish impairments based on *Enterococci* and fecal coliform contraventions. It is slated for TMDL development by 2017. In addition to this, monitoring conducted by the SCDHEC near the swash (as part of its larger beach monitoring program), detected high fecal indicator bacteria (FIB) levels that resulted in the issuance of beach closing advisories.

To better understand and address potential sources of FIB in this area, Horry County sponsored a multi-disciplinary, watershed-based study to assess the potential contribution of septic tank discharges to high FIB levels.

BACKGROUND AND RELATED WORK

Beach closings along the Grand Strand in Horry County historically have resulted from high levels of bacterial contaminants being detected in the surf zone of the Atlantic Ocean, mainly during periods of rainfall and runoff. Horry County commissioned a county-wide study (D&F, 2002) to identify sources of contamination and to recommend options for improvement to water quality at stormwater outfalls to the Atlantic Ocean. This study identified the septic tanks within the White Point Swash watershed (particularly those within the Town of Briarcliffe Acres) as a potential source of the FIB impairment.

Due to its wide geographical scope, the county-wide study included only limited analysis and sampling of the White Point Swash and contributory watershed. However, the limited sampling (using antibiotic resistance profiling and randomly amplified polymorphic DNA testing of fecal streptococcus bacteria) did indicate a relatively large human component of the measured FIB impairment.

To better understand the potential sources of FIB impairment of the White Point Swash, Horry County commissioned Thomas & Hutton, along with collaborators from Coastal Carolina University and Virginia Tech University, to conduct a bacterial source tracking study concentrating on the Town of Briarcliffe Acres tributary to White Point Swash.

METHODS

The multi-disciplined, watershed-based bacterial source tracking study included a review of previous studies and water quality monitoring data, a septic tank system survey, a watershed assessment, and a water quality sampling program conducted under wet and dry conditions.

Historical Data / Previous Studies. Previous studies (including some documenting previous and ongoing FIB sampling programs) were obtained and assessed for information relevant to this study. Studies and data were collected from various sources including the following: Meher Spiritual Center, Town of Briarcliffe Acres, Horry County, and SCDHEC. Relevant data and information were extracted and summarized.

Septic Tank System Survey. An anonymous septic system survey of Briarcliffe Acres property owners was conducted. In collaboration with project stakeholders, a 20-question, multiple-choice answer septic tank system survey was prepared and mailed to all property owners. Of the 246 surveys successfully delivered, 151 were completed and returned, representing a 61% response rate.

The survey was designed to assess the community's general knowledge of their septic tank systems, assess operation and maintenance activities, and gage the loadings to the septic tank systems. Survey responses were recorded and tabulated for analysis.

In addition to the Briarcliffe Acres septic tank system survey, the management and maintenance staff of the adjacent Meher Spiritual Center provided mapping and information concerning 30 septic tank systems located on the Center's site. Also, a detailed site visit was conducted to gather more information concerning the septic tank systems, document facilities contributing to each system, interview staff as to the operation and maintenance of the systems, and to observe and document the location of each system.

The known (Meher Spiritual Center) and estimated (parcels within the Town of Briarcliffe Acres not served by a centralized sewer system) septic tank system locations were geographically located for mapping purposes. The septic tank systems were mapped in relation to the watershed and estimated groundwater potentiometric divide (see Watershed Assessment below).

Watershed Assessment. A limited watershed assessment of the 489-acre watershed was conducted. Utilizing LiDAR derived 1-foot contours, the total watershed and six monitoring station sub-basins were delineated (Refer to Figure 1). The groundwater potentiometric divide (the divide by which groundwater is estimated to generally flow towards the lakes in the study area or towards the Intracoastal Waterway - away from study area) was also established from available data.

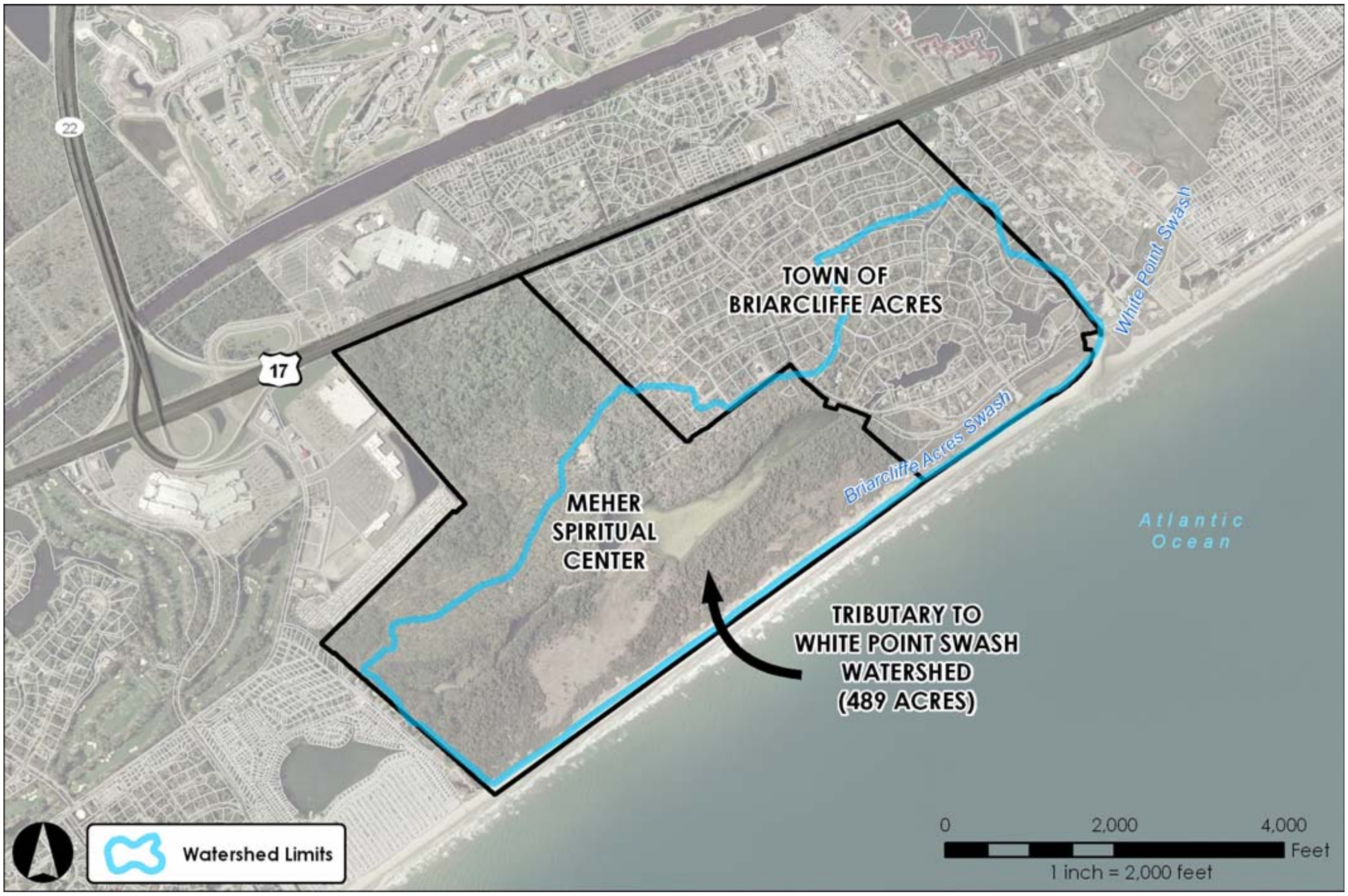

Figure 1 - Study Area

Next, land use / land cover were established from available data and summarized for each sub-basin and for the watershed. Using building footprint and impervious area GIS data supplemented with aerial photography interpretation, a homogeneous impervious cover data set was constructed for the watershed. From this, the percentage of impervious cover within each sub-basin and for the watershed was estimated.

Finally, the soils within the watershed were determined and assessed using readily available USDA National Resource Conservation Service (NRCS) soils mapping and reports.

Water Quality Sampling. A conceptual sampling plan was developed at the scoping phase of the project to test the hypothesis that septic tank effluent is a significant source of FIB. This required tracking markers of FIB contamination through space and time and differentiating amongst sources.

We elected to use a weight of evidence approach in which multiple proxies for FIB contamination were measured, including ones specific for human sources. Following recommendations of the Center for Watershed Protection and Pitt (2004), the following putative tracers of sewage are being measured: ammonia, color, conductivity, FIB, pH and turbidity. We also elected to measure Biological Oxygen Demand (BOD), Total Suspended Solids (TSS), and Volatile Suspended Solids (VSS). Two additional tracers that are specific to human sources are also being measured - optical brighteners and *Bacteroidetes* gene markers.

Onsite measurements of temperature, conductivity, dissolved oxygen, and pH are being used to track water mass sources and transport pathways. The data also afford an assessment of the degree of eutrophication in tributary to White Point Swash arising from nutrient loading associated with fecal inputs. Finally, two FIBs are being measured: *Enterococci* (via Enterolert Defined Substrate Technology ™ by IDEXX) and fecal coliform bacteria (via SM 9221 C E-99 (MPN)), as both are used as the measurement for water quality standards.

Water quality sampling is being conducted at six locations in the watershed. Sampling locations were chosen for location in the watershed, contributing land uses, accessibility, and other factors. Two sites are located in the tributary to White Point Swash and are sampled during mid ebb tide. Three sites are located in various ponds in Briarcliffe Acres and the adjacent Meher Spiritual Center. Although these ponds are natural, they presently collect nonpoint source flows from stormwater. They lay 400 to 800 feet upland of the swash. One site is being sampled only during wet weather flows to capture street runoff.

Sampling is being conducted during three wet weather events and four times during dry weather. The wet weather sampling threshold is approximately 0.75-inch accumulated rainfall. During each event, each site is sampled three times at 12 hours interval starting during the first ebb tide following initiation of rainfall. Rain data are being collected from four nearby rain gages.

Grab samples are collected, preserved, and transported to the Coastal Carolina University (CCU) Environmental Quality Laboratory (EQL) in Conway, SC for analysis. Preserved samples are shipped to the Virginia Tech Microbial Source Tracking Laboratory for optical brighteners (OBs) and gene marker analyses.

The gene marker analyses include a general fecal *Bacteroidetes* gene markers and human-specific *Bacteroidetes* gene markers. The *Bacteroidetes* gene markers are reported in a presence/absence format; however, the relative contribution of the human component is estimated from the amount of human-specific DNA recovered from each water sample (see Human-specific *Bacteroidetes* Gene Markers section below).

DISCUSSION

This study is ongoing but nearing completion. As of the submittal date of this paper (July 2010), all relevant historical studies and data have been obtained and assessed, the septic tank system survey has been conducted and results tabulated, septic tank systems (and other wastewater infrastructure) have been mapped, and the watershed assessment has been completed, including the land use / land cover and percent impervious estimations. Water quality sampling is partially complete, with three of four dry weather events completed and two of three wet weather events completed.

Although water quality results are available only for a portion of the planned sampling, the use of the specific water quality parameters in this study – particularly the testing for OBs and human-specific *Bacteroidetes* gene markers - have proven very beneficial in understanding potential bacteria sources.

Optical Brighteners. The identification of OBs via fluorometry offers a fairly rapid and comparatively inexpensive method of identifying human waste pollution. OBs, also known as fluorescent whitening agents, are organic compounds (used in household detergents to "whiten clothing") that absorb long-wave ultraviolet light (365 nm) and re-emit it most strongly within the blue portion of the visible spectrum (415nm to 435nm). This re-emission of blue light from the OBs that bind to clothing in a wash helps to balance the natural yellow color of cotton fabrics, making them appear bright white (Hagedorn and Weisberg, 2009).

Anywhere from 25-95% of the OBs used in a wash cycle are bound to clothing during the wash (Poiger et al. 1998), while the remainder is discharged into wastewater. The mixing of wastewater and grey water in household plumbing systems allows for the detection of OBs in both septic systems (Close et al. 1989; Boving et al. 2004) and untreated sewage (Poiger et al. 1998). In addition, OBs are used in a variety of other home products, including toilet papers (Hagedorn et al. 2005), providing additional OB input into wastewaters. Since there is no known natural source of OBs, their detection is indicative of human wastewater.

However, some naturally occurring organic matter my exhibit fluorescence, interfering with the positive detection of OBs. To address this, water samples that appear to be positive for OBs are exposed to intense ultraviolet (UV) light and the amount of photodecay is determined. OBs typically exhibit much higher photodecay (>30%), while organic matter's photodecay is much less (<10%). This method of separating OBs and organic matter fluorescence is described by Hartel, et. al. (2007).

Human-specific *Bacteroidetes* Gene Markers. The use of host-associated markers to classify the sources of fecal contamination has emerged as a widespread strategy for bacterial source tracking studies (Stoeckel and Harwood, 2007). *Bacteroidetes* are one of the most numerous members of the normal flora of the human gut, present at concentrations of $1 – 10 \times 10^{10}$ cells/g dry feces (Franks et al., 1998). The *Bacteroidetes* host-specific marker sequences, originally developed by Bernhard and Field (2000), are located on the 16S rRNA of these obligate anaerobes. Host-specificity and sensitivity for each primer pair was tested against non-target and target feces, respectively, by several researchers during and after the initial evaluation of the markers. The human-specific marker, HF183, was detected in 70 – 100% of human-origin wastewater samples in multiple studies (Stoeckel and Harwood, 2007). The markers are currently utilized in a presence-absence format; however, using a protocol described by Seurinck et. al. (2005), the relative load contribution of the human-source bacteria can be estimated with confidence and that protocol is being used as part of this study.

CONCLUSIONS

This study is not fully completed, and thus a definitive conclusion as to the source of the FIB impairment in the watershed cannot be made at this time. However, early indications are that methods employed in this study have successfully differentiated portions of the FIB as being attributable to a human source.

Preliminary results from initial sampling events indicate a correlation between high fecal coliform and *Enteroccoci* concentrations and the presence of human-specific *Bacteroidetes* gene markers and OBs during wet-weather events, indicating (at least partially) a human source of the FIB impairment during these times.

At one site in the tributary to the White Point Swash, very high FIB levels have coincided with high OBs and positive identification of human-specific gene markers in all wet weather (n=6) and two out of the three dry weather samples. Color measurements suggest this site is most influenced by flows from and possibly through adjacent salt marshes.

At several sites, high concentrations of BOD, TSS and VSS along with high percentage of VSS suggest a significant loading of organic rich particles. Evidence for eutrophication has also been observed at some sites suggesting significant loading of nutrients. In some of these cases, elevated ammonia concentrations were observed. This demonstrates that FIB contamination can be accompanied by other water quality impairments that are likely of similar origin, thus providing further impetus for remediation via elimination and/or reduction of the pollutant source(s).

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