EVALUATION OF FECAL INDICATOR BACTERIA CONCENTRATIONS AND EXPORTS IN THE BOATHOUSE CREEK PORTION OF THE LOWER WHITE OAK RIVER

By

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December 2017

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Major Department: Health Education and Promotion

The Boathouse Creek portion of the Lower White Oak River is listed as an impaired water because of elevated fecal indicator bacteria (FIB) concentrations. It has been estimated that 61% of the bacteria is delivered via urban storm water runoff. The goal of this project was to gain a better understanding of the spatial and temporal variability of FIB in the Boat House Creek watershed and determine if FIB concentrations posed environmental health threats. Monthly water quality monitoring began in March 2015 and ended in April 2017 at 8 locations within the watershed. Six stormflow samples were also analyzed. Monitoring included the analyses of stream samples for *Escherichia coli (E. coli)* and enterococci. In addition, physical and chemical parameters were also monitored, including: pH, temperature, dissolved oxygen, oxygen-reduction potential, specific conductivity, stream velocity, stream discharge, and turbidity. Concentrations of *E. coli* and enterococci frequently (> 75% of times sampled) exceeded recommended water quality standards. FIB concentrations in streams were typically higher closer to the estuary and stormflow concentrations of FIB were elevated relative to base flow concentrations for each sampling location. Microbial source tracking analyses indicated that animals were the most likely origin of the bacteria. Stormwater best management practices including a rain garden, water control structures (5 installed total), rock check dams (4), and various drainage way modifications were implemented in the watershed. More stormwater BMP implementations and educational outreach activities are suggested to improve water quality at the watershed-scale.

EVALUATION OF FECAL INDICATOR BACTERIA CONCENTRATIONS AND EXPORTS IN THE BOATHOUSE CREEK PORTION OF THE LOWER WHITE OAK RIVER

A Thesis

Presented to

The Faculty of the Department of Health Education and Promotion

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

Masters of Science in Environmental Health

By: Nicole Lyons

December 2017

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Introduction and Background

Water Quality and the US Environmental Protection Agency

The U. S. Environmental Protection Agency (EPA), with authority granted via the Clean Water Act (CWA) of 1972, has set a goal to protect water quality and public health by establishing water quality standards and enforcing environmental regulations to ensure water resources meet the standards. Section 303 (d) of the CWA includes requirements for identifying and listing impaired waters within a state. An "impaired water" is any water that is too degraded or polluted to meet designated uses such as recreation and aquatic habitat. Common causes of impairment include excess bacteria, nutrients, mercury, and sediment from various point and non-point sources. State regulatory agencies typically monitor and characterize the quality of water resources and compare conditions to standards set by Federal and State agencies (EPA 2016). If water quality is considered impaired, then mitigation is required for the major point and non-point sources of pollution.

The National Pollutant Discharge Elimination System Act (NPDES) was developed and implemented to help control and regulate point sources of pollution such as direct discharges from wastewater treatment plants. In 1987, the EPA amended the CWA to include non-point source pollution control and storm water permitting. Non-point sources include diffuse pollution such as septic systems, agricultural runoff, and stormwater runoff that is not piped directly into receiving waters. The EPA requires the development of Total Maximum Daily Loads (TMDL) for waters that are on the CWA 303(d) list (EPA, 2001). TMDLs are calculated allowances for pollutants to enter the water and still allow the water to meet water quality standards. The development of TMDLs requires locating the source of pollutants, which is a necessary step in identifying BMPs that will reduce the pollutants from entering the surface waters (Cabrera-Stagno, 2007).

There are many impaired waters in North Carolina including portions of major watersheds including the Neuse River, Tar-Pamlico River, Falls Lake, Jordan Lake, High Rock Lake and White Oak River. This study was conducted in the White Oak River (Figure 1).

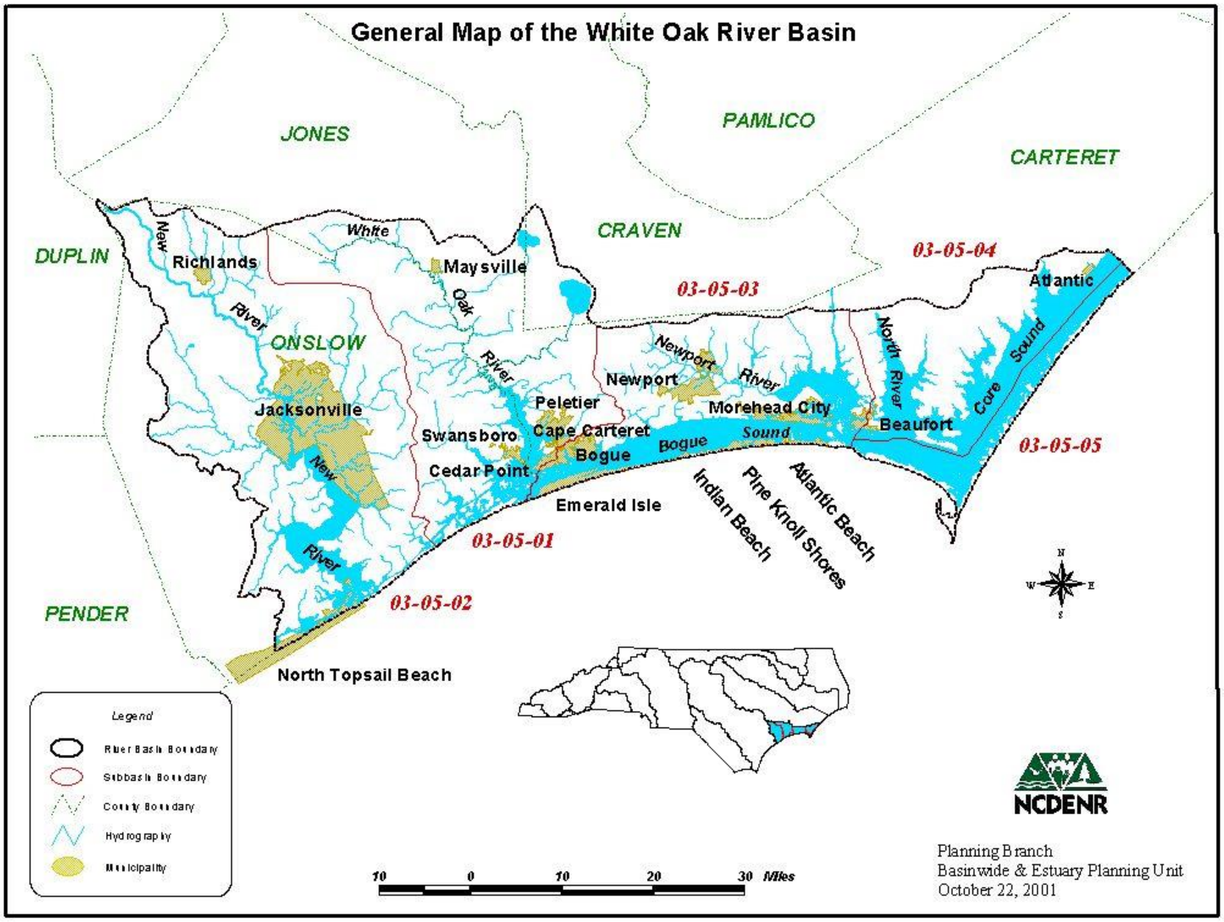


Figure 1: White Oak River Basin including the New, Newport, North and White Oak Rivers and associated drainage areas.

White Oak River Watershed, North Carolina

The White Oak River is a 42-mile-long, predominately black water river due to the high organic matter content within the river (Frankenberg, 1999), with almost 12,000 acres that drain into the estuarine system of NC (Figure 1). The lower White Oak River was previously very popular for shell fishing, but as development in the watershed increased sections of the waters became contaminated. Bacteria pollution led to the closure of 42% of the clam and oyster beds. Approximately 67% of shellfish beds are currently closed temporarily after storm events because of concerns with stormwater-related spikes in bacteria concentrations (Tursi, 2009).

The Boathouse Creek portion of the lower White Oak River, near Cedar Point, NC is listed as an impaired water under the CWA section 303(d) with fecal coliform being listed as the cause of impairment (Tursi, 2009; EPA, 2014). This area has had a human population increase of 40% from 2000 to 2015 (US Census, 2015). With the increase in population, there was a corresponding increase in construction of housing, roads and impervious surfaces and related decrease in natural areas to buffers and filter stormwater.

The loss of natural areas contributes to stormwater runoff and pollutant transport (Figure 2) (Paul and Meyer, 2001). Approximately 40% of the land within the watershed was for urban/NCDOT usage (Tursi, 2009).

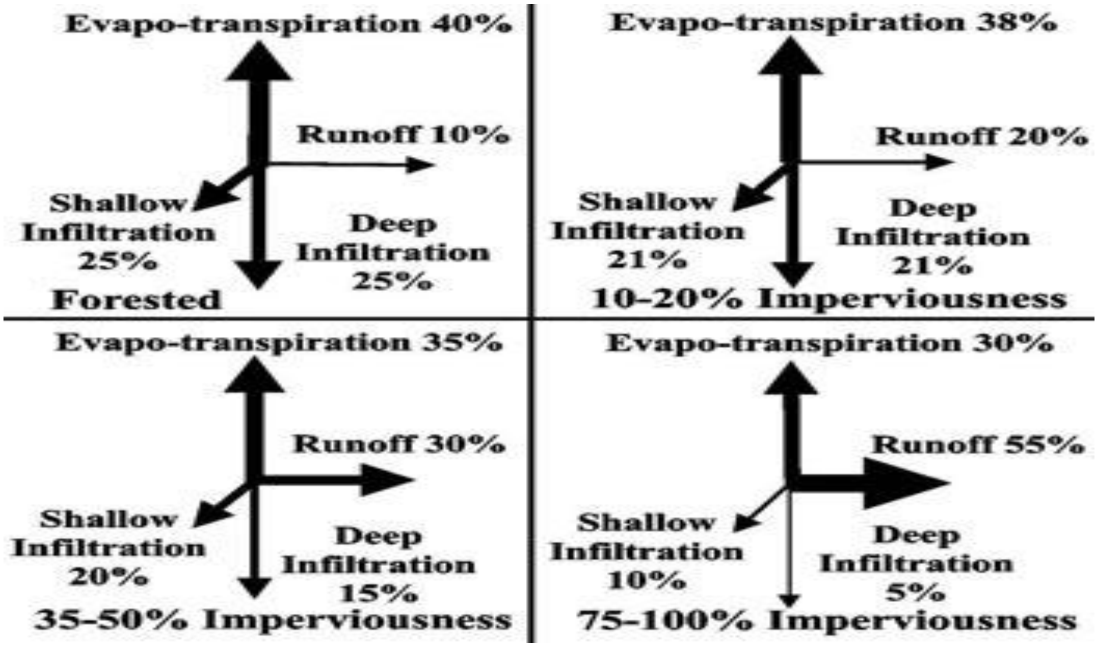


Figure 2: Change in water transport with respect to percent impervious area

During storm events, rain may overload sewer systems, or over-saturate drain fields of septic systems in urban and suburban areas (Mallin, 2006). The runoff eventually enters nearby surface waters transmitting harmful enteric bacteria from the wastewater. The Town of Cedar Point is urbanizing, but still contains many acres of wetlands that serve as habitat for wildlife. Bacteria from pet and wildlife waste that is deposited on impervious surfaces may be transported to surface waters during storms via the storm drains and curb and gutters (Gaffield et al., 2003). The increase in urbanization and erosion of the streams in response to storms may increase the transport of wildlife waste that was deposited adjacent to the streams. Stormwater runoff is also capable of transporting sediment to surface waters, creating turbid conditions and degrading aquatic habitat (EPA, 2003).

Fecal Indicator Bacteria

A commonly used analysis for water quality characterization is to determine the presence and concentration of fecal indicator bacteria (FIB). The EPA (1986) suggests using the FIB, *Escherichia coli (E. coli)* and enterococci. These bacteria typically live in the guts of warm-blooded animals, and although they themselves are usually not virulent, their presence means that there could possibly be fecal-borne pathogens in the water that could cause harm. Enterococci are gram positive, non-spore forming spherical bacteria (Fraser et al, 2017). Enterococci live in a variety of environments, with a temperature range of 5° C to 50° C and a pH range of 4.6-9.9, with an optimum pH of 7.5 for growth (Fisher and Phillips, 2009). Enterococci infections commonly include urinary tract and wound, with endocarditis as a more concerning infection (Cabral, 2010).

E. coli are rod shaped, gram-negative bacteria whose primary environment is the lower intestines of warm blooded animals. *E. coli* may persist once excreted to the outside environment, surviving a range of temperatures (7.5-49° C). Because it is a heterotrophic bacterium, the availability of nutrients encourages growth in temperate environments (Ishii and Sadowsky, 2008). Significant positive correlations have been observed between water enterococci and *E. coli* concentrations and swimmer gastrointestinal illness (GI) in recreational freshwater and between enterococci concentration and GI in marine waters that are subject to urban/stormwater runoff (Boehm and Sassoubre, 2014; EPA 1986). The EPA established the Recreational Water Quality Criteria (RWQC) to protect waters used for recreation including swimming, boating, and/or kayaking (EPA, 2015). In addition to the concern of ingesting the actual water, ingesting shellfish contaminated with fecal bacteria can lead to illness, and occasionally even death (Iwamoto, 2010). Economic loss via closure of shellfishing waters may also be associated with the excess bacteria concentrations (Mallin et al., 2016). In 2009, 113 samples were taken from Boathouse Creek, and 110 samples did exceed the bacterial standard for shellfish waters (Tursi, 2009).

Microbial Source Tracking

Waste from humans and animals may contain various pathogens that pose environmental health risks. Examples of pathogens include bacteria such as salmonella, viruses, such as swine hepatitis E virus, or parasites, such as *Ascaris*, which can infect humans (Sobsey et al., 2006). Determining the major sources of pathogens in water resources is important for developing and implementing focused strategies to improve water quality. Microbial source tracking (MST) at its simplest is the assumption that some characteristics of feces from the "host species" are specific and identifiable (Field and Scott, 2008). Molecular, or genotypic, MST allows researchers to use the genetic makeup of an organism or a cell in environmental samples for comparison to a database of microbial isolates, or "fingerprint". A match suggests the origin of the fecal bacteria (Sargeant et al., 2011). The Polymerase Chain Reaction (PCR) is used to copy the gene making billions of replicates (National Center for Biotechnology Information [NCBI] 2014). This allows for the identification and detection of gene sequences based on size and charge of the DNA. During the PCR process, target strands go through multiple cycles of heating and cooling to amplify the DNA. At the beginning of the reaction, high heat (approximately 95˚C) is applied to separate the double-stranded DNA molecule. This is the denaturing step. The second step consists of lowering the temperature to approximately 55˚ C to allow annealing of primers. Primers are short DNA sequences between 15 and 30 nucleotides long that are used to bind at the start and the end of the target strand. Primers are made by identifying the DNA sequence of the gene to be amplified. In the final cycle, DNA polymerase (Taq) is added to the strand of DNA for elongation at 72°C. The polymerase adds complimentary deoxynucleotides to the 3-prime end of the single strand of DNA on the primer, which then generates a section of double stranded DNA in the region of the gene of interest. This three-step process occurs between 30 and 40 times allowing many copies of the gene to be made. These DNA fragments usually have a dye or radioisotopes added to them to identify the gene of interest (Phillips, 2017). In qRT-PCR, an oligonucleotide probe is designed and used to hybridize to the target DNA sequence. These probes are fluorescently labeled at their 5′ ends. Taq polymerase's 5′ nuclease activity causes cleavage of the probe to generate a detectable signal. This allows for measurements of the products generated during each cycle of the PCR process (Heid et al. 1996).

Stormwater Best Management Practices

Best management practices are any practice or combination of practices that are determined to be "effective and practicable means (including technological, economic, and institutional considerations) of reducing the amount of pollution generated by non-point sources to a level compatible with water quality goals" (NC Forest Service, 2017). Stormwater best management practices (BMPs) are designed and implemented to reduce urban runoff and the mass loading of bacteria and other pollutants to receiving waters during rain events. Stormwater BMPs generally capture, store, and use various physical, chemical, and biological mechanisms to treat contaminants in runoff prior to discharge to surface waters. Physical mechanisms include retention/detention of runoff and sedimentation. Chemical treatment includes use of flocculants to enhance sedimentation, and biological treatment includes plant and microbial uptake and transformation of pollutants. Stormwater BMPs vary in size based on the drainage area, design storm, and configuration of the BMP. Common stormwater BMPs include using controlled drainage with flashboard risers, rain gardens, and check dams. Water control structures were designed and fabricated to fit into existing driveway culverts of volunteered properties. The structures included a box-shaped frame with slots to allow flashboards to be added (to raise the outlet elevation and reduce outflow) or removed (to lower the outlet elevation and release flow). When flashboards are in place, the water in the ditch must pond to a height above the boards for outflow to occur. This increases the time for infiltration, reducing runoff and bacterial loads introduced to surface waters. Controlled drainage has been used mainly in agriculture to reduce nutrient, sediment and pollution outflows (Cessti et al., 2003).

Check dams are another BMP that function similar to controlled drainage. Check dams are built with various size stone and gravel placed in the drainageway to slow runoff and increase infiltration. Check dams do not allow for easy adjustments to the outlet elevation as flashboards do, but are easier to install (NCDENR, 2013).

Another BMP that has been shown to be cost-effective and efficient is the rain garden, or bio-retention basin. Rain gardens are installed down-gradient from impervious surfaces and up-gradient from receiving waters. They are excavated to provide 7.5 to 30 cm of ponding depth/storage, and are typically lined with mulch and planted with vegetation that can withstand saturation extremes such as frequent ponding and dry conditions. Rain gardens should be installed in conductive soils with seasonal-high water tables at least 60 cm below the bottom of the rain garden (Liu et al., 2014).

Low-impact developments (LID) are generally constructed in watersheds that are very close to impaired or environmentally sensitive waters. The LID concept includes the integration of BMPs such as rain gardens (Figure 3), rainwater harvesting, and diffuse stormwater management throughout a subdivision (Tilman et al., 2011).

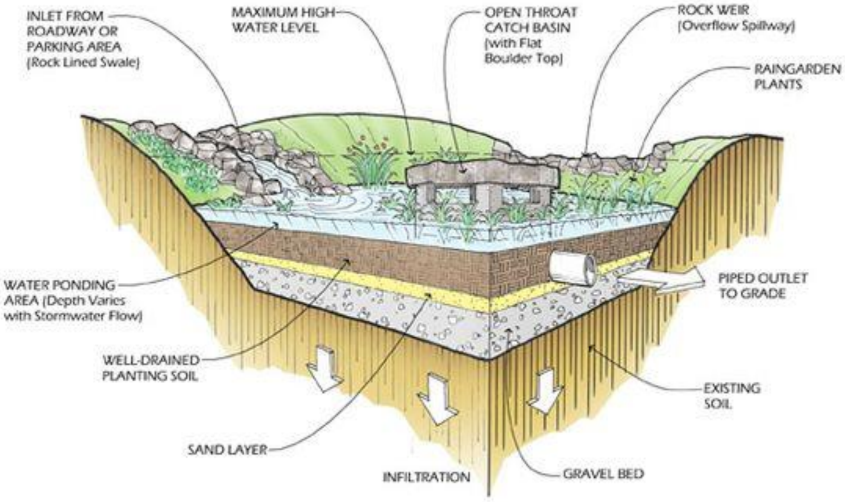


Figure 3: Rain garden best management practice for reducing stormwater runoff

Septic Systems and Non-Point Source Pollution

Many coastal areas rely upon septic systems for the treatment of their wastewater. Approximately 49% of North Carolinians rely on septic systems, compared to national average of about 24% (Naman & Gibson, 2015). Septic systems are usually composed of four primary components: the septic tank, effluent distribution device, the drain field trenches, and soil. Septic tanks have large capacities 3785 liters (1000 gallons) and are typically constructed using concrete. Septic tanks receive wastewater from all plumbing fixtures in the home/business they serve. Wastewater in the septic tank is divided into three layers including the top "scum" layer, a clear middle layer, and a solid bottom layer or sludge layer.

Septic tanks have baffles in them to slow down the water and to hold back more of the solid, giving it time to sink to the bottom (Figure 4). Microbes that live in the human gut are responsible for much of the breakdown of the organic material. Effluent from the tank is piped to a distribution mechanism such as a distribution box. The box distributes the septic tank effluent to drain fields. These drain fields are usually gravel filled beds that surround perforated pipes. Septic tank effluent flows out of the pipes, the gravel provides storage space for the effluent until it infiltrates the soil. As the effluent percolates through the soil, important microbes within the soil help break down bacteria, and the soil helps percolate the water (Vogel, 2005; Sowah et al.,2014).

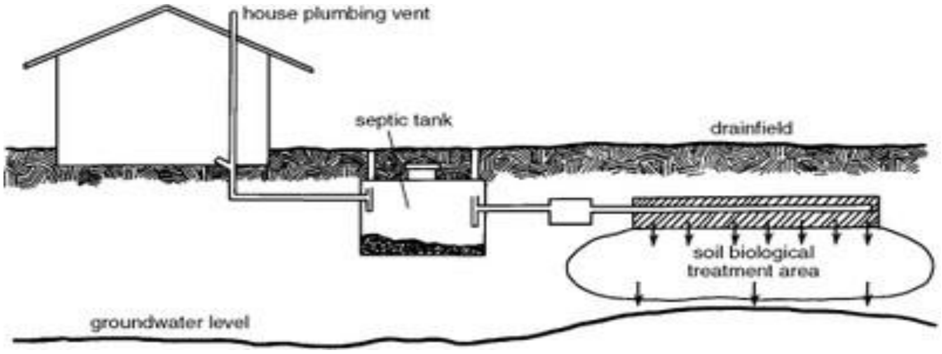


Figure 4: Schematic of septic system

There are many factors involved with the pollutant treatment efficiency of septic systems including the soil type, separation distance from the drainfield trenches to groundwater, and distance from the system to surface waters (Hygnstrom, 2008; Humphrey et al., 2015). Coastal areas tend to have sandy, hydraulically conductive soil that transmits effluent quickly, potentially limiting opportunities for bacteria treatment (filtration, adsorption, predation, etc.) (Cooper, 2016). Vertical separation from drainfield trenches to groundwater is another factor that may influence bacteria treatment in soils beneath septic systems (Humphrey et al., 2011). Systems installed in areas close to the water table don't allow for distance between the discharge point of the drain field and the water table to let the aerated soil do its job of treating the effluent (Gustafsen et al., 2000; Schneeberger et al., 2015). Proximity to surface waters is another factor related to bacteria contributions to surface waters (Anderson, 2010). Setbacks are required to protect nearby bodies of water, and they vary according to local ordinances (Mallin, 2013).

Goal and Objectives

Coastal North Carolina's tourist and permanent human populations continue to grow, and accompanying this growth are alterations to the natural environment. Increases in impervious surfaces have led to an increase in the volume of urban runoff delivered to surface waters during storms, degraded water quality, and water use impairment. The goal of this study was to gain a better understanding of the temporal and spatial variability of water quality of the lower White Oak River with regards to fecal indicator bacteria and determine if the FIB concentrations were elevated relative to recommended standards. Four specific objectives were outlined.

Objective I: Determine the frequency at which concentrations of *E. coli* and enterococci exceeded recommended water quality standards.

Objective II: Determine if differences in fecal indicator bacteria concentrations for stormflows versus baseflow were statistically significant ($p \leq 0.05$).

Objective III: Determine if statistically significant differences in fecal indictor bacteria concentrations for relatively warm and cold seasons were observed.

Objective IV: Estimate the volume of runoff and microbial loading that was reduced by the implementation of stormwater BMPs.

Materials and Methods

Study Site

Sampling locations (n = 8) were identified for routine monitoring within the Boathouse Creek watershed, where prior reports suggested the majority of FIB loading to the Lower White Oak was occurring.

Three monitoring locations were in the Ocean Spray community (WO-1 to WO-3), three were in Marsh Harbor (WO-4 to WO-6), one was near the congruence of streams draining Ocean Spray and Marsh Harbor (WO-7), and one in the estuary at the US Forest Service boat ramp (WO-8) (Figure 5).

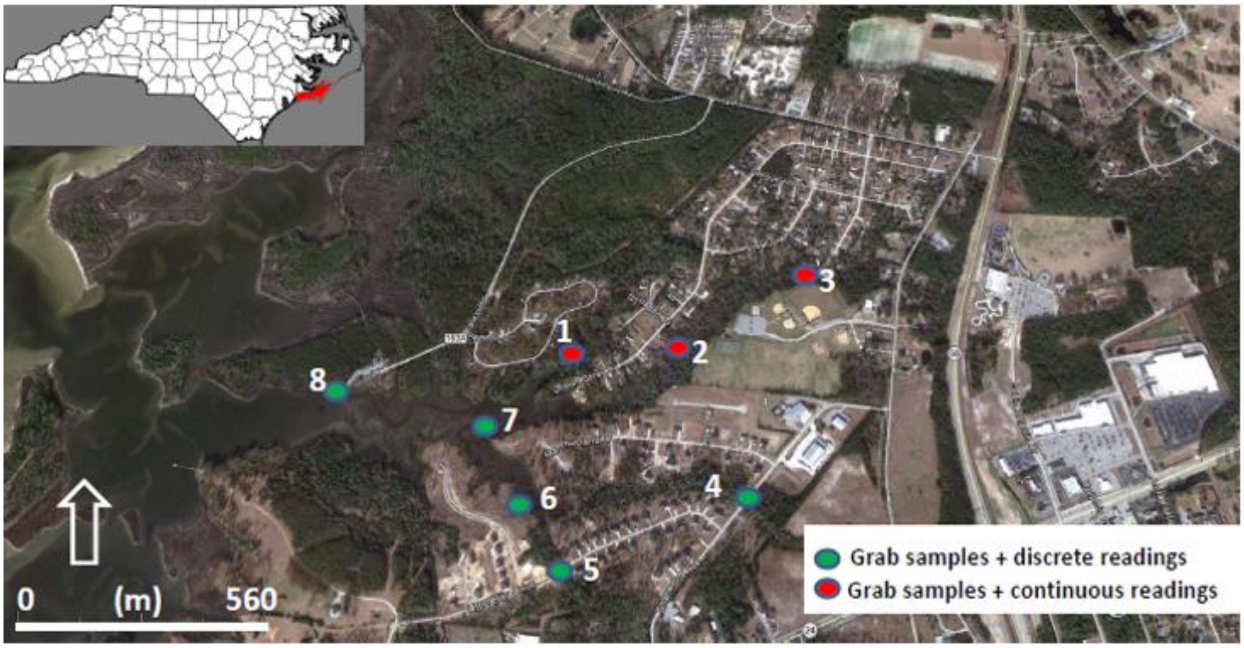


Figure 5: Aerial view of the Boathouse Creek watershed and the 8 sampling locations

Background samples (n = 5) were also collected from a pond and stream in a relatively undeveloped section of the Boathouse Creek watershed for FIB analyses and comparison to the other sample locations in more developed areas (Figure 6).

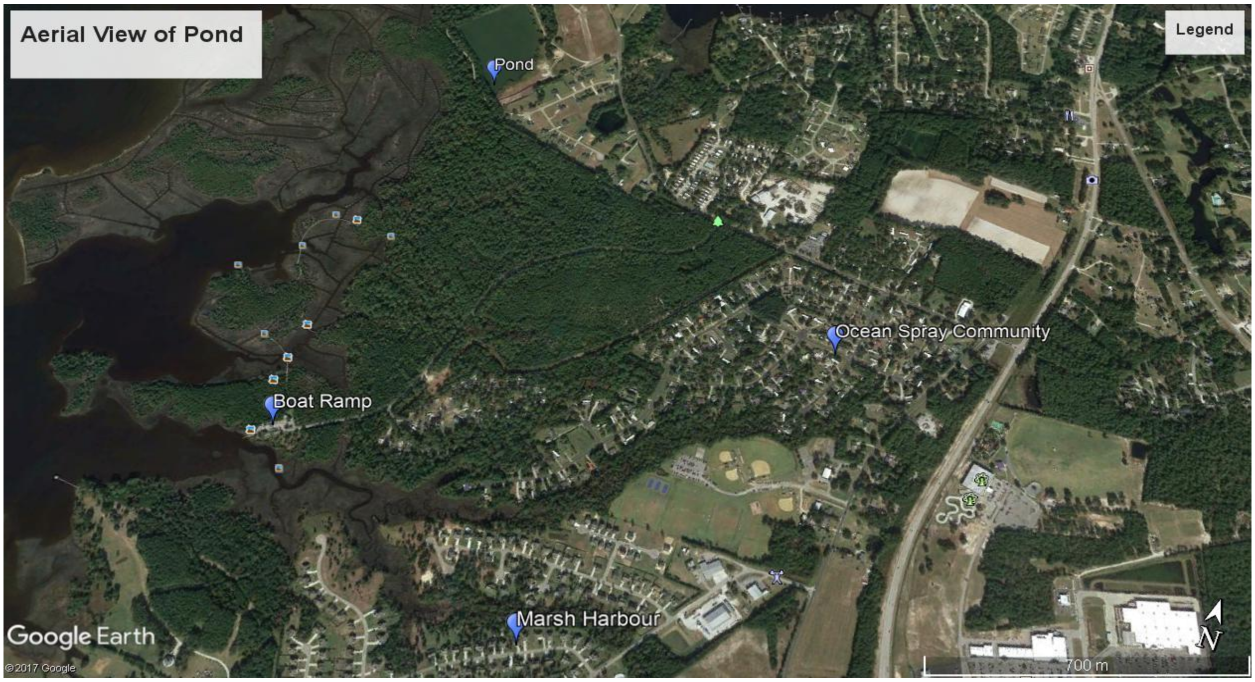


Figure 6: Pond located off of Holland Road that was sampled

Between May 2015 and April 2017, chemical, physical, biological parameters of water were obtained from the 8 sampling locations and were monitored on an approximately monthly basis (Figure 5). Water quality parameters were also monitored at the pond during the months of February 2016 to June 2016 (Appendix 5).During the study, there were 6 storm events during which sampling occurred at the 8 locations for storm samples.

Physical and chemical parameters including specific conductance, temperature, dissolved oxygen, oxidation-reduction potential, and pH were measured at each sampling location using an Yellow Spring Instrument (YSI)™ (Yellow Springs, OH) 556 Multiparameter Instrument. The YSI meter was calibrated prior to each sampling. Turbidity was also measured for all samples using the *Hach*™ (Loveland, CO) 2100p turbidimeter. These measurements were compared to standards for pH, DO, temperature, and turbidity listed in the North Carolina Department of Natural Resources "Redbook" (2007) of water quality parameters. For WO-1 through WO-5, the active stream depth, stream width, and velocity were measured and discharge (L/s) was calculated during each site visit. Velocity was measured using either the floating object method, or with a dye due to the typically low velocities (Michaud, J.P. and Wierenga, M., 2005).

The fecal indicator bacteria (FIB) enterococci and *E. coli* were analyzed for collected water samples. During each sampling event (n = 24), two 100-mL samples were collected from each site via the dip method; one for *E. coli* and one for enterococci. The samples were kept on ice in coolers for transport to the East Carolina University (ECU) Environmental Health Sciences Water Lab. Dilution factors between 2.5 and 10 were often used for samples so the maximum undiluted Most Probable Number (MPN) (2119) was not exceeded, and to allow for a better calculation of the concentrations of FIB. *IDEXX Colilert*™ and *Enterolert*™ with *Quanti-tray 2000*™ methods were used to enumerate *E. coli* and enterococci, respectively. The media were added to the appropriately diluted samples, then shaken vigorously to ensure proper mixing and dissolution. After all samples were mixed thoroughly, the 100 mL samples were poured into the Quantitrays. The Quantitrays were labeled with the time, sample identification number, the dilution factor, and the bacteria being tested (*E. coli* or enterococci). The trays were heat sealed and then placed into incubators for 24 hours. The trays tested for *E. coli* were incubated at 37°C and the trays tested for enterococci were incubated at 41°C. In a dark room, a black light was utilized to determine the number of wells that luminesced for each *E. coli* and enterococci tray.

A chart provided by IDEXX was used to determine the MPN of *E. coli* and enterococci that corresponded to the number of large and small wells that illuminated for the trays. The MPN for the samples were then multiplied by the dilution factor to determine the actual MPN.

Concentrations of *E. coli* and enterococci were compared to EPA (1986) standards for recreational waters to determine frequency of exceedance and thereby gain a better understanding of the environmental health risks associated with these waters (Table 1). This study utilized the EPA single sample maximum allowable density since sampling occurred monthly. Samples collected from freshwater locations were analyzed for *E. coli* and enterococci and compared to the EPA (1986) standards for freshwater. Samples collected from brackish or salt water locations were analyzed and compared to EPA standards for marine waters (Table 1).

Table 1: Recommended water quality standards for E. coli and enterococci in fresh and marine waters

Single Sample Maximum Allowable Density (cfu/100 mL)	*E. coli* Standards	Enterococci Standards Freshwater	Enterococci Standards Marine Water
Beach Designated Areas	205 cfu/100 mL	61 cfu/100 mL	104 cfu/100 mL
Lightly Used Full Body Contact Recreational	406 cfu/100 mL	108 cfu/100 mL	276 cfu/100 mL
Infrequently Used Full Body Contact Recreation	576 cfu/100 mL	151 cfu/100 mL	500 cfu/100 mL

Freshwater and Brackish Water Boundary Determination

The boundary between salt water and freshwater was determined by measuring specific conductivity (SC) during each sampling event and collecting water samples for chloride analyses for comparison to SC readings. Conductivity is a measure the capability to pass an electrical flow, which is directly related to the concentration of ions in water. As salts and inorganic materials break down, they dissociate into ions, either positively charged (cation) or negatively charged (anion). Electrical flow passes more easily through water with high concentrations of ions, while water with few ions results in lower conductivity (CWT, 2004). Conductance may be affected by temperature, but instruments measure specific conductance adjust and normalize the readings to 25° C. Salinity is the total concentration of all dissolved salts in the water. Salinity may be inferred from conductivity based on their strong direct relationship (Fondriest Environmental, 2014). The formula for calculating salinity from chloride concentrations is salinity part per thousand(ppt) = $0.0018066 \times \text{Cl}^{-}$ (mg/L) (Fondriest Environmental, 2014).

The National Oceanic and Atmospheric Association (NOAA) states that freshwater salinity is near 0 ppt (parts per thousand), while those that are considered brackish range between 0.5 to 35 ppt (NOAA, 2017). Based on this range, freshwater and marine waters were identified (Figure 7, Table 2).

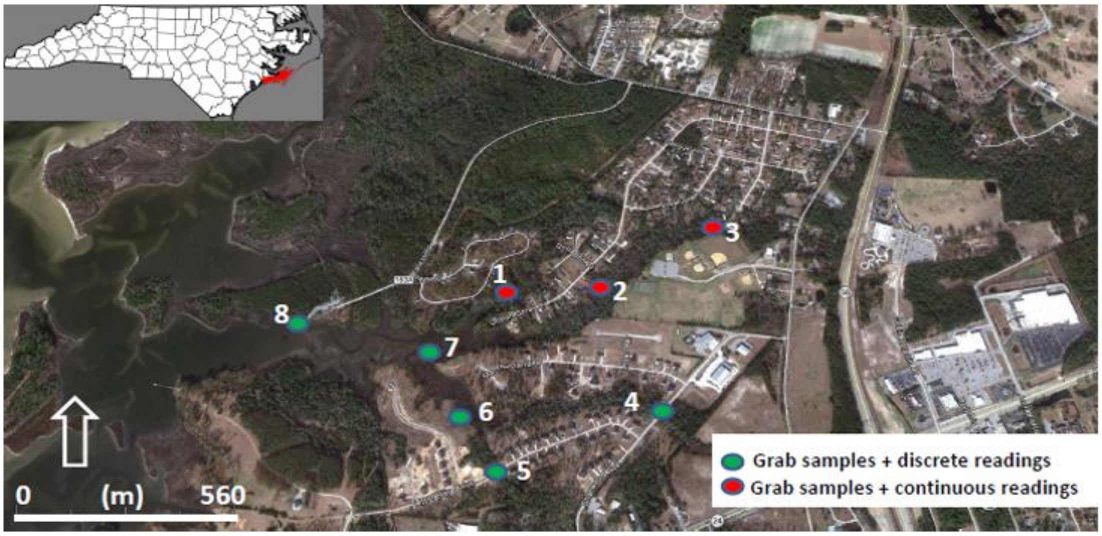


Figure 7: Sampling sites for chloride and specific conductivity

Table 2: Averages and standard deviations for chloride and specific conductivity at each of the 8 sampling sites

Site	Average Chloride Concentration	Chloride Standard Deviation	Average Specific Conductivity (µs/cm)	Specific Conductivity Standard Deviation
WO-1	39	28	1063	2207
WO-2	34	20	311	52
WO-3	33	11	336	55
WO-4	24	7	411	147
WO-5	23	16	327	66
WO-6	4282	5389	14703	14931
WO-7	4401	2645	18431	14550
WO-8	5169	3613	29364	12855

Watershed Exports of Fecal Indicator Bacteria

Watershed exports of FIB for WO-1 through WO-5 were calculated. The discharge in liters per second was multiplied by the concentrations of *E. coli* and enterococci (MPN/L) to determine the MPN per second of FIB. The drainage areas for the sites were obtained using United States Geological Survey’s Streamstats Version 4.0. Exports were then divided by the watershed size to normalize the data for area (MPN per hectare per second (Appendix 4). These analyses were conducted to provide insight into stream segments that were contributing the most FIB to estuarine waters.

Microbial Source Tracking

Quantitative real-time Taqman™ reverse transcriptase polymerase chain reaction (PCR) was used as a genotypic source-tracking tool to determine if human waste was a significant contributing source of bacteria. This method utilized fluorescent dye to amplify the DNA. Ms. Avian White, the Environmental Health Sciences Program Lab technician performed the analyses by using the Qiagen™ (Hilden, Germany) and UNEX protocol to extract DNA from the samples. The DNA extraction began by filtering 100-mL of sample through 0.45 micron (µm) filter using Fisher™ Thermoscientific™ (Hampton, NH) analytical filter unit 150-mL. (Lot # 1167103). The filter was then place into a 2-mL microcentrifuge tube of Unex™ buffer (Microbiologics Lot# 6354105). The buffer was used to stop any side reactions that might occur. After the sample was incubated for 10 minutes, the filter was removed and 200-µL of ethanol was added to the sample and pulse vortexed for 15-seconds, and then centrifuged briefly to remove drops from outside of the lid. The ethanol was added as an antisolvent to purify/concentrate the DNA, RNA, and polysaccharides. This mixture was transferred to QIAamp mini spin column and centrifuged at 8000-rpm for 1-minute. The mixture was then transferred to a new 2.0-mL collection tube and the old filtrate was discarded. 500-µL of Buffer AW1 was added, the mixture was centrifuged at 8000-rpm for 1-minute then put into a new 2.0-mL collection tube and the old filtrate was discarded. 500-µL of Buffer AW2 was added to the mixture, which was then centrifuged at 14000-rpm for 3-minutes. The mixture was transferred to a new 2.0-mL collection tube and the old filtrate was discarded. The mixture was then centrifuged once more at 14000 rpm for one minute and transferred to a new 2.0-mL collection tube and the old filtrate was discarded. 200-µL of Buffer AE was added to the solution and then incubated at room temperature for one minute, then centrifuged at 8000-rpm for one minute. The filtrate was stored at -20°C until testing (∼48 hours).

Testing of the sample was performed on Lightcycler® 480 II. The first cycle was a prep cycle that occurs one time. The cycle occurs at 50°C for 2 minutes at a ramp rate of 4.4 °C. The second cycle occurs one time at 95°C for 10 minutes at a ramp rate of 4.4 °C. This was when the initial template denaturing/enzyme activation occurred. The third cycle was a cooling stage. There were 45 cycles ran at 95°C for 15 seconds at a ramp rate 4.4 °C. This stage is when denaturation of template, annealing of primers, and extension of Taq occurred. The final cycle was at 60°C for 1 minute at a ramp rate 2.2 °C.

The samples were first compared against general indicator Bacteriodales. If the general indicator Bacteriodale was detected, then the sample was ran against the human Bacteriodales .A positive human control was used, which was a sample from a septic tank and a negative human control used, which was a dog waste sample.

Stormflow and Baseflow

Concentrations and exports of FIB during baseflow and stormflow conditions were compared to determine any statistically significant differences. Most of the data generated during the study did not follow a normal distribution, so non-parametric Mann-Whitney tests were used to determine if the differences (baseflow and stormflow) were statistically significant ($p \leq 0.05$). These comparisons were made to determine if runoff was a major contributor of FIB to surface waters.

Fecal Indicator Bacteria Concentrations during Warm and Cold Months

Data from each location for the warm months and cool months were displayed and summarized using line plots, box plots, and/or tables. The State Climate Office of North Carolina Cronos database was utilized to retrieve historical climate data to identify the months of the year that were historically the warmest and coldest months. Warm months were identified as June (mean 26.3 °C) July (mean 27.2 °C), August (mean 26.4 °C), and September (mean 24.1 °C) with a mean temperature of 26 °C. The cold months were identified as December (mean 9.4°C), January (mean 7.6°C), February (mean 8.7 °C) and March (13 °C) with a mean temperature of 9.7 °C. Non-parametric Spearman's coefficient correlations were used to determine if statistically significant correlations were observed between FIB concentrations and temperature, and flow. Mann Whitney tests (non-parametric) were used to determine if statistically significant differences in concentrations and exports of FIB were observed between warm relative to cold months. P-values of less than or equal to 0.05 were considered to be statistically significant.

Best Management Practices

The BMPs installed for this project included rain gardens, diversion of water into wooded/vegetative areas, curb and ditch bank modifications to allow water to flow into ditches, water control structures, rock check dams and rain water harvesting. The goal of the BMPs was to slow and/or divert the storm water runoff so that it did not enter the nearby surface waters during rain events without some treatment.

A bio-retention cell (Figure 8) was installed at the boat ramp near sampling location 8.

As discussed earlier, the bio-retention cell acts to reduce bacteria by reducing the actual amount of water entering the surface water and allowing the water more time to infiltrate the ground.

Figure 8: Bio-retention cell at the Cedar Point boat ramp storing runoff after a rain event.

The sidewalk at the boat ramp had a slight incline along the edge closest to the woods. The incline prevented drainage from the parking lot to runoff the walkway and into the woods. Instead, runoff was flowing along the walkway towards to the estuary. The sidewalk was removed and reconstructed so that drainage could flow from the parking area across the walkway and into the woods for infiltration (Figure 9).

Figure 9: New sidewalk at the boat ramp that was graded to allow runoff to enter the woods.

Water control structures were installed in 5 locations in the Ocean Spray community. The structures allowed for the use of flashboard risers to manipulate the outlet elevation of the culvert pipes. The structures were constructed so that they could be inserted into the 38-cm diameter culvert pipes of most driveways. The front of the structures had a frame where flashboards could be inserted to slow runoff and increase infiltration of stormwater entering the ditches (Appendix 1) (Figure 10).

Figure 10: Water Control Structure installed

Other BMPs included installing sod in eroded areas of the ditch banks of the Ocean Spray Community where focused runoff was overwhelming the vegetation. There were several locations in the Ocean Spray community where the grass along the shoulder of the road had grown higher than the road, thus preventing runoff from entering the ditches throughout the community. Runoff was moving along the side of the road to lower locations, and then spilling into the ditches and causing erosion. The ditch bank and road edges were re-graded, and then sod was installed on the bank (Figure 11).

Figure 11: Ocean Spray Community BMPs including water control structures (blue icons) and roadside modifications (green icons).

The intent was to allow runoff to enter the ditches in more locations, and to stabilize the ditch bank with rooted vegetation. This was intended to decrease the volume of runoff. The water control structures were intended to retain the water to a certain level thereby increasing infiltration.

The rain garden was designed to store 100% of the runoff during a 1-yr 24 hr. storm event. All of the runoff for a 1-yr 24 hr. storm from the parking lot that flows across the walkway and into the forested area should also infiltrate Prior research has shown that outflows can be reduced by more than 30% using controlled drainage. This value (30%) was used as an estimated for outflow and FIB loading reductions.

Bacteria Export Reduction

The simple method (Schueler, 1987) was utilized to estimate runoff volumes for the watershed up-gradient of each implemented BMP**.** The information needed for this calculation includes the watershed area, impervious cover, and rainfall amount. The Simple Method estimates runoff from a watershed with known impervious area and uses that information to create a curve-fitting relationship of the fraction of rainfall converted to runoff (the runoff coefficient) to the percent of impervious area (NCDENR, 2009). Once the volume of runoff is calculated, the volume can be multiplied by FIB concentrations to estimate watershed exports of FIB during storms. The simple method is calculated using the following formula:

$Rv=0.05+0.9*I_A$

Where: Rv= Runoff coefficient [storm runoff (in)/storm rainfall (in)] (unitless)

I_A= Impervious fraction [Imperious portion of drainage area (ac)/drainage area (ac)], (unitless)

Once Rv is determined, the volume of runoff can be calculated using the following formula:

$V=3630*R_D*R_V*A$

Where: V=Volume of runoff that must be controlled for the storm design (ft^3)

R_D= Annual storm rainfall depth (in)

A= Watershed area (ac)

Once the volume of runoff was calculated, it was converted from cubic feet to liters. Water samples from each of the BMPs were analyzed for *E. coli* and enterococci concentrations. The raingarden and sidewalk were estimated to reduce 100% of the runoff, and the water control structures (WCS) and check dams were estimated to reduce 30% of the runoff. A 30% reduction was chosen due to the percent reductions observed in agriculture from water control structures (Sunohara et al., 2016). The volume of runoff in liters was multiplied by the median *E. coli* and enterococci concentrations obtained from the BMPS to obtain the bacterial concentration load that each BMP received. That number was then multiplied by either 100% for the rain garden and sidewalk, or by 30% for the WCS and check dams to estimate the amount of bacteria load reduction.

Results and Discussion

E. coli Concentrations and Environmental Health

Sampling locations WO-1 through WO-5 are small freshwater streams that eventually discharge into the estuary, where water-based recreation is common.

Water samples collected from WO-1 through WO-5 exceeded the *E. coli* water quality standards for beach access in 71-100% of the samples analyzed and the infrequently used water standards in 24-100% of the samples analyzed. The median concentrations of *E. coli* for WO-1 through WO-5 exceeded the beach access standard, and all but WO-3 and WO-4 samples exceeded the infrequently used full body contact standard (Figure 12). Table 3 summarizes the standards and stats for the three thresholds, as well as the bacteria exceedances.

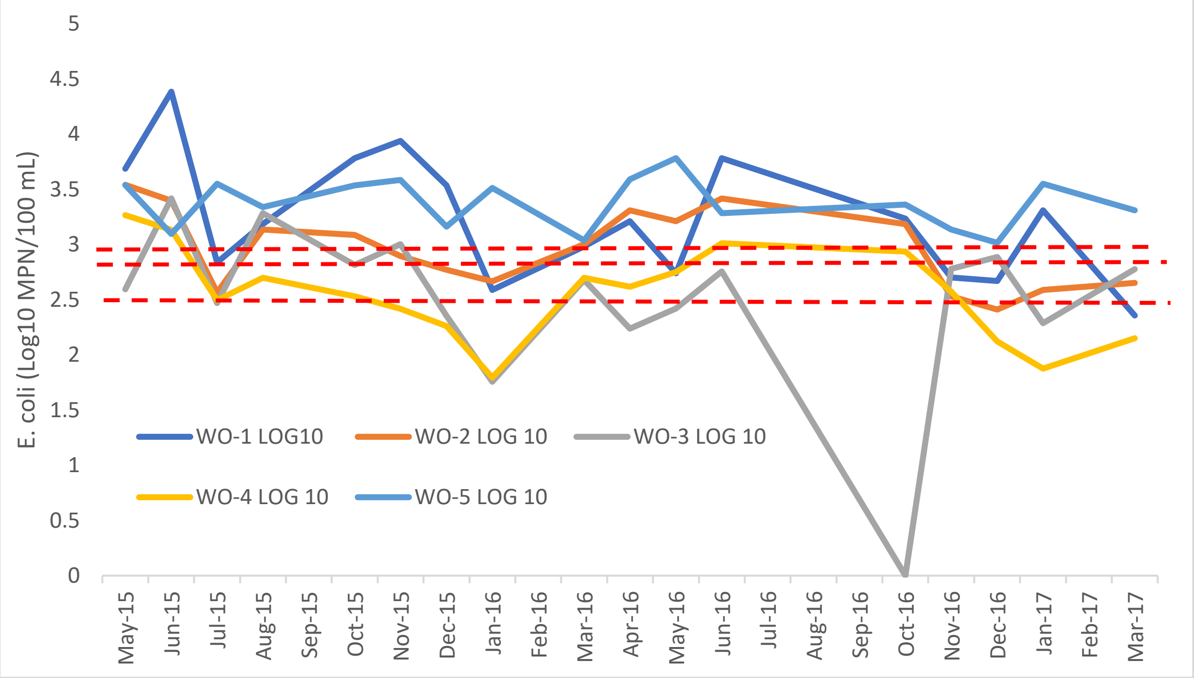


Figure 12:Concentrations of E. coli for monitoring sites WO 1-5 in relation to E. coli standards for recreational waters (fresh).

Table 3: Summary of freshwater E. coli concentrations and standard violations

***E. coli* Statistics**	**WO-1**	**WO-2**	**WO-3**	**WO-4**	**WO-5**
Median (Log10 MPN/100 mL)	3.21	2.89	2.68	2.53	3.36
Standard deviation (Log10 MPN/100 mL)	0.49	0.32	0.93	0.37	0.23
Frequency of Exceedance Beach Designated Area (2.37)	16/17 (94%)	17/17 (100%)	12/17 (71%)	12/17 (71%)	17/17 (100%)
Frequency of Exceedance Lightly used full body contact recreation (2.61)	15/17 (88%)	13/17 (76%)	9/17 (53%)	8/17 (47%)	17/17 (100%)
Frequency of Exceedance Infrequently used full body contact recreation (2.76)	12/17 (71%)	11/17 (65%)	7/17 (42%)	4/17 (24%)	17/17 (100%)

There is not a recommended standard for *E. coli* for marine waters. WO-6 through WO-8 are considered to be brackish waters. *E. coli* freshwater standards were compared to sites WO-6 through WO-8 with the understanding that if the *E. coli* concentrations were found to exceed the standard, that did not necessarily indicate a public health threat (EPA, 1986). Samples collected from WO-6 to WO-8 exceeded the single sample maximum allowable density for beach designated area in 88-100% of samples analyzed. (Figure 13). The standard for infrequently used full body contact was exceeded in 65-88% pf samples analyzed. Each of the three site medians exceeded sample standards for each of the three standards. Summary statistics are displayed in Table 4.

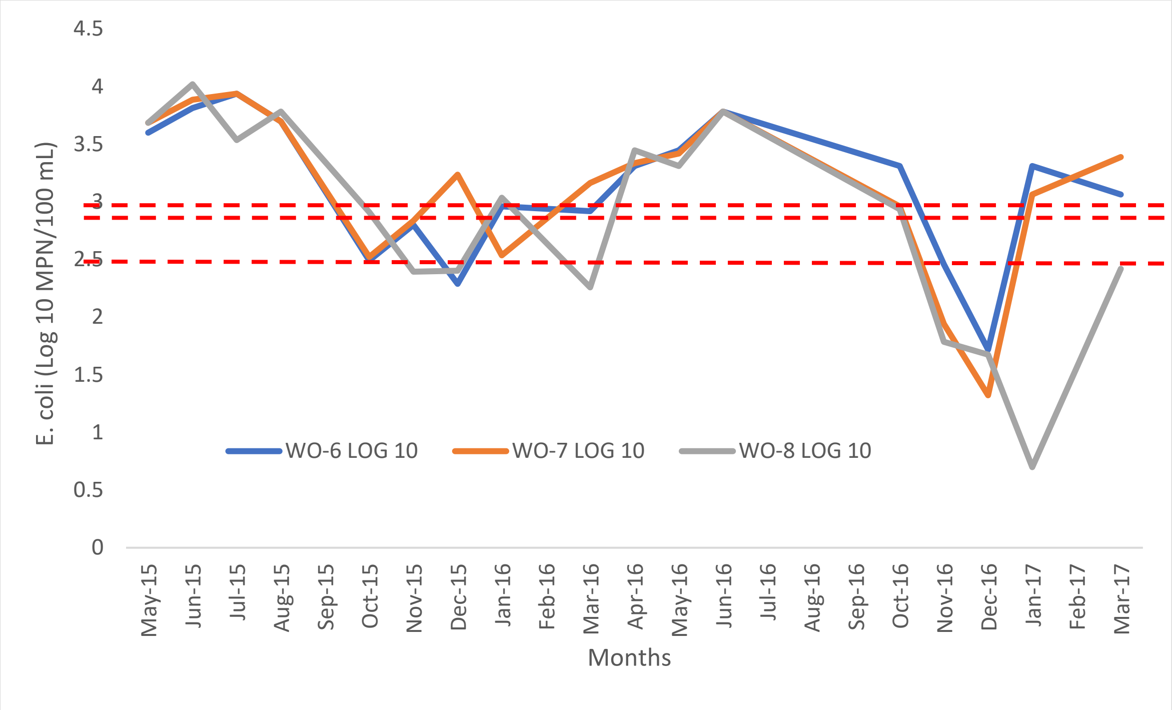


Figure 13: Concentrations of E. coli for monitoring sites WO 6-8 in relation to E. coli standards for recreational (salt)

Table 4: Summary of E. coli concentrations and standard violations

***E. coli* Statistics**	**WO-6**	**WO-7**	**WO-8**
Median (Log10 MPN/100 mL)	3.31	3.24	2.94
Standard Deviation (Log10 MPN/100 mL)	0.60	0.65	0.83
Frequency of Exceedance Beach Designated Area (2.37)	17/17 (100%)	17/17 (100%)	15/17 (88%)
Frequency of Exceedance Lightly Used Full Body Contact Recreation (2.61)	15/17 (88%)	15/17 (88%)	11/17 (65%)
Frequency of Exceedance Infrequently Used Full Body Contact Recreation (2.76)	15/17 (88%)	15/17 (88%)	11/17 (65%)

Enterococci Concentrations and Environmental Health

Water samples collected from stream sites WO-1 to WO-5 exceeded beach water quality standards in 71-100% of samples analyzed and exceeded the infrequently used full body contact standard in 35-77% of samples analyzed (Figure 14, Table 5). Median concentrations of enterococci from sites WO-1, WO-2, and WO-5 exceeded the single sample standard for infrequently used waters. Table 5 includes the standards and frequencies of exceedance for the 3 single sample maximum allowable densities of FIB.

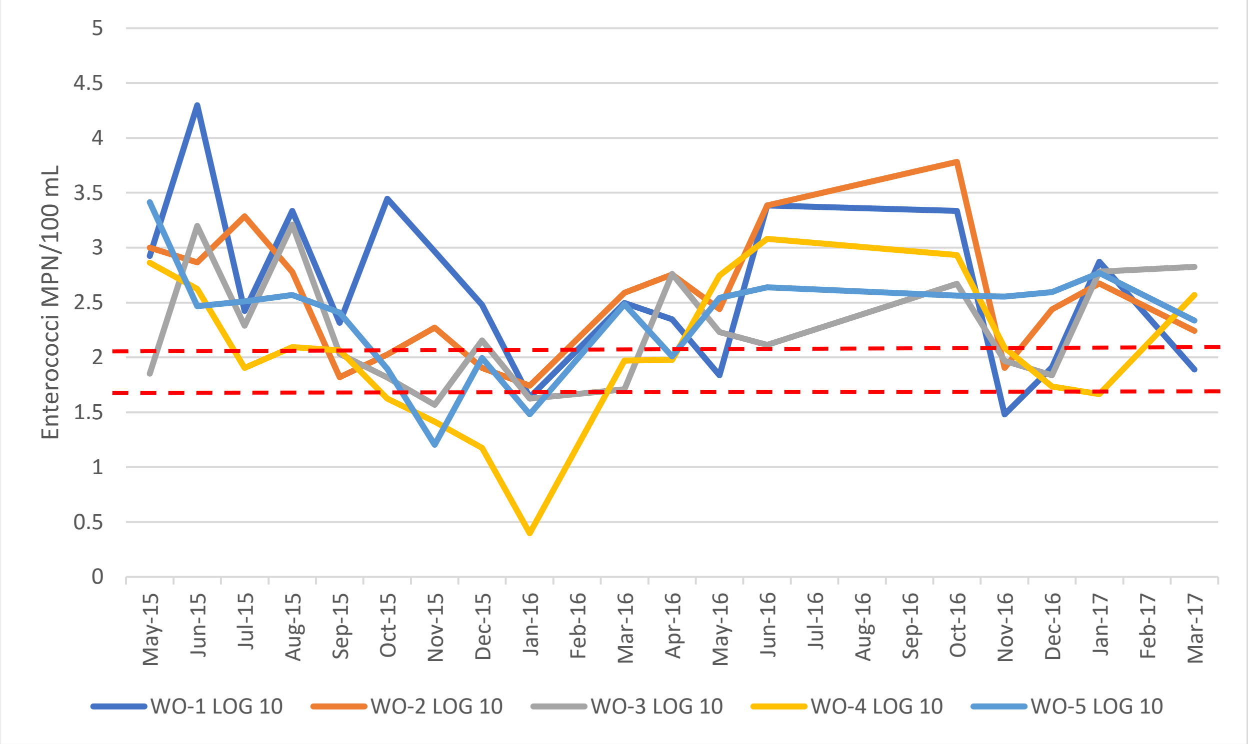


Figure 14: Concentrations of enterococci for monitoring sites 1-5 in relation to enterococci standards for recreational (fresh)

Table 5: Summary of freshwater Enterococci concentrations and standard violations

Enterococci Statistics	WO-1	WO-2	WO-3	WO-4	WO-5
Medan (Log 10 of MPN/100 mL)	2.49	2.51	2.13	2.02	2.50
Standard Deviation (Log10 of MPN/100 mL)	0.90	0.77	0.69	0.77	0.69

Frequency of Exceedance Beach Designated Area (1.79)	16/17 (94%)	17/17 (100%)	15/17 (88%)	12/17 (71%)	16/17 (94%)
Frequency of Exceedance Lightly Used Full Body Contact Recreation (2.03)	13/17 (77%)	13/17 (77%)	11/17 (65%)	9/17 (53%)	13/17 (77%)
Frequency of Exceedance Infrequently used Full Body Contact Recreation (2.18)	13/17 (77%)	13/17 (77%)	8/17 (47%)	6/17 (35%)	13/17 (77%)

Samples collected from WO-6 and WO-7 exceeded the beach standard in more than 75% of samples analyzed while samples from WO-8 exceeded the beach standard in 29% of samples analyzed. For infrequently used full body contact, WO-6 and WO-7 exceeded the standards 71% and 59% of the times sampled respectively, while WO-8 only exceeded the standard in 6% of samples analyzed (Figure 15, Table 6). Median concentrations of enterococci at WO-6 and WO-7 exceeded the beach designated use standard and the lightly used full body contact, but not the infrequently used full body contact standard.

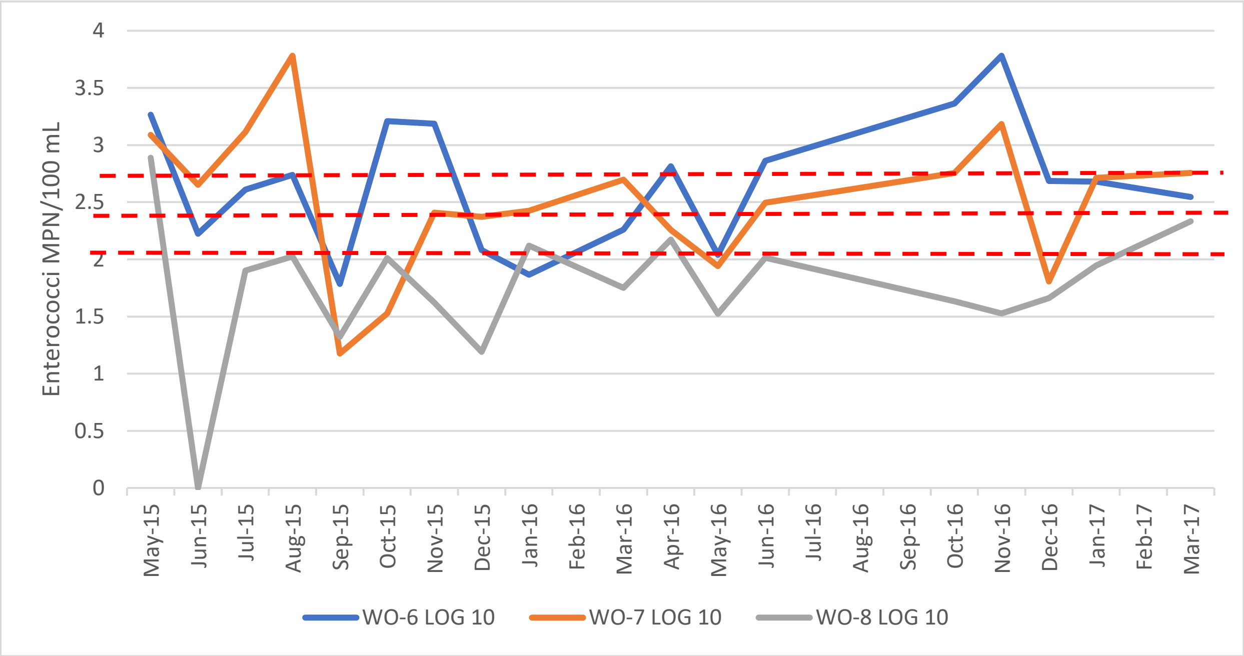


Figure 15: Concentrations of enterococci for monitoring site 6-8 in relation to enterococci standards for recreational (salt)

Table 6: Summary of saltwater enterococci concentrations and standard violations

Standards and Stats	WO-6	WO-7	WO-8
Median (Log10 MPN/100 mL)	2.68	2.57	1.83
Standard Deviation (Log10 MPN/100 mL)	0.54	0.61	0.57
Frequency of Exceedance: Beach Designated Area (2.02)	16/17 (94%)	14/17 (82%)	5/17 (29%)
Lightly Used Full Body Contact Recreation (2.44)	12/17 (71%)	10/17 (59%)	1/17 (6%)
Infrequently Used Full Body Contact Recreation (2.70)	8/17 (47%)	7/17 (42%)	1/17 (6%)

Spatial Distribution of *E. coli*

The concentrations of *E. coli* were typically elevated in sampling locations close to the estuary relative to locations further upstream (Figure 16). For example, concentrations of *E. coli* were higher at WO-1 (median=1670 MPN/100-mL, $\log_{10}$= 3.22) than at WO-2 (median= 893 MPN/100-mL, $\log_{10}$= 2.95) and WO-3 (median= 523 MPN/100-mL, $\log_{10}$= 2.72) and *E. coli* concentrations at WO-6 (median= 2041 MPN/100-mL, $\log_{10}$= 3.31) where higher than WO-4 (median= 395 MPN/100-mL, $\log_{10}$= 2.60) and WO-5 (median= 1943 MPN/100-mL, $\log_{10}$= 3.29). Sampling location WO-8 (median= 838 MPN/100-mL, $\log_{10}$= 2.91) is located in the estuary and down gradient of the other sites (WO 1-7). Stream segments that had dense vegetation, such as WO-1, WO-6, and WO-7 (median= 1589 MPN/100-mL, $\log_{10}$= 3.20) may provide habitat for wildlife which could lead to an increase in bacterial concentrations. For example, a study completed by the Virginia Department of Environmental Quality (2002) on the Accotink Creek discovered that geese contributed more of the total fecal coliform to surface waters (24%) than humans (20%) and dogs (13%). Location WO-4 had the lowest median concentration of *E. coli* (374 MPN/100-mL) and the concentrations at WO-4 were significantly lower than concentrations at WO-2 to WO-7 (Figure 15). Table 7 shows the *E. coli* concentration comparisons using Mann-Whitney tests for the 8 sampling locations. The pond that was used as a background comparison was sampled from February 2016 to June 2016. The median *E. coli* concentration for that time frame from the pond was 12 MPN/100-ml. The lowest *E. coli* concentrations during that time frame from the 8 sampling sites was at WO-3, with a median *E. coli* concentration of 263 MPN/100-mL.

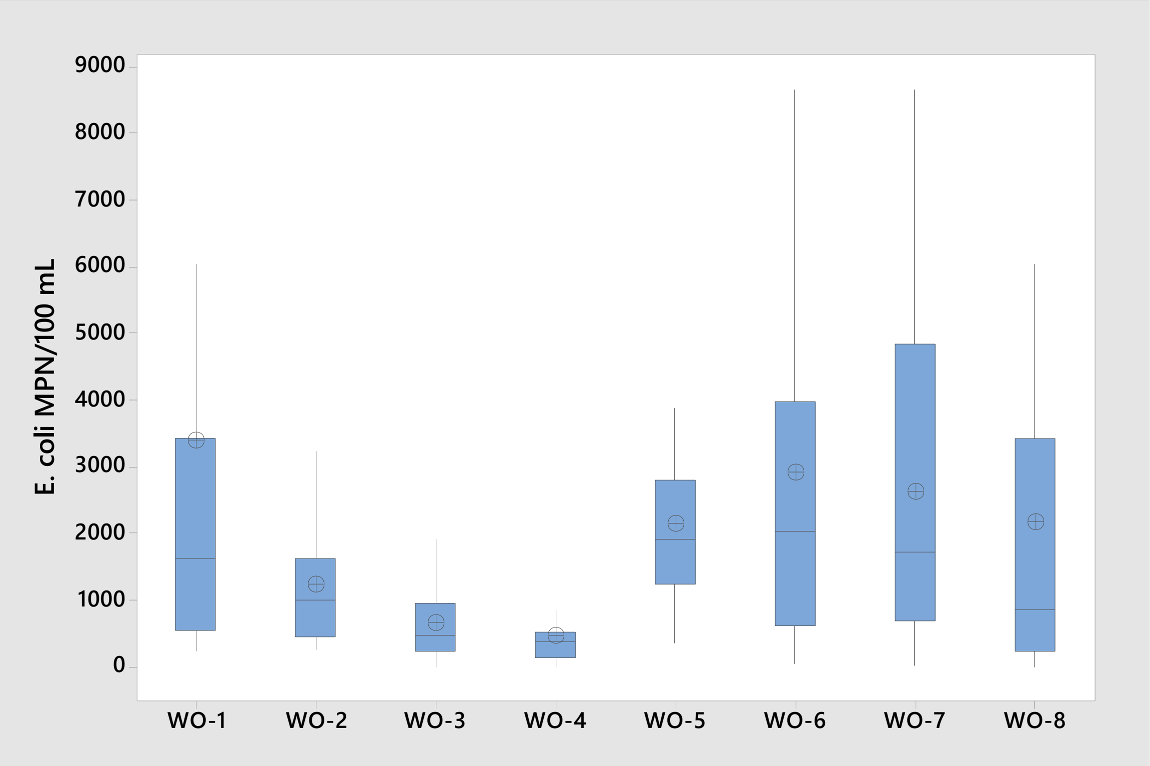


Figure 16: E. coli concentrations for each of the 8 sampling sites

Table 7: Outcomes of Mann-Whitney tests to identify statistically significant differences (p≤.05) in E. coli concentrations between sampling locations

Site	WO1	WO2	WO3	WO4	WO5	WO6	WO7
WO1							
WO2	*p=0.0990*						
WO3	*p=0.0022*	*p=0.0246*					
WO4	*p=0.0001*	*p=0.0021*	*p=0.3247*				
WO5	*p=0.7621*	*p=0.0136*	*p=0.0000*	*p=0.0000*			
WO6	*p=0.9070*	*p=0.1404*	*p=0.0032*	*p=0.0008*	*p=1.0000*		
WO7	*p=0.9651*	*p=0.1526*	*p=0.0032*	*p=0.0009*	*p=0.8609*	*p=1.0000*	
WO8	*p=0.2673*	*p=0.8495*	*p=0.1841*	*p=0.1116*	*p=0.1989*	*p=0.2426*	*p=0.2801*

Spatial Distribution of Enterococci

Sampling locations in close proximity to the estuary had higher concentrations of enterococci relative to locations upstream (Table 8 and Figure 17). For example, the median concentrations of enterococci for downstream locations WO-2 (median= 301 MPN/100-mL, $\log_{10}$: 2.49) and WO-6 (median= 480 MPN/100-mL, $\log_{10}$: 2.68) were higher than upstream locations WO-3 (median= 134 MPN/100-mL, $\log_{10}$: 2.14) and WO-4 (median= 106 MPN/100-mL, $\log_{10}$: 2.02). WO-2 is downstream from WO-3, and WO-6 is downstream from WO-4. Table 6 shows the statistically significant differences in enterococci concentrations among the sites. Enterococci concentrations from WO-8 showed to be statistically significantly different from all other sites with the exception of WO-4. WO-8 (median= 68 MPN/100-mL, $\log_{10}$: 2.33) is an open body water, so even though it is receiving drainage from WO-1 to WO-7, there is opportunity for dispersal of the FIB concentrations and attenuation via exposure to sunlight (EPA, 2010). The pond that was sampled and used as a background from February 2016 to June 2016 had a median enterococci concentration of 8 MPN/100 mL. In comparison, the lowest enterococci concentrations recovered from the 8 sites were at WO-8, with a median of 97

MPN/100-mL.

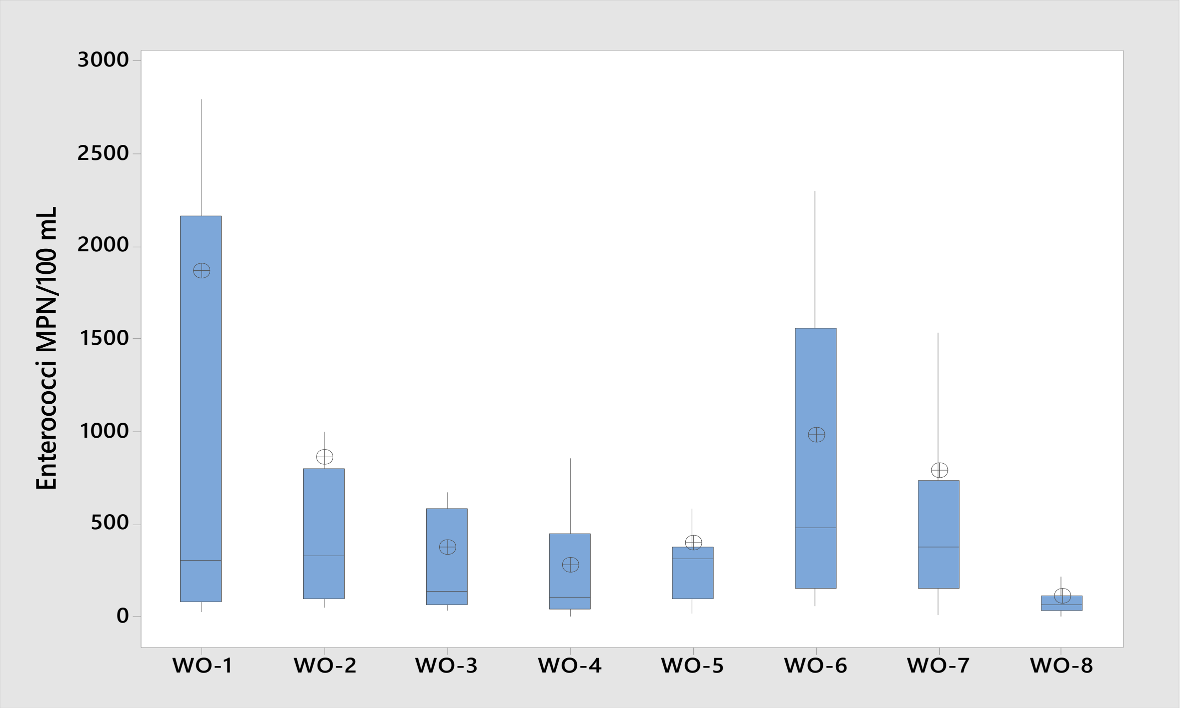


Figure 17: Enterococci concentrations for each of the 8 sampling sites

Table 8: Outcomes of Mann-Whitney tests to identify statistically significant differences (p≤.05) in concentrations of enterococci between sampling locations

Site	WO1	WO2	WO3	WO4	WO5	WO6	WO7
WO1							
WO2	*p=0.7157*						
WO3	*p=0.0875*	*p=0.1329*					
WO4	*p=0.0480*	*p=0.0480*	*p=0.4107*				
WO5	*p=0.5064*	*p=0.4198*	*p=0.4964*	*p=0.2614*			
WO6	*p=0.9118*	*p=0.5798*	*p=0.0397*	*p=0.0119*	*p=0.0791*		
WO7	*p=0.7278*	*p=0.9118*	*p=0.2614*	*p=0.0619*	*p=0.3930*	*p=0.4765*	
WO8	*p=0.0012*	*p=0.0005*	*p=0.0209*	*p=0.1839*	*p=0.0025*	*p=0.0001*	*p=0.0011*

Microbial Source Tracking

Microbial source tracking (MST) analyses were conducted, and while the Order Bacteriodales was detected at most sites, human sources of Bacteriodales were not observed in any of the samples collected from WO-1 to WO-8. The general control was a dog-waste sample that tested negative for human, but positive for general. For the human sample, the positive control was a septic tank sample, which was positive for human specific Bacteriodales. These data suggest that animals, most likely wildlife that live within the sampling area who frequent the waters as a drinking source, were major source of bacteria in the waterways.

Stormflow and Baseflow *E. coli* Concentrations and Watershed Exports

Stormflow samples were collected during the months of October 2015, December 2015, February 2016, September 2016, January 2017 and February 2017 for comparison to base flow samples collected during September 2015, October 2015, December 2015, January 2016, January 2017, and March 2017. Stormflow events were considered storm flow if the samples were collected during or right after a storm. Baseflow sampling occurred when there had not been rain within 48 hours.

Median concentrations of *E. coli* were significantly elevated during stormflow relative to baseflow for every sampling location and for the pooled data (Figure 18). Differences in concentrations for WO-2 during baseflow (median= 527 MPN/100-mL) and stormflow (median= 2321 MPN/100-mL) were statistically significantly (p= *0.0082)* and differences in WO-3 base (median= 351 MPN/100-mL) and WO-3 storm (median= 925 MPN/100-mL) were statistically significantly different (p = *0.0202)*.

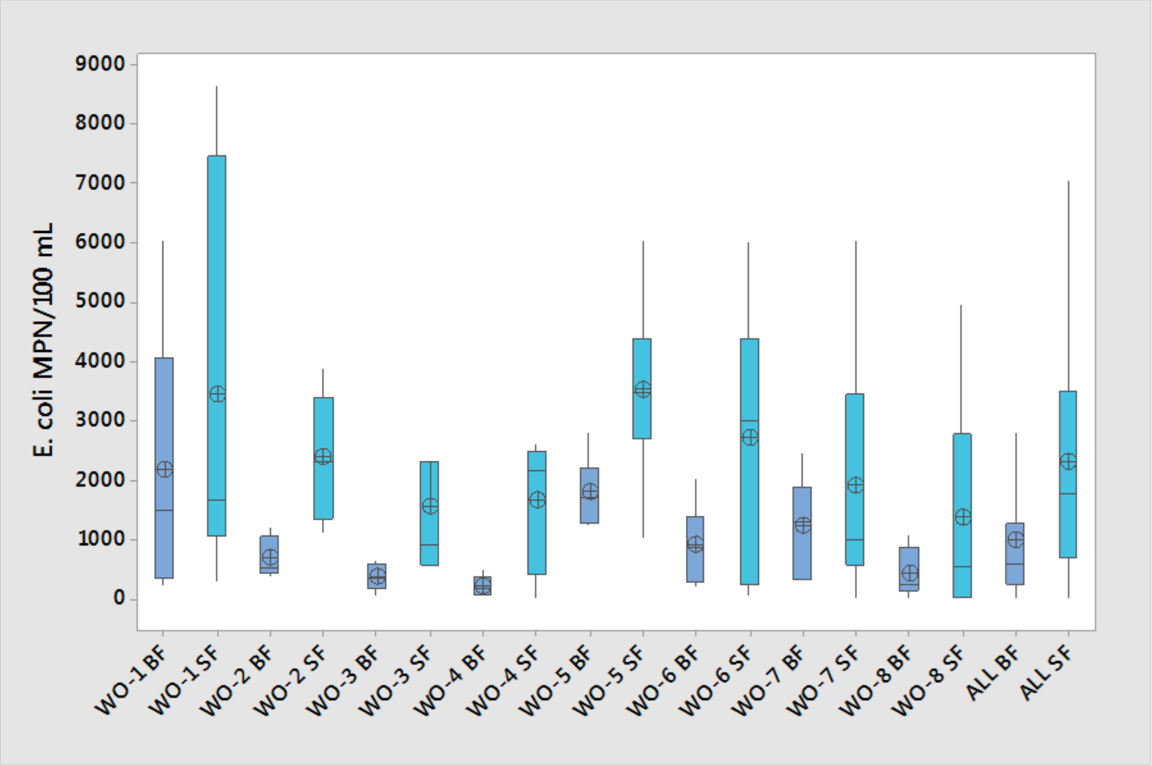


Figure 18: Concentrations of E. coli during baseflow and stormflow for each of the 8 sampling sites

Differences in concentrations for WO-4 baseflow (median= 162 MPN/100-mL) and WO-4 stormflow (median= 2172 MPN/100-mL) as well as WO-5 baseflow (median= 1705 MPN/100-mL) and WO-5 stormflow (median= 3484 MPN/100-mL) were non-significant at *p value = 0.0656*. Pooling the baseflow and stormflow data for all sites, concentrations of *E. coli* during base flow (median=625 MPN/100-mL) were significantly *(p = 0.0016*) lower relative to stormflow (median=1780 MPN/100-mL). Stormwater runoff transports contaminants such as FIB that collect on impervious surfaces to receiving waters, increasing the concentration and loading of microbial pollutants. Also, as the stream flow and stage increase in response to the runoff, FIB deposited adjacent to the stream banks may be transported with the floodwater downstream, increasing FIB concentrations and loadings to recreational waters. During storms, FIB concentrations often exceed the standards set forth by the EPA, thereby posing a public. Gregory and Frick (2000) found that bacteria concentrations in the storm samples collected from the 8 tributaries of the Chattahoochee River in Georgia, US used in the study were up to 10 times higher than the base flow samples collected.

Figure 18 gives a visual representation of the differences in *E. coli* concentrations for storm flow and base flow for this study.

Bacterial export for the storm flow and base flow conditions were also analyzed. As with *E. coli* concentrations, *E. coli* export for storm water conditions were much higher than for base flow.

WO-1 baseflow (median= 3735 MPN/s/ha) and WO-1 stormflow (median= 27788 MPN/s/ha) were significantly different at $p = 0.0453$ and the WO-5 baseflow (median= 10123 MPN/s/ha) and WO-5 stormflow (median= 52926 MPN/s/ha) were s significantly different at $p = 0.0306$. All baseflow (median= 1517 MPN/s/ha) and stormflow (median= 15486 MPN/s/ha) concentrations significantly different with a *p-value = 0.000.* This indicates that WO-1 and WO-5 could experience consistently higher bacteria loadings during storms, potentially making the waters potentially unsafe (Figure 19). It was anticipated that each of the sites would have higher bacteria loadings after a rain event due to runoff transporting FIB to receiving waters and increases in stream flow.

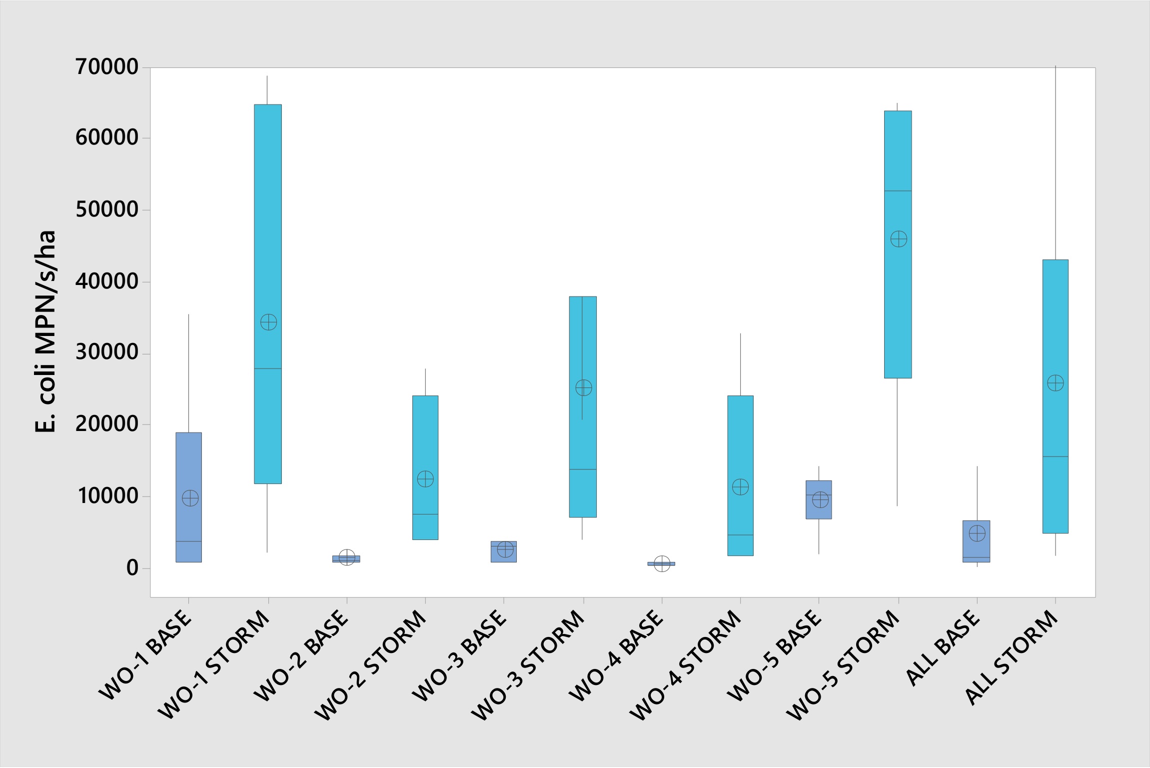


Figure 19: Watershed exports of E. coli for sites 1-5 during storm flow and base flow

Stormflow and Baseflow Enterococci Concentrations and Watershed Exports

Median enterococci concentrations were significantly higher in stormflow compared to baseflow samples for each of the 8 sites (Figure 20). Enterococci concentrations found in WO-3 stormflow (median=125 MPN/100-ml) were significantly higher (but differences in concentrations for other individual locations were not *($p > 0.05$)*. All stormflow enterococci concentrations found (median=570 MPN/100-mL) were significantly higher *($p<0.001$)* than pooled baseflow concentrations.

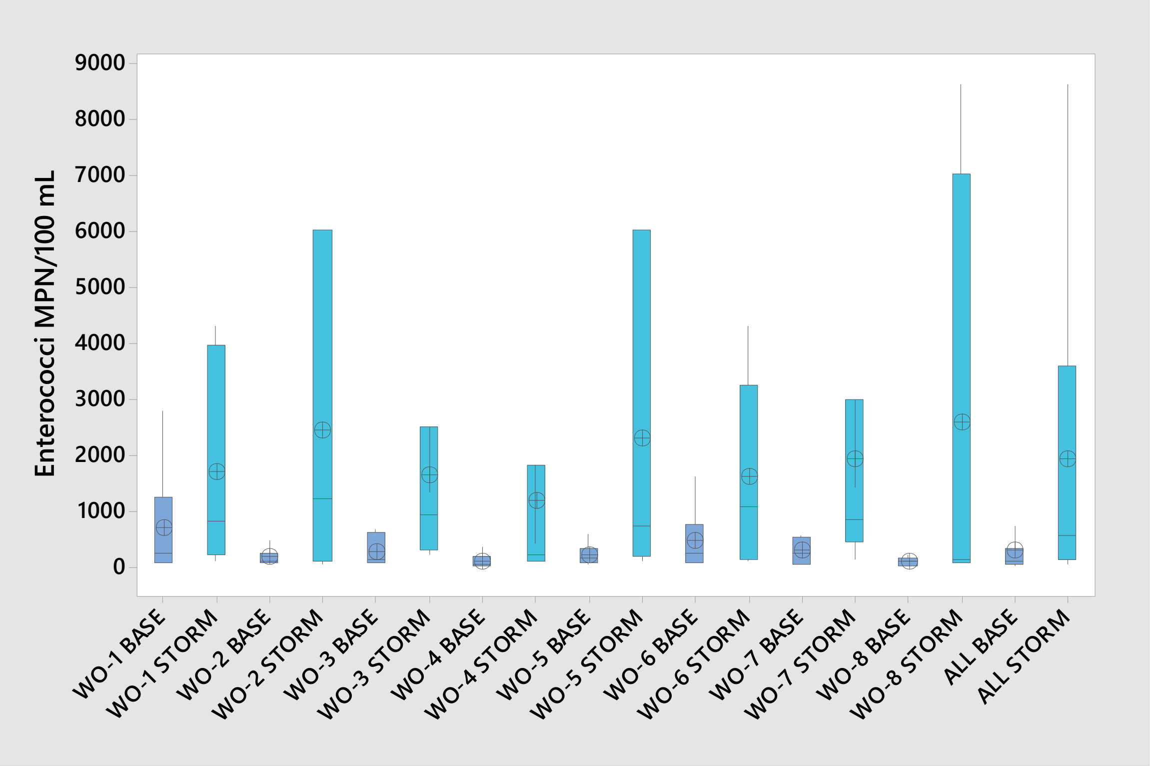


Figure 20: Concentrations of enterococci during stormflow and baseflow for each of the 8 sampling sites

As with enterococci concentrations, enterococci loadings per hectare (ha) were also significantly higher for storm samples in contrast to base flow samples for each location (Figure 21). Enterococci bacterial loadings were significantly higher for stormflow than for baseflow for all sites except WO-2. More specifically, the WO-1 baseflow (median: 567 MPN/s/ha) and stormflow (median: 10408 MPN/s/ha) were statisically significally different, *p = 0.0453;* site WO-3 baseflow median (1181 MPN/s/ha) and stormflow median (9654 MPN/s/ha) were statistically significantly different, *p = .0082*; WO-4 baseflow (median: 71 MPN/s/ha) and stormflow (median: 914 MPN/s/ha) were statistically significantly different, *p = 0.0131*; WO-5 baseflow (median: 1222 MPN/s/ha) and stormflow (median: 9832 MPN/s/ha) were statistically significantly different, *p = 0.0306*; all baseflow (median: 345 MPN/s/ha) and all stormflow (median: 5250 MPN/s/ha) were statistically signifcantly different, *p = 0.000*.

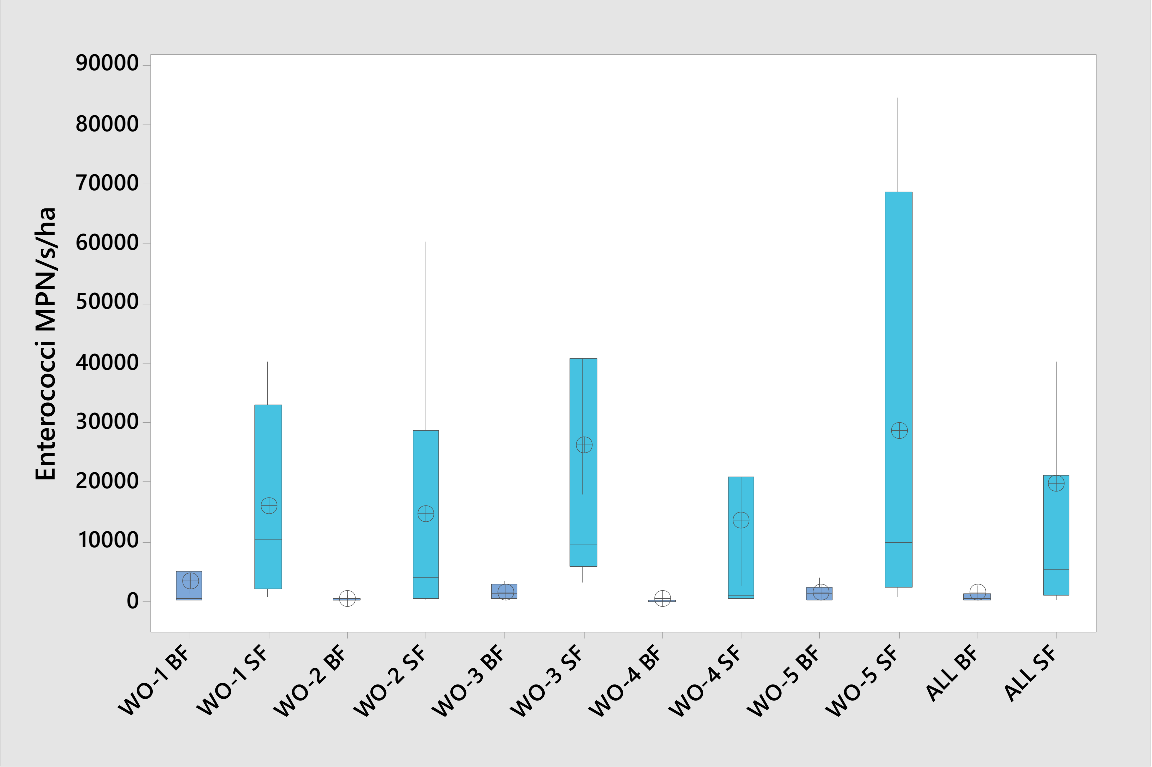


Figure 21: Watershed exports of enterococci for sites 1-5 during stormflow and baseflow

Seasonal and Temporal Variation in Fecal Indicator Bacteria Concentrations

Water temperature was measured at each site for each sampling event. The temperatures ranged from 2.2° C in January 2016 to 36.3° C in July 2015, with the warmest water temperatures occurring during the summer and the coldest during the winter. The water temperature trends tended to correlate with air temperature (Figure 22).

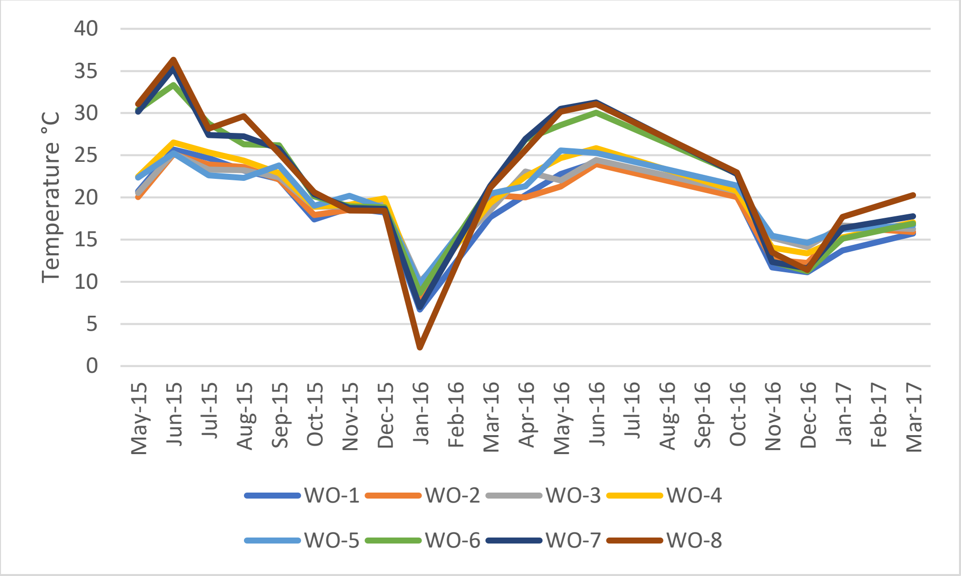


Figure 22: Range of temperatures for each of the 8 sampling sites over the course of the study

More specifically, the warmest months for air temperatures historically are June, July, August and September, and the coldest months December, January, February, and March (State Climate Office, 2017). Bacterial concentrations during winter months were typically lower than the limit for infrequently used full body recreation (575 MPN/100-mL, $\log_{10}$= 2.76). At WO-8 (151 MPN/100- mL, $\log_{10}$= 2.18), concentrations tended to stay under the beach access standards (234 MPN/100-mL, $\log_{10}$= 2.37) in the colder months as well. Strong positive correlations were observed between *E. coli* concentrations and temperature at WO-2 (*p=0.014*), WO-4 (*p=0.001*), WO-6 (*p=0.000*), WO-7 (*p=0.000*) and WO- 8 (*p=0.000*) (Table 9).

Table 9: Spearman's correlations to assess relationships between temperature and E. coli concentrations

Site	Spearman's Correlation	*p-value*
WO-1	0.451	0.069
WO-2	0.583	0.014
WO-3	0.172	0.510
WO-4	0.718	0.001
WO-5	0.205	0.430
WO-6	0.757	0.000
WO-7	0.772	0.000
WO-8	0.784	0.000

Strong positive correlations between enterococci and temperatures were also observed for many of the sampling locations including WO-1 (*p=0.041*), WO-2 (*p=0.006*), WO-3 (*p=0.030*) and WO-4 (*p=0.012*) (Table 10).

Table 10: Spearman's correlations to assess the relationship between temperature and enterococci concentrations

Site	Spearman's Correlation	*p-value*
WO-1	0.485	0.041
WO-2	0.624	0.006
WO-3	0.511	0.030
WO-4	0.579	0.012
WO-5	0.234	0.349
WO-6	0.176	0.498
WO-7	0.092	0.717
WO-8	-0.059	0.817

Temporal Trends in *E. coli* Concentrations

Median *E. coli* concentrations were significantly higher at all 8 sites during warm months compared to the cold months (Figure 23). Mann-Whitney tests were performed for the 8 sites to see if there were statistically significant differences in concentrations between warm and cold seasons. Enterococci concentrations for the warm months were significantly higher than enterococci concentrations during the cold months. Differences between WO-4 warm (median= 454 MPN/100-mL) and WO-4 cold (median= 137 MPN/100-mL), W0-6 warm (median= 5727 MPN/100-mL) and WO-6 cold (median= 674 MPN/100-mL) and WO-8 warm (median= 4742 MPN/100-mL) and WO-8 cold (median= 150 MPN/100-mL) were significantly different (*p < 0.05*).

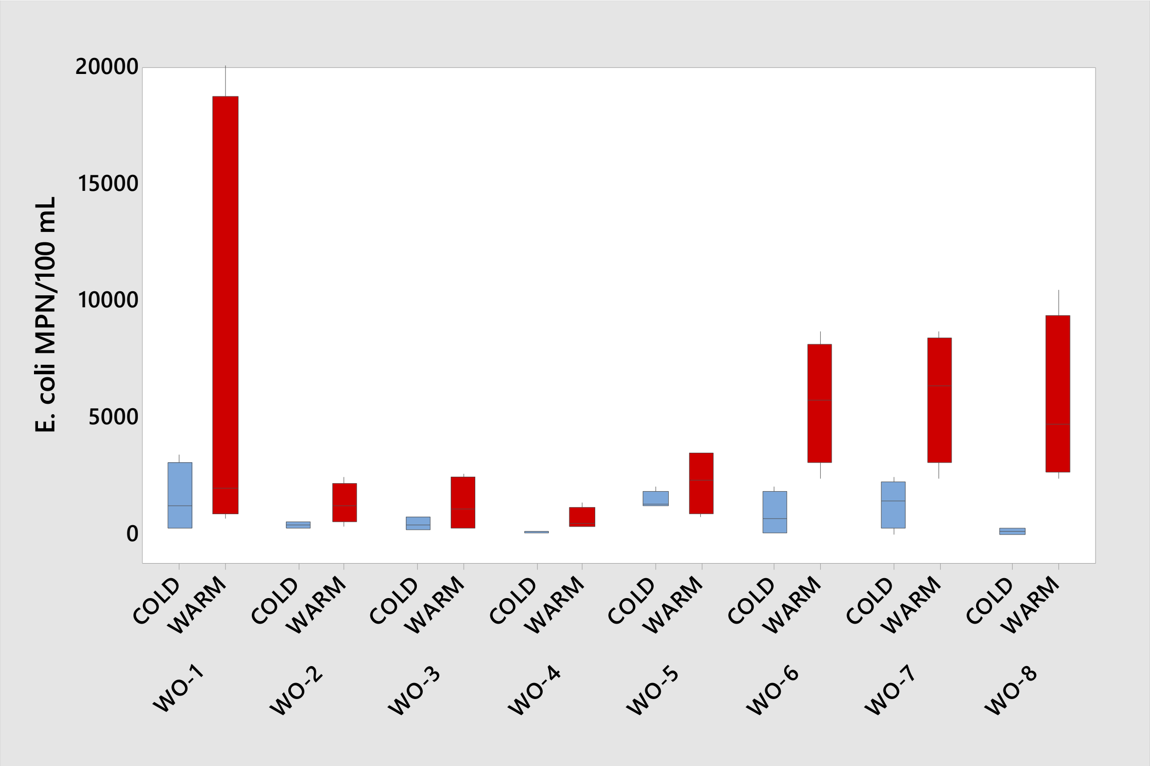


Figure 23: E. coli concentrations for warm and cold months for each of the 8 sampling sites

Moderate temperatures promote an environment conducive to the growth and survival of bacteria, so warmer months should experience an increase in bacterial concentrations (Campos and Kershaw, 2013). Additionally, warmer months tend to experience more storm events and increase sedimentation due to erosion which not only aide in depositing more bacteria into the water, but also provide a place to hide from predators (Lucena, 1994). This is a concern for public health as Cedar Point tends to experience an increase in tourism during the warm months, putting not only the local residents at risk, but those who visit the crystal coast.

Median watershed exports of bacteria were higher during the warm months for most sites than in cold months (Figure 24) however differences in concentrations were not statistically significant (all $p > 0.05$).

WO-4 had the lowest normalized exports of *E. coli* for the watershed, due to relatively low bacterial concentrations and flow for warm and cold months. WO-1 (median= 1804 MPN/s/ha) had the third lowest exports for the warm months even though it had the highest concentration for the warm months. Exports were low for WO-1 because of low normalized flow. The watershed export of *E. coli* was greatest for WO-5, which indicates that the relative normalized stream flow and *E. coli* concentrations were greatest at this location.

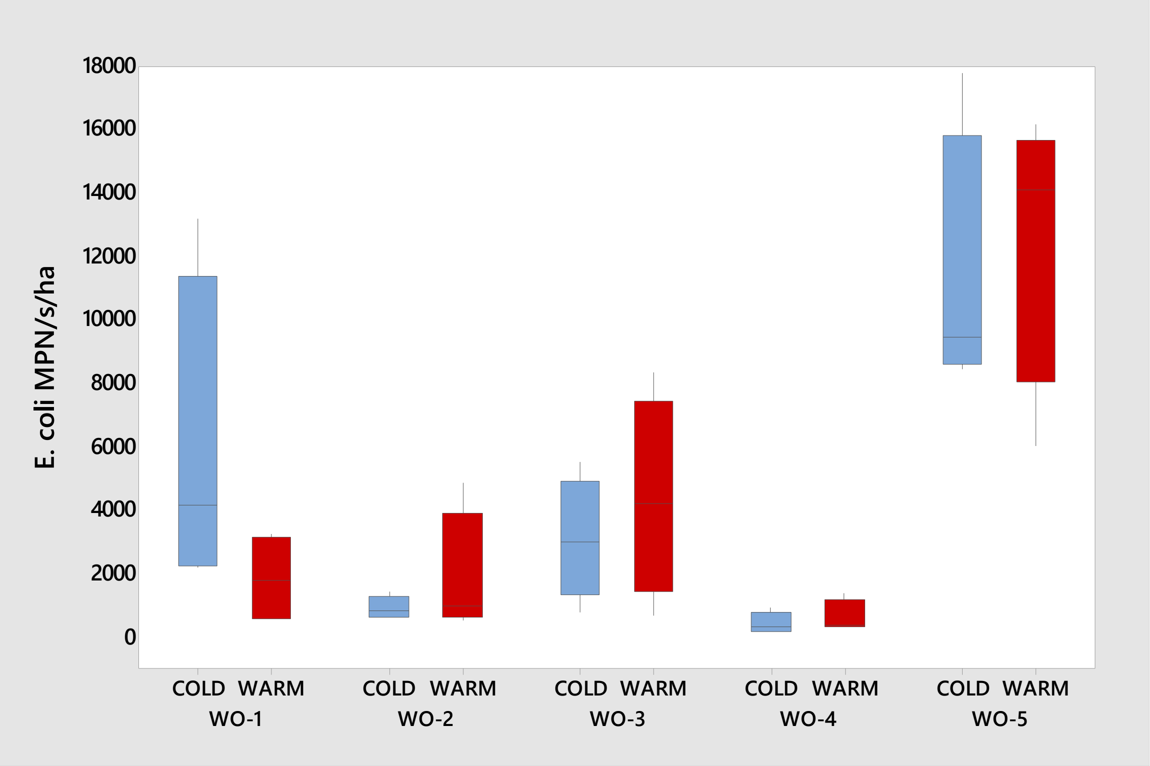


Figure 24: E. coli bacteria export for warm months and cold months for sites 1-5

Temporal Trends in Enterococci Concentrations

Median enterococci concentrations were higher in the warm months than the cold months for each location (Figure 25). However, the differences in concentrations was only statistically significant at WO-2, (p= 0.0304). As with *E. coli* concentrations, the concern for enterococci pathogen indicators in the surface waters increases in the warm months. Increased human exposure occurs in the warm months as does increased runoff and sedimentation.

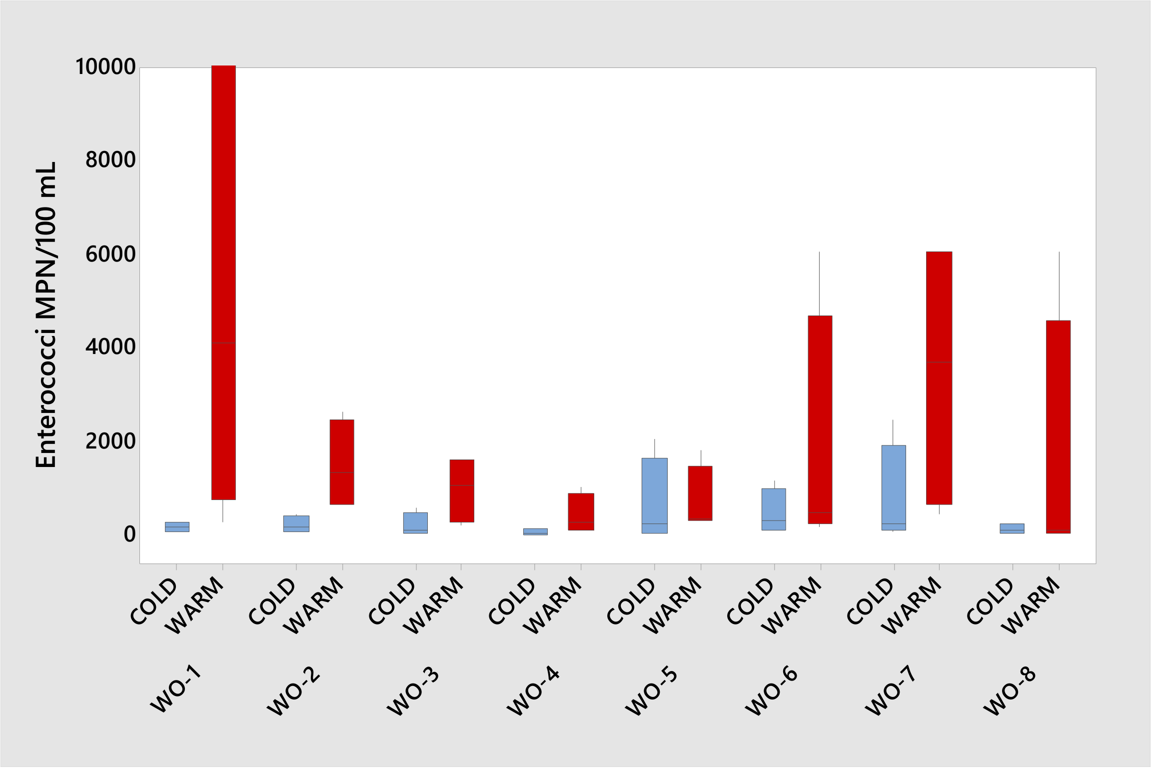


Figure 25: Enterococci concentrations for warm months and cold months for each of the 8 sampling sites

Enterococci exports were higher during warm months relative to cold months at most of the 5 sites (Figure 26), but differences were not statistically significant. As with *E. coli*, WO-4 concentration and loadings were the lowest. However, enterococci concentrations and loadings for WO-1 were both highest.

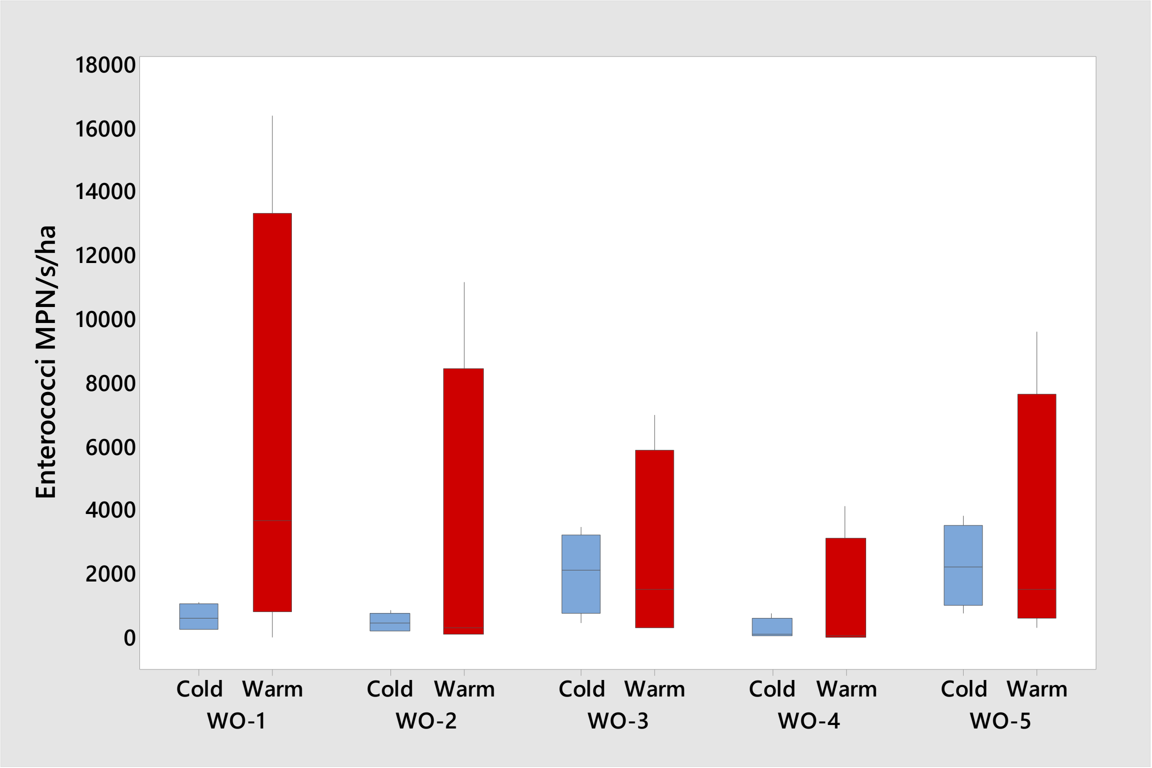


Figure 26: Enterococci loadings for warm months and cold months for sites 1-5

Stormwater Runoff Reductions

The Simple Method was used to compute the amount of runoff reduced for the raingarden, the re-graded sidewalk, the check dams, and water control structures (WCS) 1-5 (Table 11). Each of the BMPs were sampled for enterococci and *E. coli*. The check dams were sampled 3 times, the rain garden two times, and the rest of the BMPs were sampled once.

The estimated 100% of the runoff from the parking lot at the boat ramp that was directed to the rain garden and to the woods (sidewalk work) was predicted to infiltrate for all storm events (over 24 hrs.) that were 3.66” or smaller. The WCS and check dams were estimated to reduce runoff by 30% based on prior research (Sunohara et al., 2016).

The WCSs reduced runoff by an average of 26,638 liters annually. The WCSs also reduced enterococci bacteria concentrations were reduced by an average of 8,089,911 MPN annually and average *E. coli* bacteria concentrations were reduced by an average of 716,274 MPN annually. The sidewalk reduced runoff by 12,335 liters annually and did the best job at reducing enterococci concentrations (21,3395,509 MPN). The rain garden stored the least amount of runoff (5,181 liters) but reduced substantial amounts of both enterococci (4,929,073 MPN) and *E. coli* (51,810 MPN) from entering surface waters.

Table 11: Simple method computations for runoff volumes and FIB loads

Best Management Practice	Total Watershed Area (acre)	Impervious Area (acre)	1 year 24 hour rainfall (inches)	Runoff Volume (ft^3/s)	Runoff Volume (liters)	Enterococci Reduced (MPN)	*E. coli Reduced (MPN)*
WCS 1	2.7	0.6	1.00	245	69383	21231230	1885637
WCS 2	1.5	0.33	1.00	1350	39238	11700822	1032426
WCS 3	0.4	0.11	1.00	399	11307	3459942	305289
WCS 4	0.3	0.06	1.00	218	6167	1887102	166509
WCS 5	0.3	0.07	1.00	250	7093	2170458	191511
Check Dams	0.13	0.09	1.00	326	9220	833949	570027
Rain Garden	0.18	0.05	1.00	183	5181	4929073	51810
Sidewalk	0.12	0.12	1.00	436	12335	213395509	123350

Physical and Chemical Water Parameters

pH

The EPA National Recommended Water Quality Criteria (NRWQC) for pH allows a range of 6 to 9, and pH values outside this range may lead to designation as an impaired water.

Only one event, March 2017 at WO-1 (pH: 9.28), exceeded those criteria (Figure 27), so these stream segments and estuary would not be considered impaired with regards to pH.

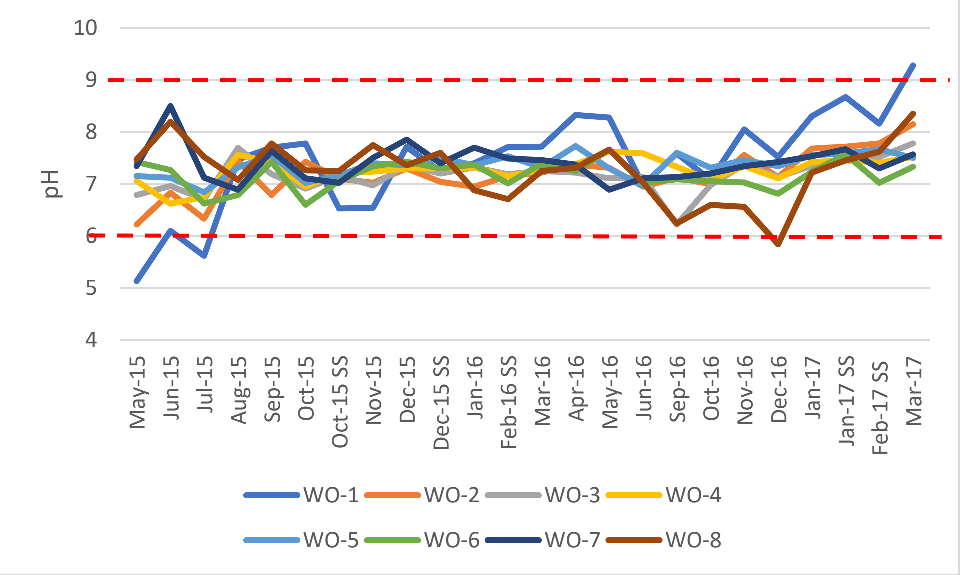


Figure 27: pH levels for each of the 8 sampling sites during the course of the study

Turbidity

The legal standard for turbidity in freshwater is 50 NTU and saltwater is 25 NTU (NC DENR, 2007). Turbidity is a concern for water because the suspended sediment provides protection for pathogens, potentially increasing their life span in the water (USGS, 2016). Pathogens that are in the surface waters are a concern for those using the surface waters for recreational use as possible ingestion may occur.

WO 1-5 did not exceed the standards for freshwater (Figure 28), however WO-6, WO-7, and WO-8 exceeded the standard for saltwater (Figure 29). WO-6 and WO-7 are brackish waters in a marsh that i is an area of interest for many of the local native fauna, which could help explain the increase in turbidity. WO-8 is at the boat ramp near the Cedar Point Campground. This is a spot where many boats, kayakers, and fishermen enter the surface waters, which could influence the turbidity due to human activities.

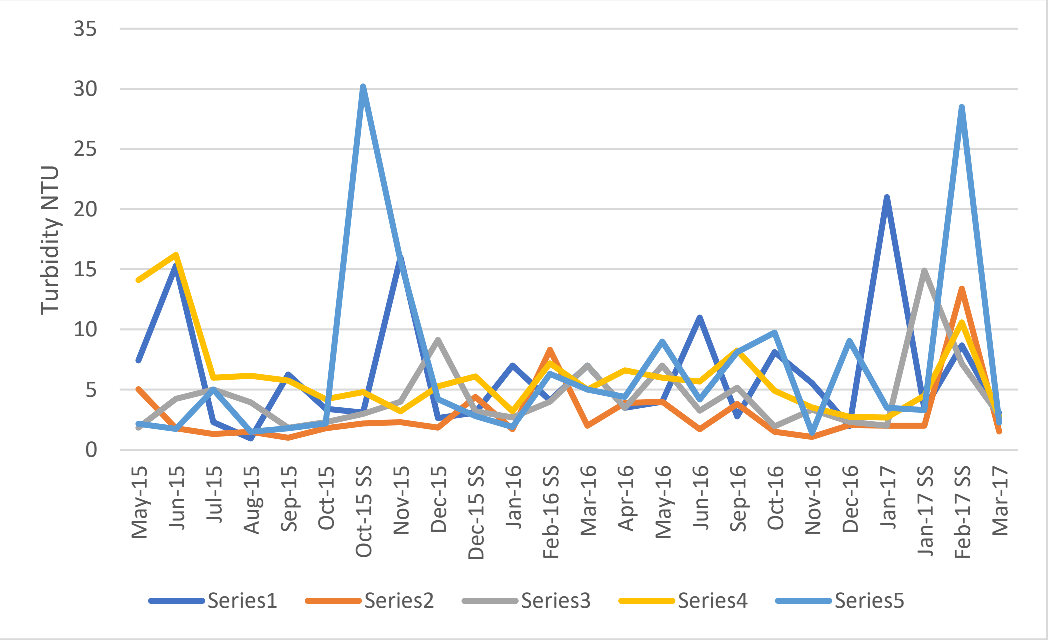


Figure 28: Turbidity readings for sites 1-5 during the course of the study

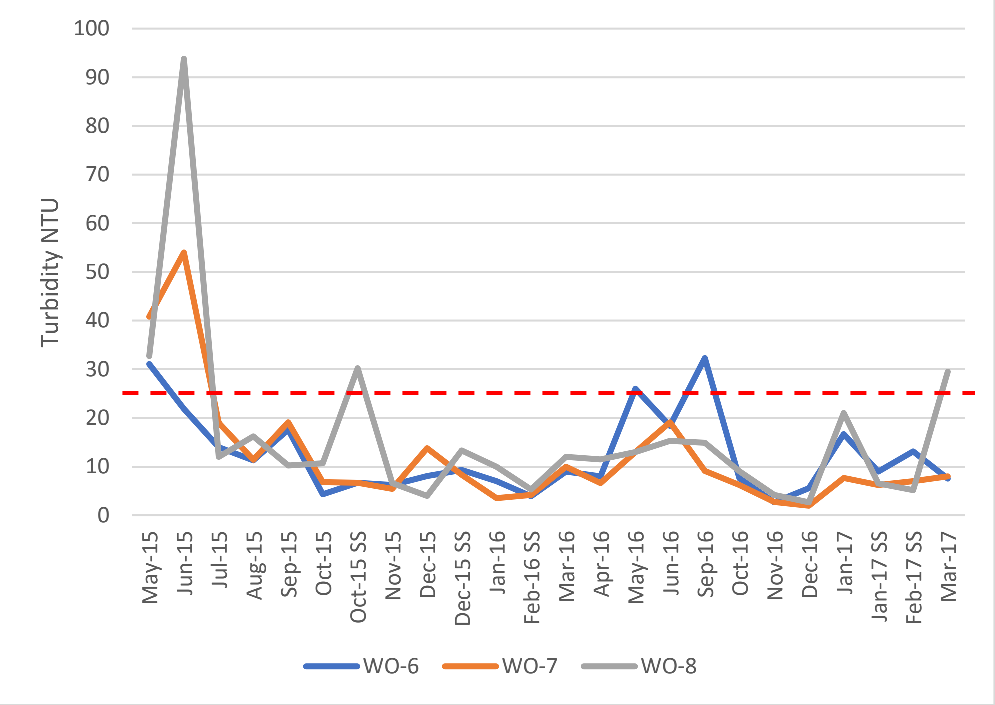


Figure 29: Turbidity readings for sites 6-8

A Spearman's correlation analysis was performed to assess the relationship between turbidity and *E. coli* concentrations. WO-1 (*p=0.050)*, WO-4 (*p=0.000)*, WO-6 (*p=0.000)*, WO-7 (*p=0.000)* and WO-8 (*p=0.020)* all showed strong positive correlations between *E. coli* concentrations and turbidity (Table 12).

Spearman's correlation was performed to assess the relationship between enterococci concentrations and turbidity with WO-8 (*p=0.005)* and WO-4 (*p=0.042)* having a strong positive correlation between turbidity and enterococci concentrations (Table 13)

Table 12: Spearman's correlations to assess the relationship between turbidity and E. coli concentrations

Site	Spearman's correlation	*p-value*
WO-1	0.469	0.050
WO-2	0.385	0.115

WO-3	0.083	0.745
WO-4	0.775	0.000
WO-5	-0.303	0.221
WO-6	0.787	0.000
WO-7	0.804	0.000
WO-8	0.541	0.020

Table 13: Spearman's correlations to assess the relationship between turbidity and Enterococci concentrations

Site	Spearman's Correlation	*p-value*
WO-1	0.347	0.158
WO-2	0.119	0.639
WO-3	0.102	0.687
WO-4	0.485	0.042
WO-5	-0.058	0.820
WO-6	-0.218	0.400
WO-7	0.082	0.748
WO-8	0.644	0.005

Dissolved Oxygen

The dissolved oxygen (DO) concentration for freshwater and tidal waters should not fall below 5.0 mg/L, with the exception of poorly flushed tidally influenced streams (ND DENR, 2007). WO-1, WO-4, WO-6, WO-7 and WO-8 would be considered poorly flushed tidally influenced streams. Low DO concentrations can be detrimental to aquatic life and may be related to excess organic and/or nutrient loading. October 2016 suffered a large loss in DO, which could be attributed the excessive rainfall and flooding conditions due to hurricane Matthew that had struck Eastern North Carolina early October 2016 (Figure 30).

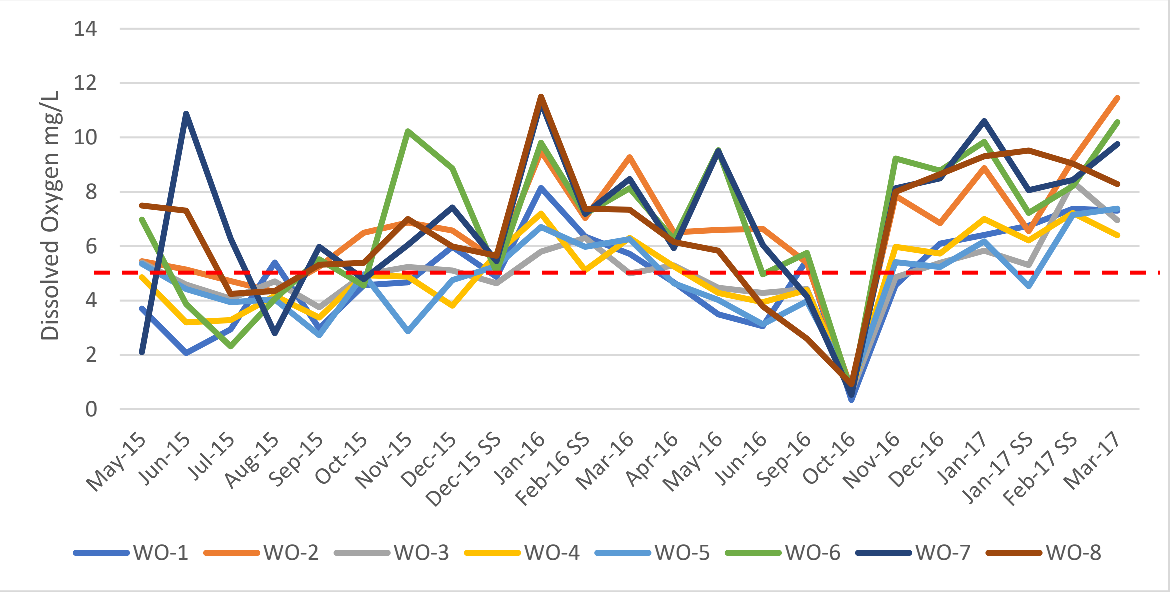


Figure 30: Dissolved Oxygen (mg/L) for each of the 8 sites over the course of the study.

Discharge and Fecal Indicator Bacteria Concentrations

Spearman's correlation analyses were used to evaluate the relationships between *E. coli* concentrations and discharge, and enterococci concentrations and discharge. For each location, and for each FIB, a negative correlation was observed (Table 14 and 15). Significant negative correlations were shown *E. coli* and discharge for WO-1 (*p=0.026)* and WO-2 (*p=0.004)* (Table 14).

A strong significant negative correlation between flow and enterococci concentrations was identified for WO-2 (*p=0.036)* (Table 15). Verhougstraete et al. (2015) also reported that *E.*

coli concentrations were higher in streams with low discharge. They hypothesized that this could be attributed to the affinity for the bacteria to persist in sediment, and low flow waters allow the bacteria a better chance to find cover in sediment. Byappanahalli et al. (2012) showed that enterococci also used sediment for persistence in the water.

Table 14: Spearman's correlations to assess the relationship between discharge and E. coli concentrations

Site	Spearman's correlation	*p-value*
WO-1	-0.523	0.026
WO-2	-0.643	0.004
WO-3	-0.036	0.887
WO-4	-0.375	0.126
WO-5	-0.251	0.330

Table 15: Spearman's correlations to assess the relationship between discharge and Enterococci concentrations

Site	Spearman's correlation	*p-value*
WO-1	-0.410	0.091
WO-2	-0.489	0.036
WO-3	-0.104	0.681
WO-4	-0.381	0.119
WO-5	-0.088	0.736

Freshwater and Brackish Water Boundary

Table 16 shows that WO-2, WO-3, WO-4, and WO-5 would be considered by NOAA to be freshwater, while WO-1 would be considered brackish and WO-6, WO-7, and WO-8 would be considered salt water. Figure 31 shows the 8 sites that were sampled for chloride. Chlorides are ions that increase conductivity, so the strong linear relationship between chloride concentrations and specific conductance is one that would be expected (Figure 32).

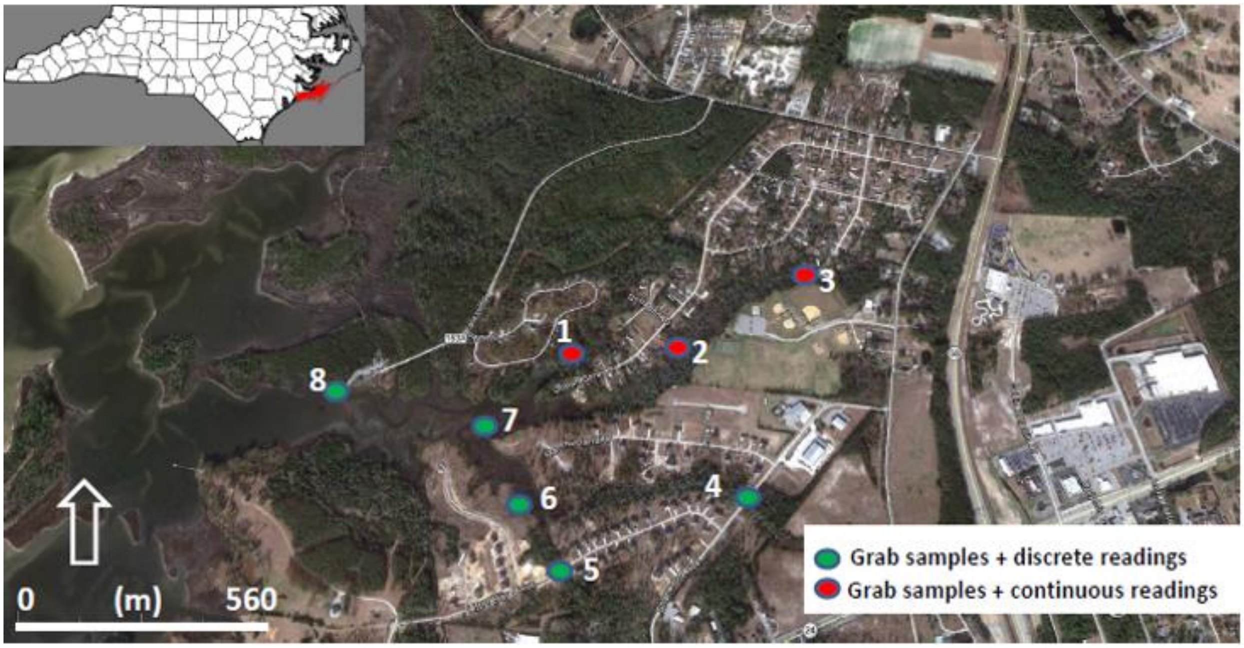


Figure 31: Chloride sampling sites

Table 16: Average chloride concentrations for each of the 8 sampling sites converted to salinity

Site	Cl- (mg/L)	Conversion Factor	Salinity (ppt)	Specific Conductance (µs/cm)
WO-1	38.90	.0018066	.070277	1063
WO-2	33.63	.0018066	.060756	311
WO-3	32.64	.0018066	.058967	336
WO-4	23.51	.0018066	.042654	411
WO-5	22.85	.0018066	.041281	327
WO-6	4282.36	.0018066	7.736512	14703
WO-7	4011.80	.0018066	7.247718	18431
WO-8	6788	.0018066	12.25778	29364

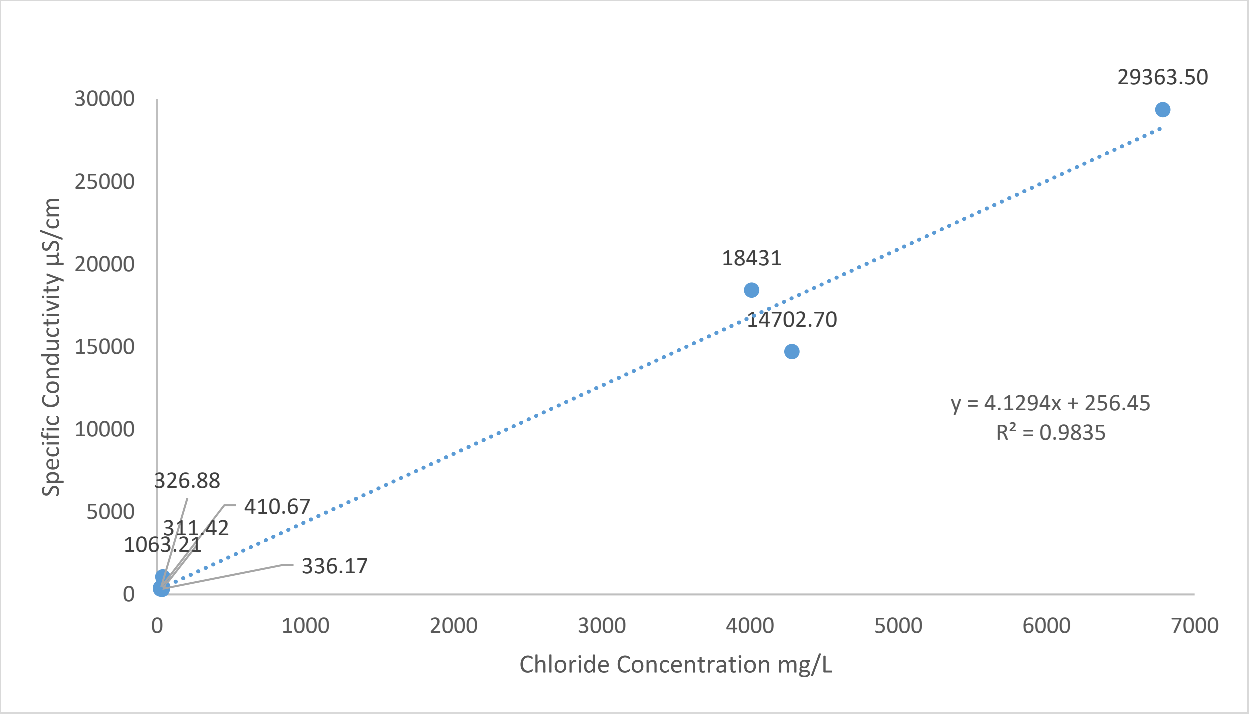


Figure 32: Linear regression specific conductivity and chloride concentration

Conclusions

This study was conducted in the Boat House Creek portion of the Lower White Oak River near Cedar Point, North Carolina. A watershed improvement plan for the White Oak River showed fecal indicator bacteria concentrations often exceeded the recreational standards set by the EPA. The plan indicated that stormwater runoff was the main contributing factor of water quality degradation. This coastal area that has experienced great increases in population during the previous decade and since the watershed plan was developed. In this project, the goal was to determine FIB concentrations frequently exceeded the EPA single standard maximum allowable density, and to investigate if best management practices would be able to reduce the amount of urban runoff and thereby reduce the amount of FIB entering the waters. Sampling was conducted over a 24-month period to investigate the effects of seasonal variability, as well as the effects of storm flow, on fecal indicator bacteria *E. coli* and enterococci. Stormwater BMPs including a rain garden, water control structures, rock check dams, drainageway modifications, and sidewalk renovations were implemented to reduce runoff.

E. coli concentrations exceeded the single sample maximum allowable density in more than 20% of the samples analyzed for all freshwater sampling locations. Enterococci concentrations exceeded water quality standards for more than 33% of the samples collected from for freshwater sites. Enterococci concentrations exceeded the marine waters standard in 42-47% of samples taken from the marsh sampling locations (WO-6 and WO-7), but only 6% of the samples collected from the estuary near the boat ramp (WO-8). Concentrations of *E. coli* and enterococci were greatest during warm periods (summer and spring) when water-based recreation is most popular. Reducing watershed exports of FIB is important for protecting environmental health.

There was spatial variability with regards to FIB concentrations. Concentrations typically increased along a down-stream gradient towards the estuary. Microbial source tracking (MST) data suggests that wildlife were the most significant source of FIB. Compared to the pond that was used for the background, both enterococci and *E. coli* concentrations were high in other water samples analyzed. This could be due to the limited human disturbances at the pond, as well as soils that promote infiltration of stormwater.

Concentrations and watershed exports of enterococci and *E. coli* were higher during storm flow relative to baseflow. The implementation of the BMPs did appear to succeed in reducing runoff, and thereby potentially reducing the amount of bacteria entering the surface water.

This study was conducted in coastal NC where many people take advantage of water resources for recreation, leisure and work. Additional efforts to reduce the volume of stormwater runoff entering the surface waters is suggested along with continued educational outreach activities.

References

Anderson, J. R. (December 15, 2010). *The Effects of High Density Septic Systems on Surface Water Quality in Gwinnett County, Georgia.* Thesis. Retrieved from: http://scholarworks.gsu.edu/cgi/viewcontent.cgi?article=1027&context=geosciences_theses

Boehm, A. B., & Sassoubre, L. M. (February 5, 2014). Enterococci as Indicators of Environmental Fecal Contamination. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection (Internet).* Retrieved from: https://www.ncbi.nlm.nih.gov/books/NBK190421/

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R., & Harwood, V. J. (2012). Enterococci in the Environment. *Microbiology and Molecular Biology Reviews : MMBR*, *76*(4), 685–706. http://doi.org/10.1128/MMBR.00023-12

Cabral, J.P.S. (2010). Water Microbiology. Bacterial Pathogens and Water. *International Journal of Environmental Research and Public Health, 7*(10), 3657-3703. doi: 10.3390/ijerph7103657

Cabrera-Stagno, V. (2007). Developing effective TMDLs: an evaluation of the TMDL process. Proceedings: Water Environment Federation TMDL 2007 Conference, Bellevue, Washington, pp. 443-453.

Campos C. J., Kershaw S. R., Lee R. J. (2013). Environmental influences on faecal indicator organisms in coastal waters and their accumulation in bivalve shellfish. *Estuaries Coast,* 36 834–853. Doi: 10.1007/s12237-013-9599-y

Cessti, R., Srivastava, J., Jung, S. (January 2003). *Agriculture Non-Point Source Pollution Control Good Management Practices Chesapeake Bay Experience.* Retrieved from: http://lshs.tamu.edu/docs/lshs/end-

Clean Water Team (CWT). (2004). Electrical conductivity/salinity Fact Sheet, FS- 3.1.3.0(EC). *The Clean Water Team Guidance Compendium for Watershed Monitoring and Assessment, Version 2.0. Division of Water Quality, California State Water Resources Control Board (SWRCB), Sacramento, CA.* Retrieved from: https://www.waterboards.ca.gov/water_issues/programs/swamp/docs/cwt/guidance/3130en.pdf

Cooper J.A., Loomis G.W., Amador J.A. (2016) Hell and High Water: Diminished Septic System Performance in Coastal Regions Due to Climate Change. *PLOS ONE, 11*(9): e0162104. doi: https://doi.org/10.1371/journal.pone.0162104

Field, K.G., and Scott, T.M. (2008). *Microbial Source Tracking.* Retrieved from: http://cws.msu.edu/documents/MicrobialSourceTrackingWhitePaper.pdf

Fisher, K. and Phillips, C. (2009). The ecology, epidemiology and virulence of Enterococcus. *Microbiology, 155,* 1749-1757. doi: 10.1099/mic.0.026385-0

Fondriest Environmental, Inc. (2014). *Conductivity, Salinity and Total Dissolved Solids*. Fundamentals of Environmental Measurements. Retrieved from: http://www.fondriest.com/environmental-measurements/parameters/water-quality/conductivity-salinity-tds/

Frankenburg, D. (1999). *The White Oak River: Introduction*. Retrieved from: http://www.learnnc.org/lp/editions/cede_blackwaterriver/47

Fraser, S.L. (2017). *Enterococcal Infections.* Retrieved from: https://emedicine.medscape.com/article/216993-overview

Gaffield, S.J., Goo, R.L., Richards, L.A., Jackson, R.J. (2003). Public health Effects of Inadequately Managed Stormwater Runoff. *American Journal of Public Health, 93*(9). doi: 10.2105/AJPH.93.9.1527

Gregory, B.M. and Frick, E.A. (2000). *Fecal Coliform bacteria concentrations in streams of the Chattahoochee River National Recreation Area, Metropolitan Atlanta, Georgia, May-October 1994 and 1995.* Retrieved from: https://pubs.usgs.gov/wri/wri004139/pdf/wrir00-4139.pdf

Gustafsen, D.M., Anderson, J.L., Heger, S.F., Liukkonen, B.W. (2000). *Choosing an alternative septic system for a homesite with a high water table.* Retrieved from: https://www.extension.umn.edu/environment/housing-technology/moisture-management/choosing-an-alternative-septic-system/index.html

Heid, C.A., Stevens J., Livak K.J., Williams P.M. (1996) Genome Methods Real Time Quantitative PCR. *Genome Research, 1996*(6), 986-994. doi: 10.1101/gr.6.10.986

Humphrey, C., Finley, A., O'Driscoll, M., Manda, A., Iverson, G. (2015). Groundwater and stream *E. coli* concentrations in coastal plain watersheds served by onsite wastewater and a municipal sewer treatment system. *Water Science Technology, 72*(10), 1851-1860. doi: 10.2166/wst.2015.411

Humphrey, C., O'Driscoll, M.A., Zarate, M.A. (2011). Evaluation of on-site wastewater system Escherichia coli contributions to shallow groundwater in Coastal North Carolina. *Water Science Technology, 63*(4), 789-795. doi: 10.2166/wst.2011.310

Hygnstrom, J. R., Skipton, S. O., Woldt, W. E. (October 2008). *Residential Onsite Wastewater Treatment: Septic Tank Design and Installation.* University of Nebraska-Lincoln Extension. Retrieved from: http://extensionpublications.unl.edu/assets/pdf/g1473.pdf

Ishii and Sadowsky, 2008. Escherichia Coli in the environment; Implications for water quality and human Health. *Microbes and Environments*, *23*(2), 101-108.
DOI: 10.1264/jsme2.23.101

Iwamoto, M., Ayers, T., Mahon, B. E., Swerdlow, D. L. (April 2010). Epidemiology of Seafood-Associated Infections in the United States. *Clinical Microbiology Review.* 23(2) 399-411. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2863362/

Liu J., Sample D.J., Bell C., Guan Y. (2014). Review and Research Needs of Bioretention Used for the Treatment of Urban Stormwater. *Water*, *6*(4):1069-1099. doi: 10.3390/w6041069

Lucena, F., J. Lasobras, D. McIntosh, M. Forcadell, and J. Jofre. (1994). Effect of distance from the polluting focus on relative concentrations of Bacteroides fragilis phages and coliphages in mussels. *Applied and Environmental Microbiology* 60(7): 2272–2277. Retrieved from: https://www.ncbi.nlm.nih.gov/pubmed/8074509

Mallin, M. A. (June 2006). Wading in Waste. *Scientific American, 294*(6), 52-59. Retrieved from: https://www.scientificamerican.com/article/wading-in-waste/

Mallin, M.A. (2013) *Septic Systems in the Coastal Environment: Multiple Water Quality Problems in Many Areas.* Monitoring Water Quality: Pollution Assessment, Analysis, and Remediation. 81-102. doi: 10.1016/B978-0-444-59395-5.00004-2.

Mallin, M.A., Turner, M.I.H., McIver, M.R., Toothman, B.R., and Freeman, H.C. (2016). Significant Reduction of Fecal Bacteria and Suspended Solids Loading by Coastal Best Management Practices. *Journal of Coastal Research*, *32*(4). 923 – 931. doi: https://doi.org/10.2112/JCOASTRES-D-15-00195.1

Michaud, J.P. and Weirenga, M. (2005). *Estimating Discharge and Stream Flow. Ecology Publication Number 05-10-070.* Retrieved from: https://fortress.wa.gov/ecy/publications/documents/0510070.pdf

Naman, J. M., & Gibson, J. M. (October 2015). “Disparities in Water and Sewer Services in North Carolina: An Analysis of the Decision-Making Process.” *American Journal of Public Health, 105*(10), 20-26. doi: 10.2105/AJPH.2015.302731.

National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine. (2014) *Polymerase Chain Reaction*. Accessed at: https://www.ncbi.nlm.nih.gov/probe/docs/techpcr/

National Oceanic and Atmospheric Administration. (2017). *Salinity*. Retrieved from: https://oceanservice.noaa.gov/education/kits/estuaries/media/supp_estuar10c_salinity.html

North Carolina Division of Environment and Natural Resources. (2013). *Erosion and Sediment Control Planning and Design Manual.* Retrieved from: https://files.nc.gov/ncdeq/Energy%20Mineral%20and%20Land%20Resources/Land%20Resources/Land%20Quality/Erosion%20and%20Sediment%20Control%20Planning%20and%20Design%20Manual/Cover%20and%20Table%20of%20Contents/CoverandTableofContents_rev%20May%202013.pdf

North Carolina Division of Environmental and Natural Resources. (2009). *NCDENR Stormwater BMP Calculations*. Retrieved from: https://files.nc.gov/ncdeq/Water%20Quality/Surface%20Water%20Protection/SPU/SPU%20-%20BMP%20Manual%20Documents/BMPMan-Ch03-SWCalcs-20090616-DWQ-SPU.pdf

North Carolina Forest Service. (March 6, 2017). *What Are BMPs?* Retrieved from: http://ncforestservice.gov/water_quality/what_are_bmps.htm

Paul, M. J., & Meyer, J. L. (November 2001). Streams in the Urban Landscape. *Annual Review of Ecology and Systematic, 32,* 333-365. doi: http://annualreviews.org/doi/abs/10.1146/annurev.ecolsys.32.081501.114040

Phillips, T. (2017). *How the Polymerase Chain Reaction Works.* Retrieved from: https://www.thebalance.com/how-the-polymerase-chain-reaction-pcr-works-375670

Sargeant, D., Kammin, W.R., and Collyard, S. (2011). *Review and Critique of Current Microbial Source Tracking (MST) Techniques.* Retrieved from: https://fortress.wa.gov/ecy/publications/documents/1103038.pdf

Schneeberger, C.L., O'Driscoll, M.A., Humphrey, C.P., Henry, K.A., Deal, N., Seibert, K.L., Hill, V.R., and Zarate-Bermudez, M.A. (2015). Fate and Transport of Enteric Microbes from Septic Systems in a Coastal Watershed. *Journal of Environmental Health. 77*(9), 22-30.

Sobsey, M.D., Khatib, L.A., Hill, V.R., Alocilja, E., Pillai, S. (2006). Pathogens in Animal Wastes and the Impacts of Waste Management Practices on their Survival, Transport, and Fate. *Animal Agriculture and the Environment: National Center for Manure and Animal Waste Management White Papers.* 609-666. Retrieved from: https://www.researchgate.net/publication/265568682_PATHOGENS_IN_ANIMAL_WAS

TES_AND_THE_IMPACTS_OF_WASTE_MANAGEMENT_PRACTICES_ON_THEIR_SURVIVAL_TRANSPORT_AND_FATE

Schueler, T.R. (1987). *Controlling Urban Runoff: A Practical Manual for Planning and Designing Urban BMPs.* Publication no. 87703. Metropolitan Washington Council of Governments. 275 pp.

Sowah, R., Zhang, H., Radcliffe, D., Bauske, E., Habteselassie, M. Y. (2014). Evaluating the influence of septic systems and watershed characteristics on stream faecal pollution in suburban watersheds in Georgia USA. *Journal of Applied Microbiology, 117*(5), 1365-2672. doi: 10.1111/jam.12614

State Climate Office of North Carolina. (2017). *Bogue Field MCALF, NC; Monthly Data Retrieval.* Retrieved from: https://climate.ncsu.edu/cronos/?station=KNJM&temporal=monthly

Sunohara, M.D., Gottschall, N., Craiovan, E., Wilkes, G., Topp, E., Frey, S.K., Lapen, D.R. (2016) Controlling tile drainage during the growing season in Eastern Canada to reduce nitrogen, phosphorus, and bacteria loading to surface water. *Agricultural Water Management, 178*, 159-179. doi: https://doi.org/10.1016/j.agwat.2016.08.030

Tilman, L., Plevan, A., Conrad, P. (June 2011). *Effectiveness of Best Management Practices for Bacteria Removal.* Retrieved from: https://www.pca.state.mn.us/sites/default/files/wq-iw8-08q.pdf

Tursi, F. (March, 2009). *Southeast White Oak River Shellfish Restoration Project.* Retrieved from: https://ncdenr.s3.amazonaws.com/s3fs-public/Water%20Quality/Planning/TMDL/FINAL%20TMDLS/White%20Oak/NC%20Coastal%20Federation%20-%20White%20Oak%20Final%20Report.pdf

United States Census Bureau. (July 1st, 2015). *Annual Estimate of the Resident Population April 1, 2010 to July 1, 2010.* Retrieved from: https://factfinder.census.gov/faces/tableservices/jsf/pages/productview.xhtml?src=bkmk

United States Environmental Protection Agency. (1986). *Ambient Water Quality for Bacteria.* Retrieved from: https://nepis.epa.gov/Exe/ZyNET.exe/00001M74.txt?ZyActionD=ZyDocument&Client=EPA&Index=1981%20Thru%201985&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C81THRU85%5CTXT%5C00000001%5C00001M74.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1

U.S. Environmental Protection Agency (USEPA). (1986). Bacteriological Ambient Water Quality Criteria for Marine and Fresh Recreational Waters. EPA 440/5-84-002. EPA Office of Water. PB-86-158-045

United States Environmental Protection Agency. (2014). *EPA Partial Approval and Decision Document for North Carolina 2014 303 (d).* Retrieved from: https://archive.epa.gov/pesticides/region4/water/tmdl/web/pdf/nc-2014-303d-decision-package.pdf

United States Environmental Protection Agency. (2016). *Impaired Waters and TMDLs: Identifying and Listing Impaired waters.* Retrieved from: https://www.epa.gov/tmdl/impaired-waters-and-tmdls-identifying-and-listing-impaired-waters

United States Environmental Protection Agency. (2015). *Recreational Water Quality Criteria.* Retrieve from: https://www.epa.gov/sites/production/files/2015-10/documents/rwqc2012.pdf

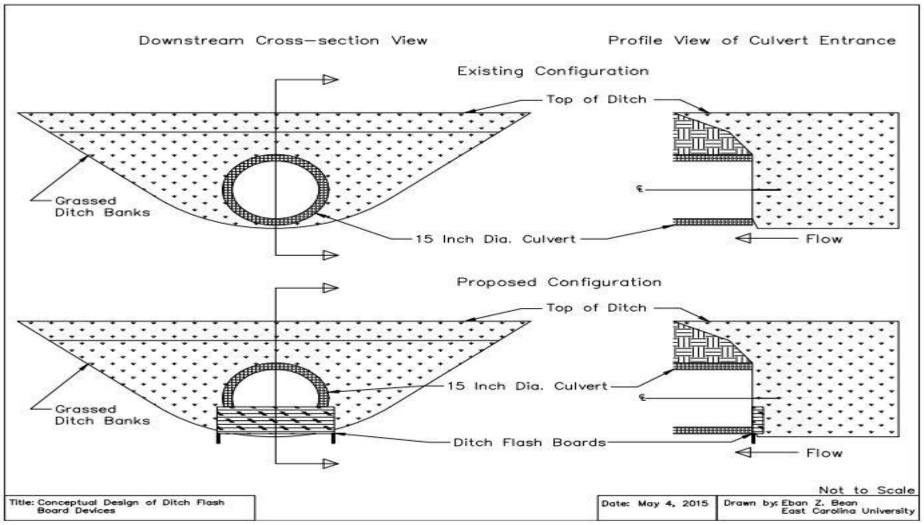
United States Geographical Survey. (2016). *Turbidity*. Retrieved from: https://water.usgs.gov/edu/turbidity.html

Verhougstraete, M. P., Martin, S. L., Kendall, A. D., Hyndman, D. W., & Rose, J. B. (2015). Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(33), 10419–10424. http://doi.org/10.1073/pnas.1415836112

Virginia Department of Environmental Quality. (2002). *Fecal Coliform TMDL for Accontink Creek, Fairfax Creek, Virginia*. Retrieved from: http://www.deq.virginia.gov/portals/0/DEQ/Water/TMDL/apptmdls/potrvr/accotink.pdf

Vogel, M. P. (August 2005). *Septic Tank and Drainfield Operation and Maintenance.* Montana State University Extension Service. Retrieved from: http://msuextension.org/publications/HomeHealthandFamily/MT199401HR.pdf

Appendix A: Diagrams



Schematic of a flashboard riser (water control structure) drawn be Dr. Eban Bean.

Appendix B: Tables

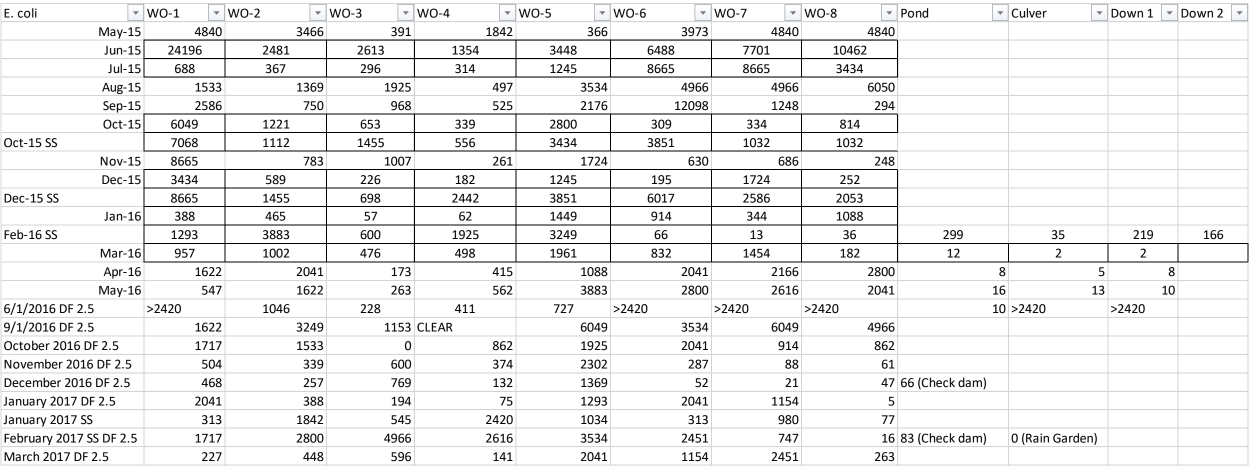
Sample (round 1)	Date Collected	Crossing Point General	Crossing Point Human
WO-1	17-Dec-15	Negative	Negative
WO-3	17-Dec-15	Negative	negative
WO-4	17-Dec-15	37.16	Negative
WO-5	17-Dec-15	Negative	Negative
WO-6	17-Dec-15	40.00	Negative
WO-8	17-Dec-15	Negative	Negative
WO-4	18-Dec-15	Negative	Negative
WO-5	18-Dec-15	Negative	Negative
WO-6	18-Dec-15	Negative	Negative
WO-8	18-Dec-15	Negative	Negative

Sample (round 2)	Date Collected	Crossing Point General	Crossing Point Human
WO-1	28-Oct-15	Negative	Negative
WO-2	28-Oct-15	Negative	Negative
WO-3	28-Oct-15	40.00	Negative
WO-4	28-Oct-15	35.81	Negative
WO-5	28-Oct-15	Negative	Negative
WO-6	28-Oct-15	30.61	Negative
WO-7	28-Oct-15	33.64	Negative
WO-8	28-Oct-15	33.35	Negative
WO-1	17-Dec-15	Negative	Negative
WO-2	17-Dec-15	Negative	Negative
WO-3	17-Dec-15	Negative	Negative
WO-4	17-Dec-15	Negative	Negative
WO-5	17-Dec-15	Negative	Negative
WO-6	17-Dec-15	Negative	Negative
WO-7	17-Dec-15	Negative	Negative
WO-8	17-Dec-15	Negative	Negative
WO-1	18-Dec-15	Negative	Negative
WO-2	18-Dec-15	Negative	Negative
WO-3	18-Dec-15	Negative	Negative
WO-4	18-Dec-15	40.01	Negative
WO-5	18-Dec-15	Negative	Negative

WO-6	18-Dec-15	Negative	Negative
WO-7	18-Dec-15	Negative	Negative
WO-8	18-Dec-15	Negative	Negative

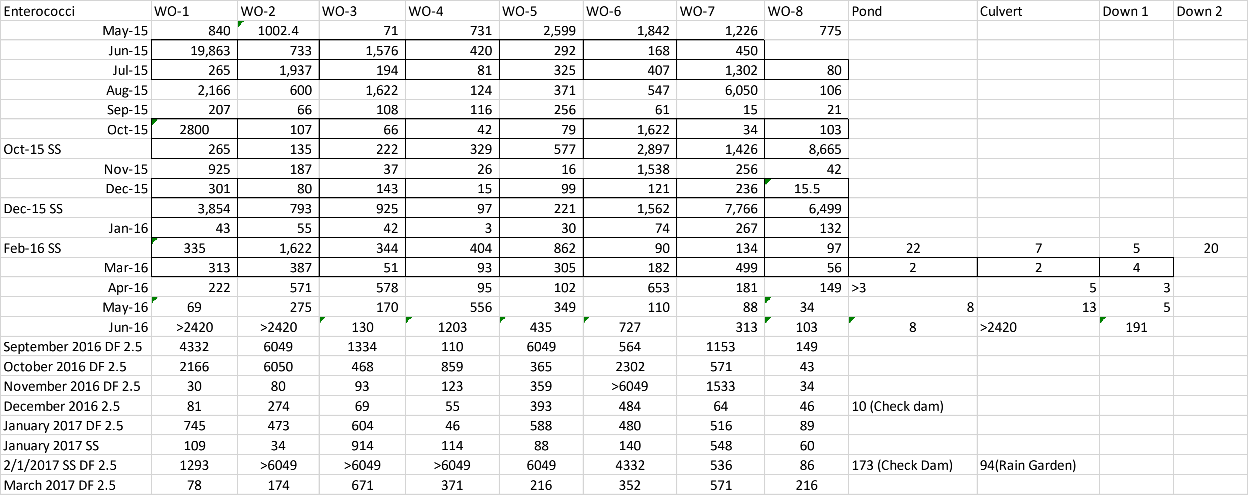
Appendix C: Raw data

E. coli (MPN/100 mL)



E. coli	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8	Pond	Culver	Down 1	Down 2
May-15	4840	3466	391	1842	366	3973	4840	4840				
Jun-15	24196	2481	2613	1354	3448	6488	7701	10462				
Jul-15	688	367	296	314	1245	8665	8665	3434				
Aug-15	1533	1369	1925	497	3534	4966	4966	6050				
Sep-15	2586	750	968	525	2176	12098	1248	294				
Oct-15	6049	1221	653	339	2800	309	334	814				
Oct-15 SS	7068	1112	1455	556	3434	3851	1032	1032				
Nov-15	8665	783	1007	261	1724	630	686	248				
Dec-15	3434	589	226	182	1245	195	1724	252				
Dec-15 SS	8665	1455	698	2442	3851	6017	2586	2053				
Jan-16	388	465	57	62	1449	914	344	1088				
Feb-16 SS	1293	3883	600	1925	3249	66	13	36	299	35	219	166
Mar-16	957	1002	476	498	1961	832	1454	182	12	2	2	
Apr-16	1622	2041	173	415	1088	2041	2166	2800	8	5	8	
May-16	547	1622	263	562	3883	2800	2616	2041	16	13	10	
6/1/2016 DF 2.5	>2420	1046	228	411	727	>2420	>2420	>2420	10	>2420	>2420	
9/1/2016 DF 2.5	1622	3249	1153	CLEAR	6049	3534	6049	4966				
October 2016 DF 2.5	1717	1533	0	862	1925	2041	914	862				
November 2016 DF 2.5	504	339	600	374	2302	287	88	61				
December 2016 DF 2.5	468	257	769	132	1369	52	21	47	66 (Check dam)			
January 2017 DF 2.5	2041	388	194	75	1293	2041	1154	5				
January 2017 SS	313	1842	545	2420	1034	313	980	77				
February 2017 SS DF 2.5	1717	2800	4966	2616	3534	2451	747	16	83 (Check dam)	0 (Rain Garden)		
March 2017 DF 2.5	227	448	596	141	2041	1154	2451	263				

Enterococci (MPN/100 mL)



Enterococci	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8	Pond	Culvert	Down 1	Down 2
May-15	840	1002.4	71	731	2,599	1,842	1,226	775				
Jun-15	19,863	733	1,576	420	292	168	450					
Jul-15	265	1,937	194	81	325	407	1,302	80				
Aug-15	2,166	600	1,622	124	371	547	6,050	106				
Sep-15	207	66	108	116	256	61	15	21				
Oct-15	2800	107	66	42	79	1,622	34	103				
Oct-15 SS	265	135	222	329	577	2,897	1,426	8,665				
Nov-15	925	187	37	26	16	1,538	256	42				
Dec-15	301	80	143	15	99	121	236	15.5				
Dec-15 SS	3,854	793	925	97	221	1,562	7,766	6,499				
Jan-16	43	55	42	3	30	74	267	132				
Feb-16 SS	335	1,622	344	404	862	90	134	97	22	7	5	20
Mar-16	313	387	51	93	305	182	499	56	2	2	4	
Apr-16	222	571	578	95	102	653	181	149	>3	5	3	
May-16	69	275	170	556	349	110	88	34	8	13	5	
Jun-16	>2420	>2420	130	1203	435	727	313	103	8	>2420	191	
September 2016 DF 2.5	4332	6049	1334	110	6049	564	1153	149				
October 2016 DF 2.5	2166	6050	468	859	365	2302	571	43				
November 2016 DF 2.5	30	80	93	123	359	>6049	1533	34				
December 2016 2.5	81	274	69	55	393	484	64	46	10 (Check dam)			
January 2017 DF 2.5	745	473	604	46	588	480	516	89				
January 2017 SS	109	34	914	114	88	140	548	60				
2/1/2017 SS DF 2.5	1293	>6049	>6049	>6049	6049	4332	536	86	173 (Check Dam)	94(Rain Garden)		
March 2017 DF 2.5	78	174	671	371	216	352	571	216				

pH

pH	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8
May-15	5.13	6.22	6.79	7.06	7.15	7.42	7.34	7.47
Jun-15	6.1	6.83	6.96	6.62	7.12	7.27	8.5	8.2
Jul-15	5.62	6.33	6.7	6.75	6.84	6.62	7.12	7.52
Aug-15	7.47	7.44	7.69	7.56	7.32	6.79	6.89	7.08
Sep-15	7.7	6.79	7.19	7.42	7.55	7.43	7.64	7.78
Oct-15	7.78	7.43	6.91	6.95	7.02	6.6	7.11	7.26
Oct-15 SS	6.53	7.1	7.16	7.21	7.17	7.06	7.02	7.25
Nov-15	6.54	7.02	6.97	7.24	7.4	7.34	7.5	7.75
Dec-15	7.71	7.3	7.36	7.3	7.36	7.43	7.85	7.36
Dec-15 SS	7.24	7.04	7.2	7.3	7.5	7.3	7.4	7.6
Jan-16	7.4	6.95	7.32	7.32	7.36	7.37	7.7	6.88
Feb-16 SS	7.71	7.15	7.19	7.15	7.53	7.01	7.49	6.71
1-Mar	7.72	7.22	7.25	7.27	7.32	7.39	7.46	7.25
Apr-16	8.33	7.37	7.22	7.38	7.73	7.25	7.37	7.31
May-16	8.28	7.31	7.11	7.62	7.3	7.66	6.89	7.66
Jun-16	6.97	6.95	7.13	7.59	6.96	25.27	30.07	
Sep-16	7.59	7.12	6.22	7.33	7.6	7.09	7.13	6.24
Oct-16	7.08	7	6.98	7.08	7.31	7.05	7.2	6.6
Nov-16	8.05	7.56	7.37	7.34	7.46	7.03	7.34	6.56
Dec-16	7.52	7.13	7.13	7.11	734	6.81	7.42	5.84
Jan-17	8.3	7.68	7.35	7.42	7.55	7.23	7.53	7.22
January 2017 SS	8.67	7.72	7.46	7.45	7.59	7.57	7.67	7.45
February 2017 SS	8.16	7.78	7.52	7.41	7.67	7.02	7.3	7.62
Mar-17	9.28	8.15	7.78	7.52	7.5	7.33	7.57	8.35

Temperature (°C)

Temp	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8	Pond	Culvert	Down 1	Down 2
May-15	20.7	20.1	20.5	22.5	22.3	30.4	30.2	31.1				
Jun-15	25.7	25.1	25.2	26.5	25.2	33.3	35.3	36.3				
Jul-15	24.7	23.9	23.3	25.3	22.6	28.8	27.4	28.2				
Aug-15	23.2	23.6	23.2	24.4	22.3	26.3	27.2	29.6				
Sep-15	2218.0	22.1	22.4	22.9	23.8	26.2	25.8	25.2				
Oct-15	17.4	17.9	18.9	18.9	19.0	20.1	20.4	20.6				
Oct-15 SS	28.7	21.2	21.3	21.5	20.7	20.7	20.9	21.9				
Nov-15	18.8	18.5	19.0	19.2	20.2	19.0	18.7	18.5				
Dec-15	18.2	18.7	19.2	19.9	18.7	18.8	18.7	18.4				
Dec-15 SS	17.6	18.1	18.6	18.2	18.5	18.5	18.3	18.4				
Jan-16	6.7	8.0	10.0	9.0	9.8	8.7	7.0	2.2				
Feb-16 SS	15.6	16.9	17.5	18.1	18.3	17.5	17.2	18.5	7.8	11.8	11.8	12.6
Mar-16	17.7	20.3	18.5	19.3	20.5	21.2	21.4	21.2	20.6	20.4	19.7	
Apr-16	20.3	20.0	23.1	22.5	21.3	26.9	27.0	25.6	24.5	24.1	23.9	
May-16	22.8	21.3	22.0	24.7	25.6	28.6	30.5	30.2	28.6	27.5	27.4	
Jun-16	24.2	24.0	24.4	25.8	25.3	30.1	31.3	31.1	29.5	28.9	28.5	
Sep-16	24.4	24.3	24.6	25.1	23.7	27.1	27.4	26.5				
Oct-16	20.2	20.1	20.7	20.8	21.4	22.8	22.8	23.0				
Nov-16	11.7	12.6	15.2	14.1	15.5	12.4	12.3	13.5				
Dec-16	11.1	12.2	14.1	13.4	14.6	11.3	11.6	11.4				
Jan-17	13.7	16.5	16.7	15.3	16.2	15.1	16.4	17.7				
January 2017 SS	14.7	15.4	17.3	16.9	18.3	17.9	18.0	15.6				
February 2017 SS	12.3	12.4	13.0	13.7	13.1	12.3	11.9	13.1				
Mar-17	15.7	15.9	16.3	17.0	16.7	16.9	17.8	20.3				

Turbidity (NTU)

Turbidity	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8	Pond	Culvert	Downstream
May-15	7.4	5.0	1.8	14.1	2.2	31.1	40.8	32.7			
Jun-15	15.3	1.8	4.2	16.2	1.7	21.9	54.0	93.8			
Jul-15	2.3	1.3	5.0	6.0	5.0	14.0	19.0	12.0			
Aug-15	0.9	1.5	3.9	6.1	1.5	11.3	11.4	16.2			
Sep-15	6.3	1.0	1.8	5.8	1.8	17.6	19.1	10.2			
Oct-15	3.4	1.8	2.3	4.2	2.2	4.3	6.8	10.7			
Oct-15 SS	3.1	2.2	3.0	4.8	30.2	6.7	6.7	30.2			
Nov-15	16.0	2.3	4.0	3.2	15.7	6.2	5.4	6.6			
Dec-15	2.7	1.9	9.1	5.3	4.2	8.1	13.8	4.0			
Dec-15 SS	3.1	4.4	3.2	6.1	2.8	9.3	8.3	13.3			
Jan-16	7.0	1.7	2.7	3.2	1.9	7.0	3.5	10.0			
Feb-16 SS	4.1	8.3	4.0	7.2	6.3	3.9	4.2	5.3			
Mar-16	7.0	2.0	7.0	5.0	5.0	9.0	10.0	12.0			
Apr-16	3.5	3.9	3.5	6.6	4.4	8.0	6.6	11.5	7.8	7.2	5.4
May-16	4.0	4.0	7.0	6.0	9.0	26.0	13.0	13.0	12	46	13
Jun-16	11.0	1.7	3.3	5.7	4.2	18.4	19.1	15.3	6.7	19	23.9
Sep-16	2.8	3.8	5.2	8.3	8.1	32.3	9.1	14.9			
Oct-16	8.1	1.5	1.9	4.9	9.7	7.6	6.3	9.0			
Nov-16	5.5	1.1	3.3	3.5	1.4	2.7	2.7	4.2			
Dec-16	2.0	2.1	2.3	2.8	9.1	5.5	2.0	2.7			
Jan-17	21.0	2.0	2.0	2.7	3.5	16.7	7.7	21.0			
January 2017 SS	3.4	2.0	14.9	4.5	3.3	9.0	6.2	6.6			
February 2017 SS	8.7	13.4	7.2	10.6	28.5	13.1	7.0	5.1			
Mar-17	3.1	1.5	2.9	2.8	2.3	7.5	8.0	29.5			

Specific Conductivity (µs/cm)

SC	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8	Pond	Culvert	Down 1	Down 2
May-15	237	355	400	407	353	1409	8770	31380				
Jun-15	260	347	388	395	235	1305	5855	24568				
Jul-15	156	285	357	309	317	35716	16046	45186				
Aug-15	293	206	285	363	419	6956	19654	32266				
Sep-15	268	359	384	391	488	43259	47603	48555				
Oct-15	264	311	332	397	317	38672	40482	41530				
Oct-15 SS	379	276	270	300	295	25180	31270	35370				
Nov-15	211	284	300	388	211	284	300	388				
Dec-15	717	282	286	19	287	36560	36320	16730				
Dec-15 SS	345	252	270	326	280	2685	5267	10260				
Jan-16	223	283	301	393	318	1189	1570	3633				
Feb-16 SS	589	245	262	398	260	34950	37720	22880	402	385	385	398
Mar-16	616	304	344	416	318	1822	3751	17280	497	396	388	
Apr-16	704	341	385	441	325	16240	19770	30930	435	425	424	
May-16	537	332	370	579	343	2745	9975	30990	430	403	402	
Jun-16	995	446	453	950	430	10540	21816	45986	540	463	460	
Sep-16	574	283	268	365	294	7184	1700	25070				
Oct-16	1249	372	378	472	433	4153	27286	31712				
Nov-16	1439	384	408	454	356	18100	27200	45400				
Dec-16	1352	319	327	407	362	40120	42530	42850				
Jan-17	11490	325	368	440	317	6064	9179	26520				
January 2017 SS	663	294	301	401	330	1270	5545	25870				
February 2017 SS	1227	245	249	398	217	14450	20700	41470				
Mar-17	729	344	382	447	340	2012	2034	27900				

Dissolved Oxygen (mg/L)

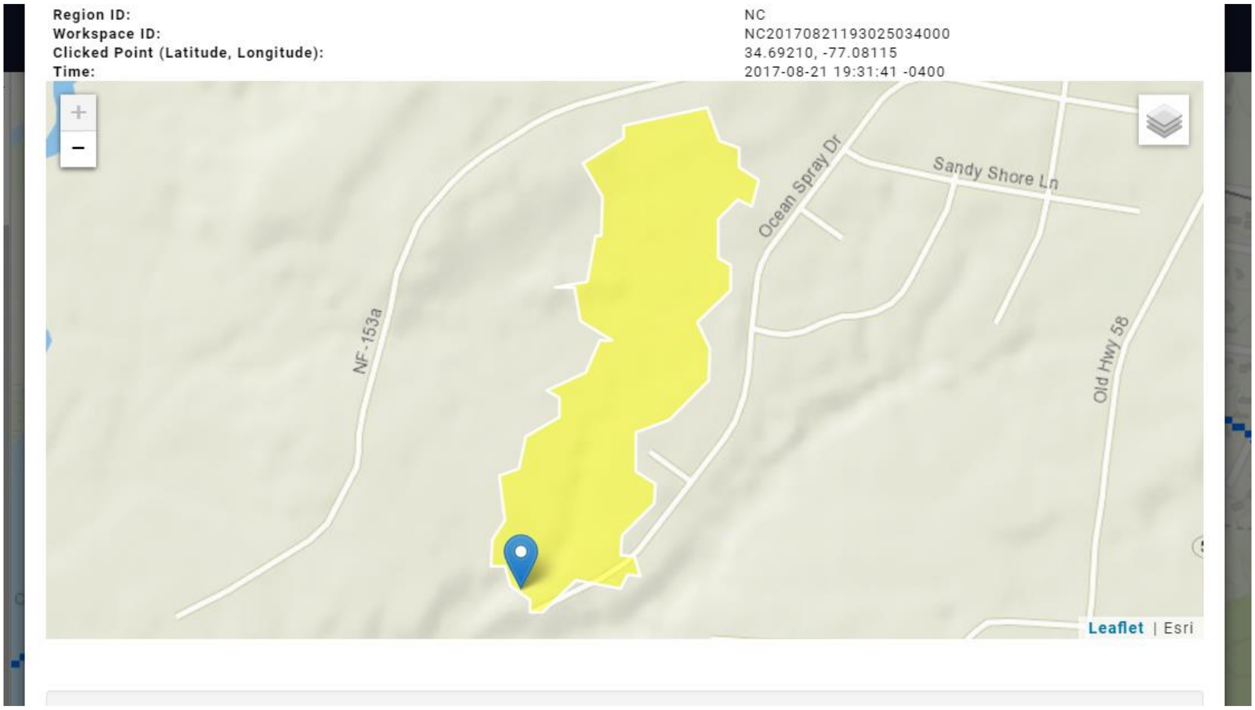
DO	WO-1	WO-2	WO-3	WO-4	WO-5	WO-6	WO-7	WO-8	Pond	Culvert	Downstream
May-15	3.7	5.4	5.4	4.9	5.4	7.0	2.1	7.5			
Jun-15	2.1	5.1	4.6	3.2	4.4	3.9	10.9	7.3			
Jul-15	2.9	4.7	4.1	3.3	3.9	2.3	6.3	4.2			
Aug-15	5.4	4.3	4.7	4.2	4.0	4.1	2.8	4.4			
Sep-15	3.0	5.2	3.8	3.4	2.7	5.5	6.0	5.3			
Oct-15	4.6	6.5	5.0	4.9	5.0	4.6	4.8	5.4			
Oct-15 SS	60.5	68.5	70.4	59.8	53.7	51.4	53.6	56.5			
Nov-15	211.0	284.0	300.0	388.0	2.9	10.2	6.1	7.0			
Dec-15	6.0	6.6	5.1	3.8	4.8	8.9	7.4	6.0			
Dec-15 SS	4.9	5.5	4.6	5.7	5.3	5.0	5.5	5.7			
Jan-16	8.1	9.5	5.8	7.2	6.7	9.8	11.3	11.5			
Feb-16 SS	6.4	7.0	6.3	5.1	6.0	7.2	7.2	7.4	7.4	8.7	8.3
Mar-16	5.7	9.3	5.0	6.3	6.3	8.1	8.5	7.3			
Apr-16	4.7	6.5	5.3	5.3	4.6	6.3	5.9	6.2	5.7	5.0	4.2
May-16	3.5	6.6	4.5	4.3	4.0	9.5	9.5	5.8	9.8	8.8	8.4
Jun-16	6.8	9.3	7.9	7.3	5.4	7.0	7.1	7.3	6.9	5.8	5.0
Sep-16	5.5	5.4	4.4	4.4	4.0	5.8	4.2	2.6			
Oct-16	0.3	0.7	0.6	0.7	0.9	0.6	0.5	0.9			
Nov-16	4.6	7.9	4.9	6.0	5.4	9.2	8.1	8.0			
Dec-16	6.1	6.9	5.4	5.7	5.2	8.8	8.5	8.7			
Jan-17	6.4	8.9	5.8	7.0	6.2	9.8	10.6	9.3			
January 2017 SS	6.8	6.6	5.3	6.2	4.5	7.2	8.1	9.5			
February 2017 SS	7.4	9.2	8.4	7.2	7.2	8.2	8.4	9.0			
Mar-17	7.3	11.5	7.0	6.4	7.4	10.6	9.8	8.3			

Discharge (ft^3/s)

Discharge	WO-1	WO-2	WO-3	WO-4R	WO-4L	WO-5
May-15	0.059	0.142	0.033	0.069		0.090
Jun-15	0.001	0.344	1.767	0.420		0.209
Jul-15	0.033	0.479	0.101	0.240		0.242
Aug-15	0.082	0.219	0.188	0.189	0.012	0.200
Sep-15	0.011	0.431	0.168	0.127	0.118	0.324
Oct-15	0.251	0.672	0.210	0.180	0.261	0.205
Oct-15 SS	0.384	1.130	0.614	0.636	0.437	0.472
Nov-15	0.006	1.269	0.483	0.413		0.256
Dec-15	0.164	0.770	0.556	1.088	1.048	0.400
Dec-15 SS	0.339	1.104	0.585	1.807	1.498	0.672
Jan-16	0.178	0.734	0.549	0.798		0.357
Feb-16 SC	0.509	1.868	0.865	1.325		0.828
Mar-16	0.031	1.514	0.278	1.911	0.000	
Apr-16	0.106	0.455	0.517	0.398		0.707
May-16	0.083	0.401	0.415	0.368		0.248
Jun-16	0.023	0.593	0.355	0.290		0.445
Sep-16	0.396	0.955	0.587	0.714		0.526
Oct-16	0.132	0.469	0.180	0.378		0.503
Nov-16	0.137	0.808	0.256	0.105		0.741
Dec-16	0.198	0.738	0.313	0.544		0.331
Jan-17	0.123	0.589	0.177	0.326		0.328
Janaury 2017 SS	0.271	0.691	0.314	0.606		0.415
February 2017 SS	0.556	3.188	0.785	2.643		0.921
Mar-17	0.466	0.674	0.229	0.451		0.437

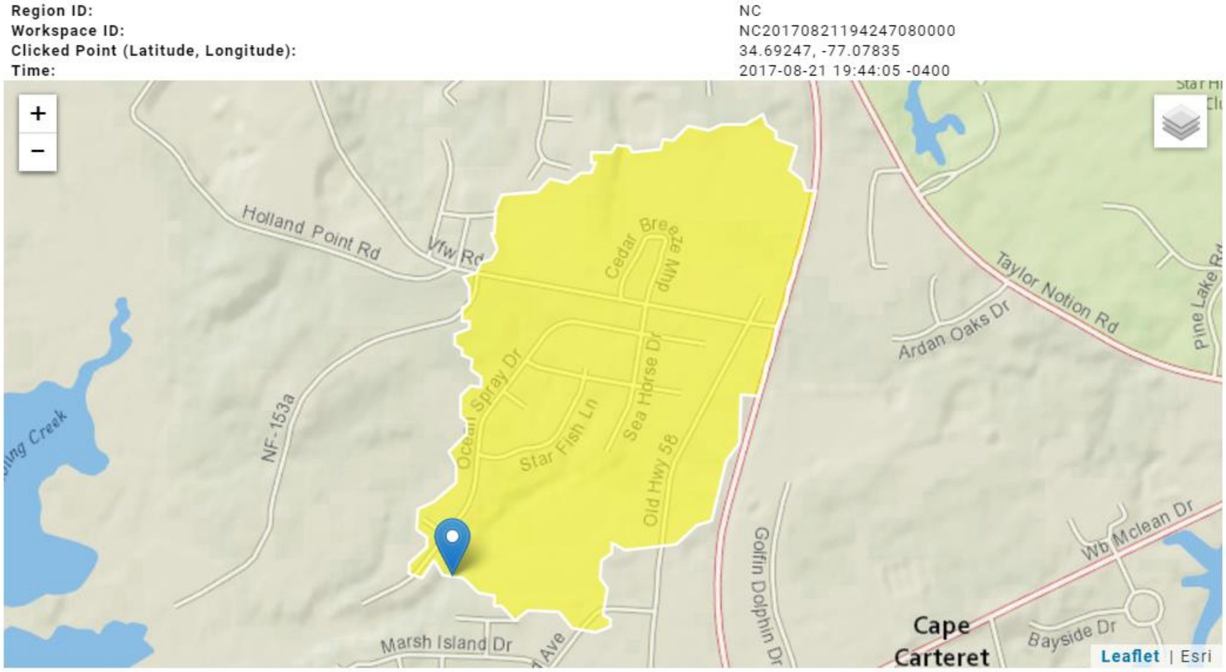
Appendix D: Watershed Area

WO-1



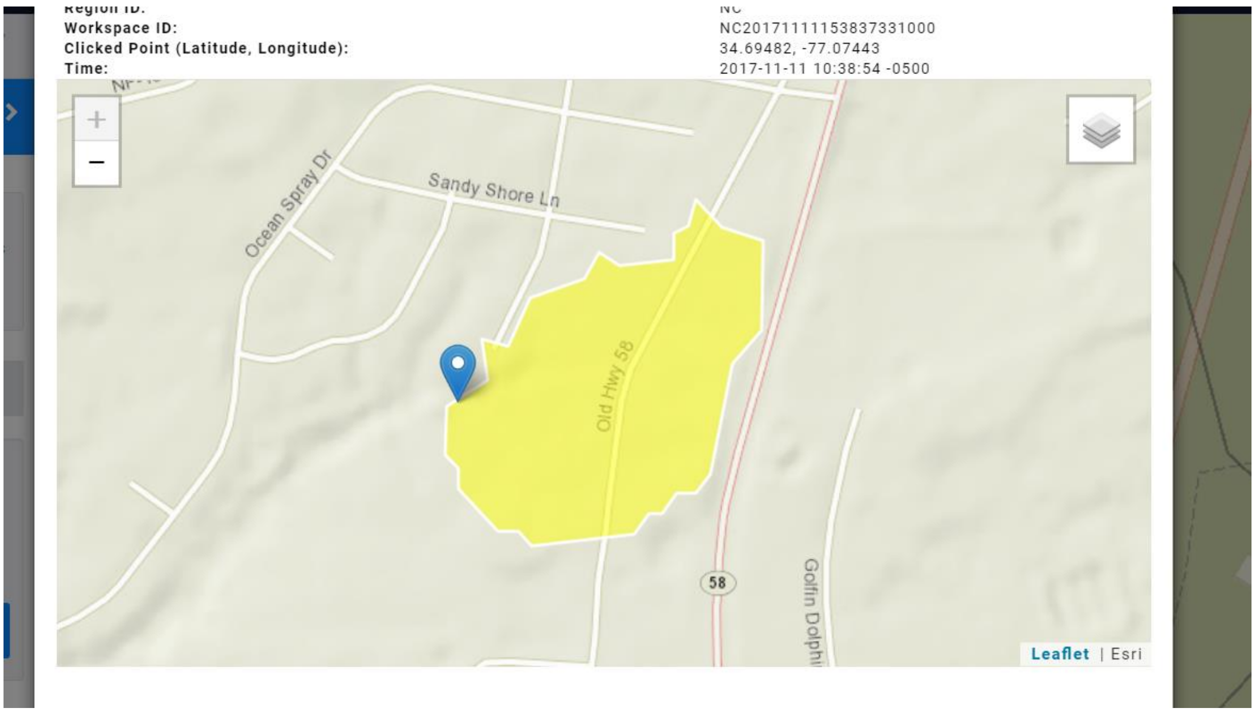
Region ID: NC
Workspace ID: NC20170821193025034000
Clicked Point (Latitude, Longitude): 34.69210, -77.08115
Time: 2017-08-21 19:31:41 -0400

WO-2



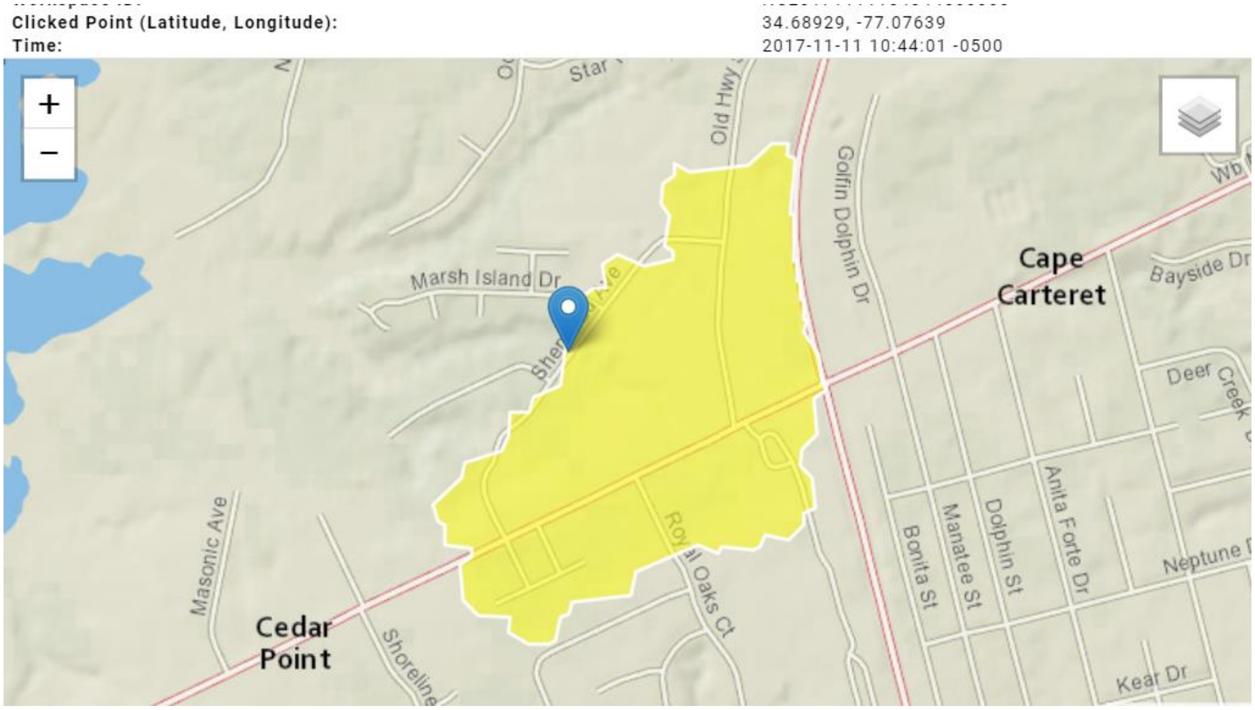
Region ID: NC
Workspace ID: NC20170821194247080000
Clicked Point (Latitude, Longitude): 34.69247, -77.07835
Time: 2017-08-21 19:44:05 -0400

WO-3



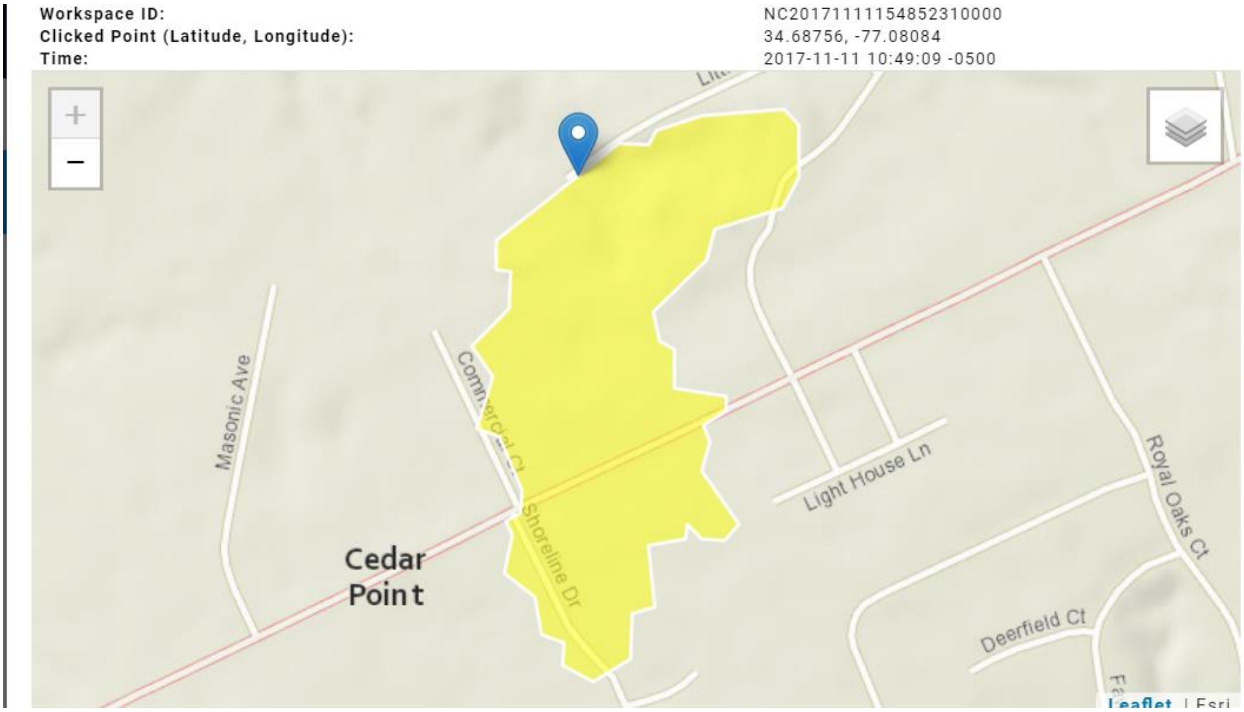
Region ID: NC
Workspace ID: NC20171111153837331000
Clicked Point (Latitude, Longitude): 34.69482, -77.07443
Time: 2017-11-11 10:38:54 -0500

WO-4



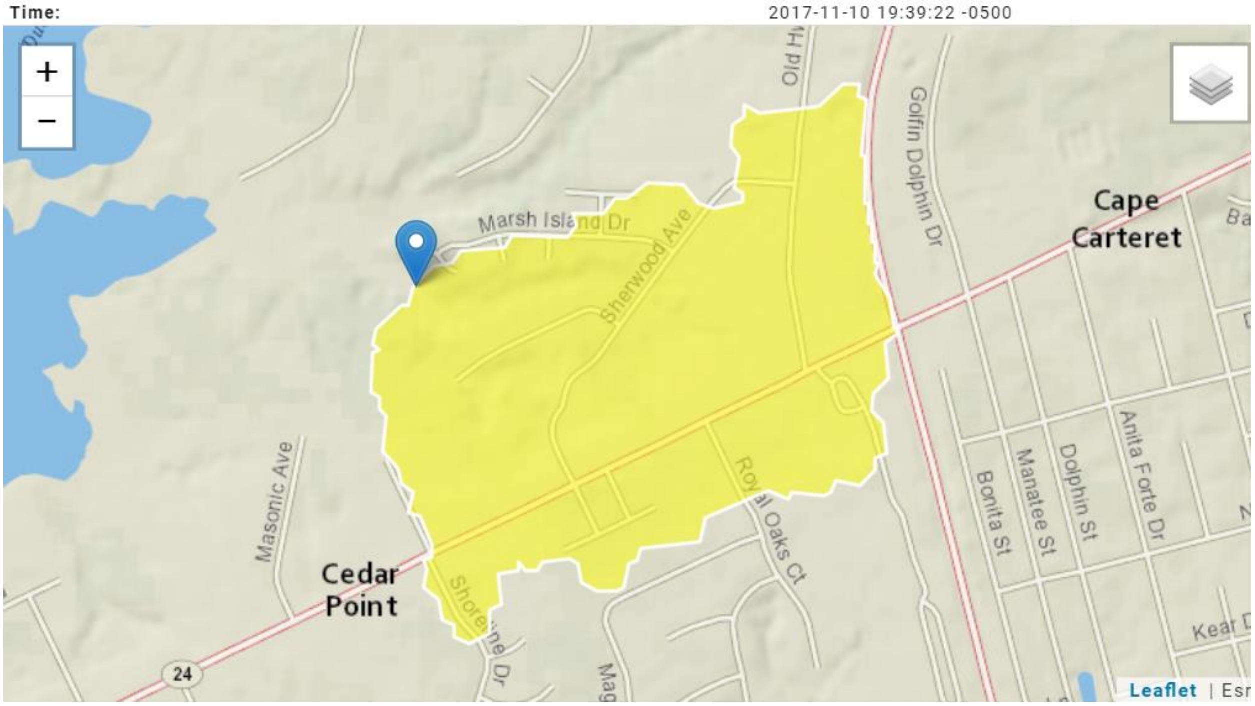
Clicked Point (Latitude, Longitude): 34.68929, -77.07639
Time: 2017-11-11 10:44:01 -0500

WO-5



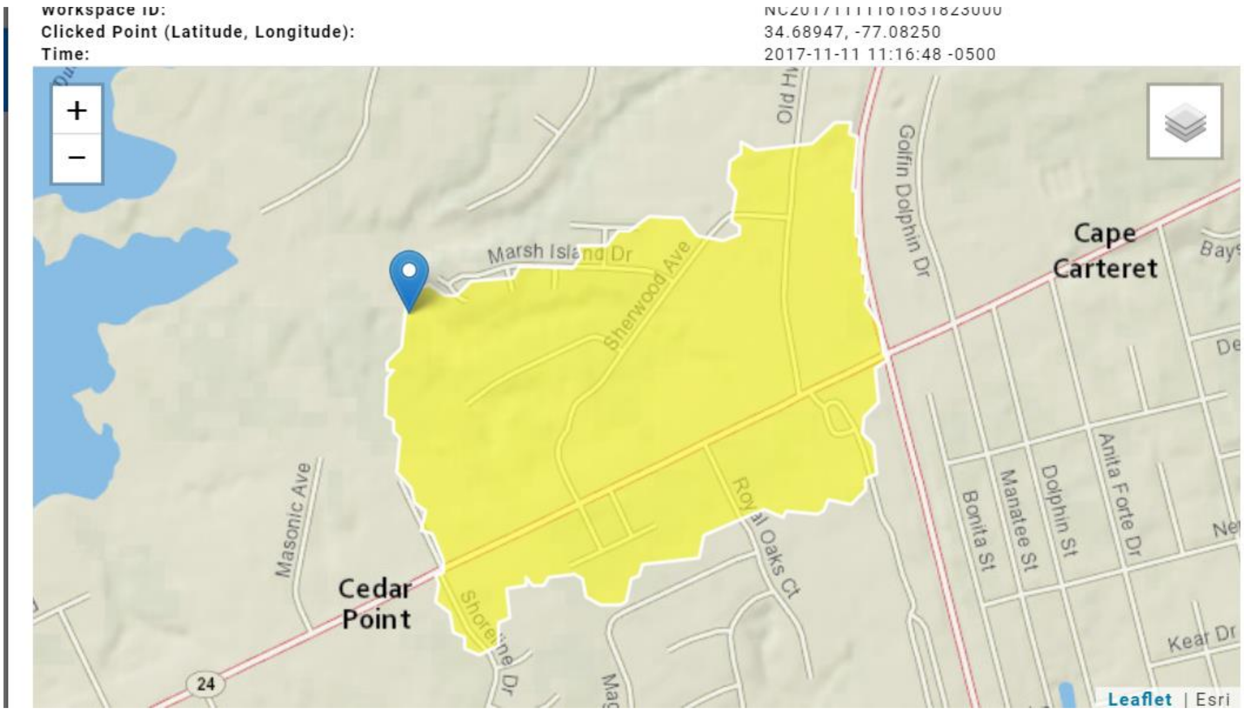
Workspace ID: NC20171111154852310000
Clicked Point (Latitude, Longitude): 34.68756, -77.08084
Time: 2017-11-11 10:49:09 -0500

WO-6



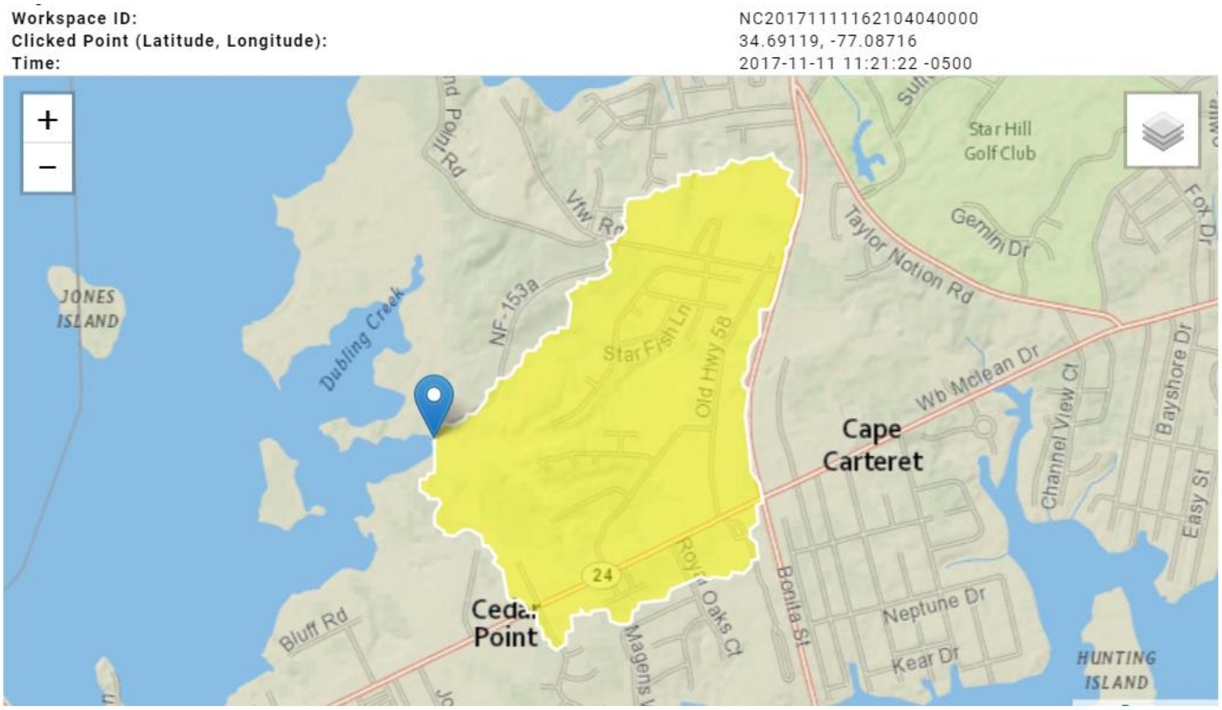
Time: 2017-11-10 19:39:22 -0500

WO-7



Workspace ID: NC20171111161631823000
Clicked Point (Latitude, Longitude): 34.68947, -77.08250
Time: 2017-11-11 11:16:48 -0500

WO-8



Workspace ID: NC20171111162104040000

Clicked Point (Latitude, Longitude): 34.69119, -77.08716

Time: 2017-11-11 11:21:22 -0500

Table 17: Watershed drainage area for each of the sampling sites

Site	Watershed area (Ha)
WO-1	12.1
WO-2	88.1
WO-3	12.4
WO-4	46.6
WO-5	14.2
WO-6	90.6
WO-7	90.6
WO-8	240.9

Appendix E: Pond Data

Date	pH	Temp (C°)	SC (µS/cm)	DO (mg/L)	E. coli (MPN/100 mL)	Enterococci (MPN/100 mL)
Feb-16	7.8	7.8	402	7.35	299	22
Mar-16		20.6	497		12	2
Apr-16	7.91	24.53	435	5.72	8	2
May-16	7.61	28.56	430	9.78	16	8
Jun-16	8.11	29.45	540	6.92	25	19
Average	7.9	22.2	460.8	7.4	72.0	10.6