

Evaluation of Fecal Indicator Bacteria Concentrations in Watersheds Served by Varying  
Densities of Onsite Wastewater Systems in the North Carolina Piedmont

By

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Onsite wastewater systems (OWS) are commonly used in North Carolina for wastewater treatment. Wastewater contains elevated concentrations of pathogens. If OWS do not efficiently treat wastewater then high concentrations of microorganisms may be contributed to water resources. The fecal indicator bacteria (FIB) *Escherichia (E.) coli* and enterococci are often used to assess the environmental health risks associated with microbial contamination. The goal of this study was to gain a better understanding of the influence that onsite system density have on FIB concentrations in piedmont streams. The studied streams included segments of Lick Creek, Laurel Creek, and Little Lick Creek and all are tributaries of Falls Lake, a water supply and recreation source for central North Carolina. Stream sampling occurred approximately monthly between January 2015 to December 2016 at 13 sample locations in the watersheds. Monitoring included the analyses of stream samples for *E. coli* and enterococci and, physical and chemical parameters including: pH, temperature, dissolved oxygen, oxygen-reduction potential, specific conductivity, stream discharge, and turbidity. The geometric mean of *E. coli* and enterococci concentrations in streams were typically elevated in smaller watersheds (< 250 ha) with high densities of conventional-style OWS. The geometric mean FIB concentrations were lower in a forested watershed, centralized sewer watershed, and larger watersheds (> 500 ha) with lower

densities of OWS. These data suggest that the density of OWS and watershed size may influence FIB concentrations in streams.



Evaluation of Fecal Indicator Bacteria Concentrations in Watersheds Served by  
Varying Densities of Onsite Wastewater Systems in the North Carolina Piedmont

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by

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## LIST OF ABBREVIATIONS

FIB	Fecal Indicator Bacteria.....	2
MCL	Maximum Contamination Limit.....	4
OWS	Onsite wastewater systems .....	4
MST	Microbial Source Tracking.....	4
RTQ-PCR	Real-time Quantitative Polymerase Chain Reaction .....	4
MSW	Municipal Sewer Works .....	8
NC	North Carolina.....	9
DEQ	Department of Environmental Quality .....	11
HD	Watersheds with a High Density of Septic Systems.....	12
LD	Watersheds with a Low Density of Septic Systems .....	12
SF	Watersheds with Sand Filter Septic Systems .....	12
ORP	Oxygen Reduction Potential .....	13
DO	Dissolved Oxygen .....	13
SC	Electrical Conductance.....	13
MPN	Most Probable Number .....	15

## **I. Chapter 1: Introduction**

### **A. Falls Lake**

Falls Lake, in central North Carolina, is located close to the head waters of the Neuse River. Falls Lake is a man made 12,000 acre reservoir that serves to reduce downstream flooding provide a source of recreation, and water supply to the Raleigh-Durham area. Drainage from the lake flows into the Neuse River, which empties into the Albemarle-Pamlico Sound, and eventually the Atlantic Ocean. Falls Lake and many of its tributaries including Lick Creek and Little Lick Creek are on the 303(d) list of impaired waters, indicating that they do not fully support their intended uses such as aquatic habitat. Watershed plans for Lick Creek and Little Lick Creek have been developed to try to identify the major sources of water use impairment and provide strategies for remediating the water quality problems.

### **B. Watershed Characteristics**

Watershed characteristics such as predominant land use, amount of impervious surface, number of wastewater treatment plant outfalls, and septic system densities may influence surface water quality (NCDEQ, 2015). Lick Creek is a 22.9 square mile watershed located on the edge of Durham and Wake County. The watershed is currently a mostly rural, undeveloped area surrounded by urban growth. About 80% of the watershed's land use is protected natural area, urban green space, forestry, agriculture, unmanaged rural lands, and undeveloped land. Approximately 37% of the land is unmanaged and undeveloped land, with about 25% of the

watershed managed for production of agricultural and forestry products (NCDEQ, 2015). The Lick Creek watershed is expected to experience significant urban development and an increasing amount of impervious cover, potentially having deleterious effects on the already impaired watershed (NCDEQ, 2015).

The Little Lick Creek watershed is 17.2 square miles and is located in eastern Durham County. The land use is about 80% agricultural, low density residential, undeveloped area, parks and open space; and 11% includes the industrial, commercial, and high density residential land use (NCDEQ, 2015). The Lick Creek and Little Lick Creek watersheds underlying geology is mainly unconsolidated Triassic Basin formed sedimentary rock. The soils contain expansive clays, which swell when wet and shrink when drying, causing low permeability, infiltration rates, and reducing groundwater transmittance (NCDEQ, 2015). Discharges from onsite wastewater systems are suspected to be a major source of nutrient and bacteria pollution in both the Lick Creek and Little Lick Creek watersheds (NC DEQ, 2015).

### C. Fecal Bacteria and Water Quality

Fecal bacteria are a common pollutant of ground and surface water (James & Joyce, 2004; Payment and Locas, 2011; USEPA, 2016). Fecal bacteria in water may include pathogenic and nonpathogenic strains and pathogenic microorganisms can cause potential adverse health effects on the public including gastrointestinal illnesses and sometimes death (James & Joyce, 2004; USEPA, 2016). The USEPA (2016) has reported that fecal indicator bacteria are a leading cause of water quality impairment. Water is tested for the presence and concentrations of fecal indicator bacteria or FIB which include fecal coliforms, *E.coli*, and *enterococci*. FIB are used because monitoring for individual pathogens was recognized as impractical, and an alternate

approach for assessing the microbial safety of water was required (Payment and Locas, 2011). High concentrations of FIB increases the likelihood that pathogens are present (Field & Samadpour, 2007; USGS, 2016). An estimated 5,281 waterbodies in the US are over the allowable limits for FIB concentrations, resulting in swim advisories, closings of recreational waters, or closing of shellfish beds to prevent illness in the population (USEPA, 2000; USEPA, 2002). Total coliforms and *E.coli* are the most commonly used FIB for water quality analyses. *E. coli* is recommended by the USEPA (1986) for assessing fresh water, while enterococci is recommended for fresh or salt water.

Many water resources are monitored for FIB including recreational waters and drinking water. Recreational water quality standards for FIB in salt water are divided into three categories for enterococci: 1) swimming areas that are used daily during the swimming season should not exceed a geometric mean of 33 enterococci per 100 milliliter of water, 2) a single sample of 61 enterococci per 100 milliliter of water for designated beach areas; 3) the moderate full body contact recreation standard should not exceed 89 enterococci per 100 milliliter of water (USEPA 1986). The USEPA recommended recreational water quality standard for *E. coli* in salt water is either a geometric mean of 126 *E. coli* per 100 mL based on several samples during dry weather conditions, or 235 *E. coli* per 100 mL for any single water sample for beach areas or 298/100 mL for moderate full body contact recreation (USEPA, 1986). The fresh water recreational water standard is 576/100 mL for *E. coli* and 151/100 mL for enterococci for a single sample in infrequently used water (USEPA, 1986). In 2012 the USEPA released statistical threshold values and geometric means for FIB in their Recreational Water Quality Criteria publication. The statistical threshold value for *E. coli* is 410 cfu/100 mL and enterococci is 130 cfu/mL. These thresholds should not to be exceeded by more than 10% of the samples collected within a 30 day

period. The geometric mean values should not exceed 126 cfu/100 mL for *E. coli* and 35 cfu/100 mL for enterococci (USEPA, 2012). Drinking water standards have a maximum contaminate level or MCL of less than 1 cfu/mL for *E. coli* (USEPA, 2016).

Research has shown that concentrations of *E. coli* and enterococci in surface waters may be influenced by temperature (Sowah et al., 2014; Meeroff et al., 2008; Plummer and Long, 2007; Young and Thackston, 1999). More specifically, studies showed increased concentrations of *E. coli* and enterococci during the summer months, and a decrease in the number in the winter (Sowah et al., 2014; Meeroff et al., 2008; Plummer and Long, 2007; Young and Thackston, 1999). A few studies reported increases in warmer months were more noticeable for *E. coli* concentrations relative to enterococci (Sowah et al., 2014; Plummer and Long, 2007). Most water-based recreation occurs in the warmer months of spring and summer when FIB concentrations are typically the greatest.

#### D. Bacteria Source Identification

FIB can originate from a multitude of warm blooded animals; and it is important to identify where the bacteria originate to understand how to mitigate address problems with excess FIB concentrations in water resources (USGS, 2016). Sources of FIB include livestock, wildlife, onsite wastewater systems (OWS), municipal wastewater facility discharges, and leaking sewers (Sowah et al., 2014). Microbial source tracking (MST) techniques have become an important tool in identifying sources of FIB in watersheds. For example, numerous studies have used quantitative-PCR or other MST methodology to try to reduce the uncertainty of origin of the FIB (Conn et al., 2012; Field & Samadpour, 2007; Habteselassie et al., 2011). Q-PCR uses analyses of DNA in water samples that may be used to track the origin of the bacteria and determine if the



source was animal or human (Conn et al., 2012). Monitoring of surface waters for FIB concentrations and characterizing the land-use of watersheds to identify potential sources of bacteria in conjunction with MST would allow improved interpretations of causes of water quality impairment.

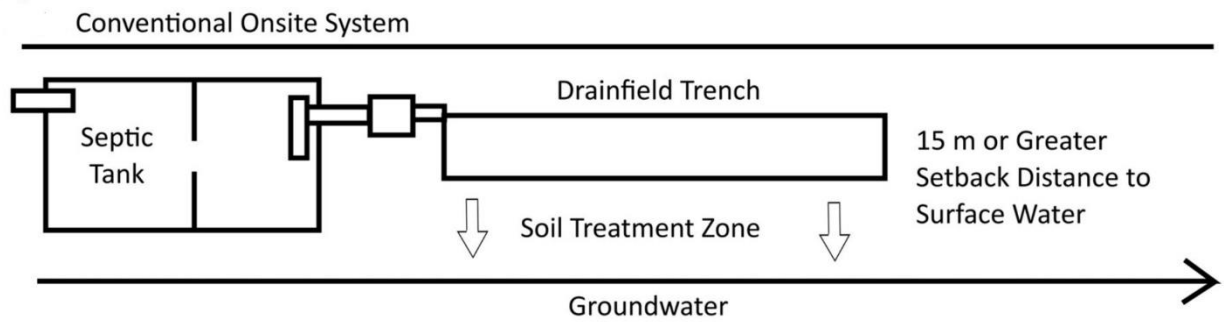
#### E. Wastewater Treatment Technologies and Microbial Water Quality

The method of wastewater treatment used in urbanizing watersheds may also be an influencing factor on water quality for some regions. For example, in a study by Humphrey et al. (2015) in the Coastal Plain of North Carolina, stream *E. coli* concentrations were found to be elevated in an urbanizing watershed served by OWS as compared to an urban watershed served by sewer. Meeroff et al. (2008) reported that in Florida during the seasonal high water table, FIB concentrations were elevated in surface waters of areas adjacent to OWS relative to areas using sewer, possibly because septic systems did not have sufficient separation to groundwater and/or were malfunctioning. In contrast, Young and Thackston (1999) found that the FIB counts were higher in the sewered areas than in the OWS area of the Cumberland River watershed of Tennessee. Trends found showed that fecal coliform including *E. coli* were directly related to many factors such as apparent domestic animal density, housing density, development, the amount of impervious area. The data indicates OWS overflows may be masked due to urban land use causing higher bacterial runoff from impervious surfaces and the high density of dogs (Young and Thackston, 1999). Sowah et al. (2014) showed that FIB counts were not significantly different for the high-density OWS watersheds as compared to low-density OWS watersheds in the piedmont of Georgia. A moderate positive trend was found between *E. coli* concentrations recovered and the percentage of agricultural land-use in low density septic system watersheds.

However, the same study found that there was a negative trend between *E. coli* in watersheds with a high-density of systems and the percentage of agricultural land-use (Sowah et al., 2014). Differences in geology may have contributed to the contrasting results reported in the studies by Humphrey et al. (2015) and Meeroff et al. (2008) in coastal plain areas and Sowah et al. (2014) and Young and Thackston (1999) in piedmont and mountain regions.

## 1. Onsite Wastewater Systems

In the United States approximately 25% of the population is served by OWS and 30% of new construction is reliant upon them (Gelting, 2007, USEPA, 2000, Zarate-Bermudez, 2014). Onsite wastewater systems are used to treat and dispose of wastewater for areas not serviced by centralized municipal wastewater treatment plants. Wastewater contains pollutants such as phosphorus, nitrogen, protozoa, bacteria, and viruses (USEPA, 2002). Onsite systems typically have five main components which include a septic tank, distribution device, drain field trenches, soil, and setbacks (Figure 1). A septic tank is watertight, usually constructed of concrete or fiberglass, where some anaerobic treatment occurs. It allows for separation of liquids, scum, and solids with the use of a baffle wall before exiting the tank. After leaving the tank effluent enters a distribution box which disperses the effluent to the drain field trenches for storage until the effluent enters the soil. The soil is where aerobic treatment occurs through filtration, adsorption, and biological processes. Time is needed for these processes which is why setback distances are used to allow for treatment to continue before reaching surface waters (Sowah et al. 2014, Zarate-Bermudez, 2014, USEPA, 2002).



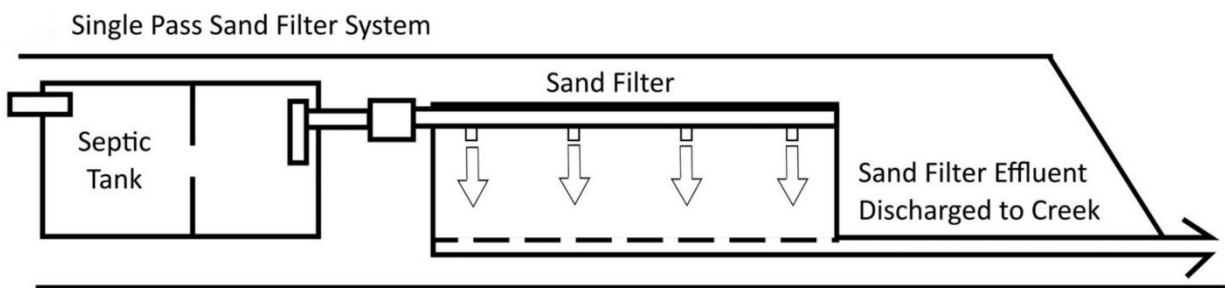
**Figure 1:** Conventional onsite wastewater system include a septic tank, distribution box, drainfield trenches, soil for treatment, and setback distances. (Humphrey et al., 2016).

When onsite systems are correctly designed, installed and maintained they can provide effective treatment of wastewater. This is shown in studies such as Humphrey et al. (2014), where FIB concentrations in septic tank effluent were reduced by more than 99.9% in soil beneath the drainfield trenches. However, if wastewater surfaces in the drainfield due to soil clogging and/or hydraulic overload, then untreated effluent may run off and contaminate nearby surface waters (Harris et al., 2013). Also, if OWS drainfield trenches are installed too close to the water table, then groundwater may become enriched in FIB concentrations (Humphrey et al., 2011; Conn et al., 2012). These hydraulic and treatment malfunctions may contaminate ground water, surface water, and aquatic habitat, potentially contributing to environmental and public health concerns (USEPA, 2015).

## 2. Sand Filter Systems

There are approved wastewater pre-treatment technologies that allow for OWS to be installed in locations where the soil is unsuitable for a conventional-style system. Single-pass sand filters are an example of an OWS that was commonly permitted in the North Carolina Piedmont region where expansive clay soils are prevalent (NC DEQ, 2015). Sand filter OWS include a septic

tank, a sand filter bed, and effluent discharge pipe (Figure 2). The effluent from the septic tank is piped to the sand filter, where it percolates through the sand filter and is then collected by an underdrain. Sand filter effluent is discharged to receiving waters via an outlet pipe (Humphrey et al., 2016). Since these systems discharge directly to surface waters they are considered point sources of pollution, whereas other OWS which discharge effluent to the subsurface are non-point sources of pollution. However, most sand filter beds are not lined, and so these systems may also influence groundwater quality during some periods and thus could also be considered non-point sources.



**Figure 2:** Single pass sand filter systems include a septic tank, sand filter bed, and a discharge pipe (Humphrey et al., 2016).

### 3. Centralized Municipal Sewer Systems

Many areas are served by centralized municipal sewer facilities, or MSW that treat thousand to millions of gallons of wastewater per day from large geographical service areas. MSW use treatment processes that involve several steps. MSW treatment steps include primary stage, secondary stage, and tertiary stage treatment. The primary stage is where solids are allowed to settle and be removed from wastewater through a process of fine screens that remove insoluble material larger than  $\frac{1}{4}$  inch, and grit removal which removes small dense inorganic materials (City of Durham, 2017). The secondary stage uses biological processes to further

purify wastewater (e.g., activated sludge), and the tertiary stage includes the addition of chemicals and disinfection. The NC treatment plant that serves the study area for this project uses a five-stage system to biologically remove nitrogen and phosphorus. These stages include aeration for nitrification and oxidation of organic matter, the addition of a carbon source to facilitate denitrification, chemical polishing by sodium aluminate to precipitate out additional phosphorus, clarifiers where biomass is separated from treated water, and tertiary filters to remove more biomass (City of Durham, 2017). The tertiary stage also includes disinfection via ultraviolet light and then reaeration to add dissolved oxygen prior to discharge of final effluent (City of Durham, 2017). These complex treatment systems are routinely monitored for water quality parameters to insure facility treatment is effective. Municipal treatment facilities are costly to build, enlarge, and maintain which is why many areas still use OWS.

#### F. Land Use Characteristics and Water Quality

Urban growth can have a negative effect on water quality by causing changes to land cover and runoff patterns related to the increase in impervious surface (e.g., parking lots, buildings, and roads) (Lewis et al., 2007). The effects of urban land cover on streams occurring at storm and base flow conditions may include decreased infiltration of water into soil and increased peak discharge into streams (Lewis et al., 2007). Urban areas generally have more impervious surfaces and utilize storm drains to transport pollutants collected from roads, lawns and other surfaces into streams, which is why concentrations of FIB often are higher during storm events than under base flow conditions. Some research has suggested that domestic pet waste may be a significant source of enteric bacteria in urban runoff to streams (Lewis et al., 2007; Mallin et al., 2000; Young & Thackston, 1999). Agricultural land use can also affect

concentrations of FIB in the watershed. In a study by Sinclair et al. (2009) export of *E. coli* from five subwatersheds was greatest for watersheds with high-intensity agricultural land uses relative to other subwatersheds in the area dominated by forestry and low-intensity agricultural use.

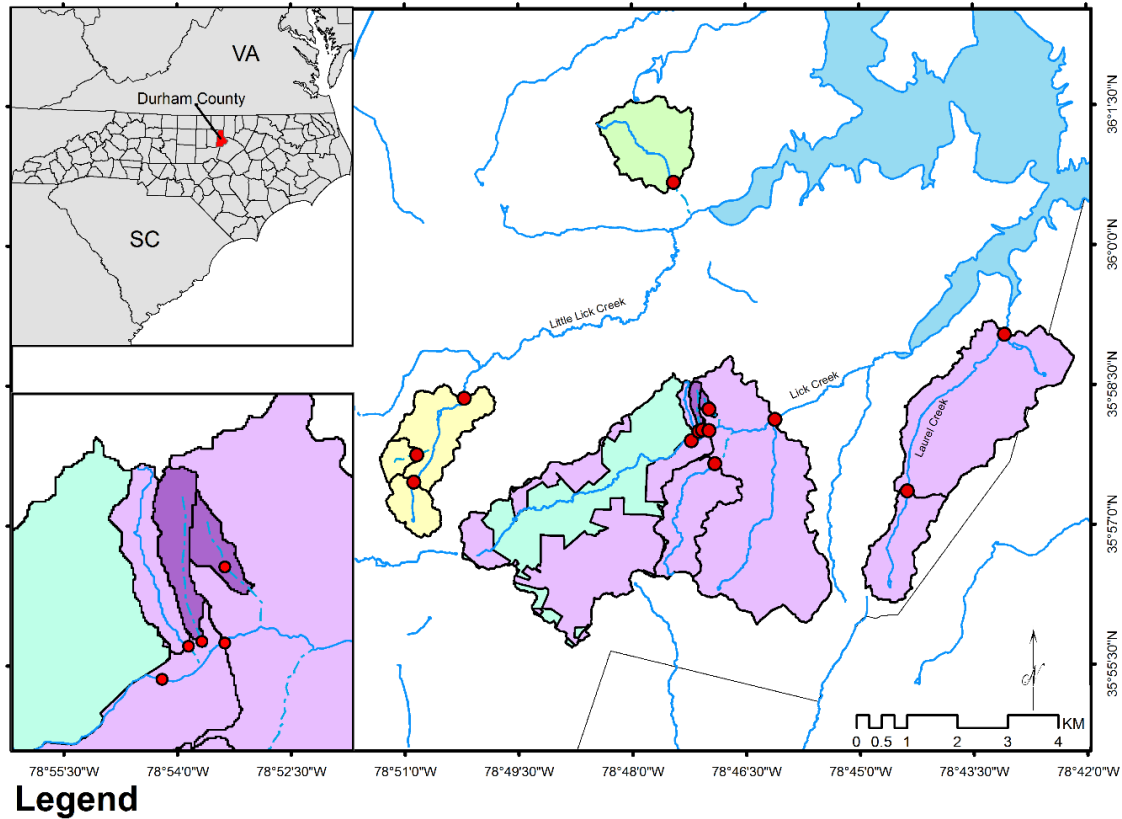
#### G. Research Goal and Objectives

The primary goal of this study was to gain a better understanding of the influence that onsite system density may have on FIB concentrations in piedmont streams. The specific objectives of this project were to 1) determine if there are statistically significant differences in FIB concentrations and exports for watersheds with high in comparison to low densities of OWS, 2) determine if there are differences in FIB concentrations and exports for watersheds served by sewer and OWS in comparison to a mostly natural watershed, 3) determine the frequency that concentrations of FIB in the monitored watersheds exceeded EPA standards and 4) determine if there are differences in FIB concentrations in warmer in comparison to colder months. During the progress of this study a secondary goal was identified: to determine if there are differences in FIB concentrations and exports for watersheds served by different wastewater treatment technologies including sand filter onsite systems, conventional onsite systems, and a centralized sewer. The results and discussion, of the secondary goal will be included in a separate chapter from the first goal.

## **II. Chapter 2: Methods**

### **A. Site Selection and Study Area**

The watersheds selected for this study are located in Wake and Durham Counties in the Piedmont of NC and include Lick Creek, Little Lick Creek, Laurel Creek (considered to be a tributary of Lick Creek), and an un-named tributary of Falls Lake. These watersheds were identified in the Lick Creek and Little Lick Creek watershed restoration plans that were approved by the NC Department of Environmental Quality, or DEQ. The watershed plans suggested that suspicious discharges from failing OWS and sand filters might be contributing to the impairment of Lick Creek and Little Lick Creek (NCDEQ, 2015). Walking surveys were conducted to identify potential sources that could be contributing FIB to the streams. During these surveys malfunctioning OWS in the watersheds were identified. Specific watersheds were selected for monitoring that encompassed different land uses, wastewater treatment technologies, and different densities of wastewater systems. There were 10 sampling locations chosen to address the first goal. These locations included a segment of Lick Creek that was served by MSW “Sewer”, a mostly forested watershed identified as “Natural”, and 8 watersheds served by OWS. For the second goal, monitoring data from three watersheds served by sand filters were compared to three watersheds with similar densities of conventional onsite systems, Sewer, and Natural watersheds.



**Figure 3:** Sampling sites along Lick creek, Little Lick Creek and Laurel Creek, which are all tributaries to Falls Lake. The subwatersheds sampled include 2 high density septic system watersheds (HD1- 2), 6 low density septic system watersheds (LD 1-6), a watershed served by MSW (Sewer), a watershed that is mostly forested with little development (Natural), and three Sand Filter watersheds (SF 1-3).

For the first goal, water quality data were collected approximately monthly for 2 years (January 2015 – December 2016) from 10 sampling locations for a total of 22 sampling events. Sampling locations (watersheds) were grouped based on their densities of conventional-style OWS, and compared to control watersheds that did not receive significant wastewater inputs (Natural and Sewer). Watersheds with more than 0.8 system/ha were considered high-density (HD) and watersheds with less than that threshold were considered low-density (LD). These thresholds were based on work by Sowah et al. (2014). Two sub-watersheds were categorized as



high-density septic (HD 1- HD 2) having a mean density of 1.87 systems/ha; six sub-watersheds were classified as low-density septic (LD 1- LD 6) having a mean density of 0.19 systems/ha.

For the second goal, FIB concentrations and exports for three watersheds served by OWS were compared to three watershed served by a similar density of sand filter OWS, the Sewer, and Natural watersheds. These watersheds were monitored approximately monthly for one year (April 2015- March 2016).

**Table 1:** Watershed characteristics for the High Density (HD), Low Density (LD), Sewered (Sewer), and Natural watersheds.

<i>Watershed</i>	<i>Stream</i>	<i>Impervious Cover</i>	<i>Area</i>	<i># of Septic</i>	<i>Septic System Density</i>
	Gradient	(%)	(ha)	Systems	(systems/ha)
<i>HD1</i>	0.0073	6% <sup>a</sup>	15	28	1.86
<i>HD2</i>	0.0099	6% <sup>a</sup>	9	16	1.88
<i>LD1</i>	0.0045	< 1%	221	20	0.09
<i>LD2</i>	0.002	7%	1179	143	0.12
<i>LD3</i>	0.002	4%	2283	280	0.12
<i>LD4</i>	0.0047	1%	184	48	0.26
<i>LD5</i>	0.0031	1%	835	144	0.17
<i>LD6</i>	0.0078	13% <sup>b</sup>	19	7	0.37
<i>Natural</i>	0.0036	2%	246	-----	-----
<i>Sewer</i>	0.002	7%	1128	-----	-----

## B. Water Quality Monitoring

Physical and chemical stream characteristics including specific conductivity (SC), pH, oxidation-reduction potential (ORP), temperature, and dissolved oxygen (DO) concentrations were measured in the field using an YSI™ 556 MPS (Xylem, Inc., Yellow Springs, Ohio). Turbidity was also recorded for each sampling event using a HACH® turbidimeter (Danaher, Corp., Loveland, Colorado). These were collected to characterize the stream physical and

chemical properties, these properties can be used to assess for water impairment. Stream width, stream velocity, and stream depth were determined using a grade rod, tape measure, and either the float method or Global Water FP101 flow meter (Xylem, Inc., Gold River, California). The mean stream velocities for each watershed for each sampling event were calculated using the flow meter by moving the meter through the water column for the width of the stream. When velocity was too low for the meter detection the float method was used. The float method consists of taking three measurements for the time, seconds, it takes for a floating object to move a certain distance, feet, in the stream. These measurements are averaged in order to determine the mean stream velocity (ft/s) (Michaud & Wierenga, 2005). The stream cross-section area was multiplied by the mean stream velocity, in order to determine stream discharge (ft<sup>3</sup>) during each sampling event. Stormflow samples and environmental readings were collected on two occasions (May 2016 and September 2016). The physical and chemical stream properties were compared to the standards for pH, DO, temperature and turbidity listed in the North Carolina 15A NCAC 02B Surface Water Quality Standards in the North Carolina Department of Natural Resource (NC DNR) “Redbook” (2007).

Concentrations of *E. coli* and enterococci in streams were enumerated using IDEXX<sup>TM</sup> test kits (IDEXX Laboratories, Inc., Westbrook, Maine) for each sampling event. Samples were collected using the dip method, and stored on ice during transport to the ECU Water Lab. Then the samples were tested for *E. coli* and enterococci using Colilert<sup>®</sup> and Enterolert<sup>®</sup>, respectively. The Colilert<sup>®</sup> dehydrated medium contains the nutrient substrate o-nitrophenyl  $\beta$ -D-galactopyranoside, which is metabolized by  $\beta$ -galactosidase, an enzyme of coliforms, and 4-methyl-umbelliferyl  $\beta$ -D-glucuronide, which is metabolized by  $\beta$ -glucuronidase, an enzyme of *E. coli*. The Enterolert<sup>®</sup> dehydrated medium contains the nutrient indicator 4-methyl-umbelliferyl  $\beta$ -

D-glucoside the enterococci utilize their  $\beta$ -glucosidase enzyme to metabolize the indicator causing the sample to fluoresce. Samples were diluted to allow for quantification of *E. coli* and enterococci at concentrations above the maximum un-diluted concentration (2419 MPN/100 mL) of the trays. Dilutions factors from 2 to 10 were made using deionized water to bring sample up to 100 mL then mixed with the Colilert® or Enterolert® dehydrated medium. After the media was completely dissolved, the mixture was poured into a sterile Quanti-Tray®/2000 tray and heat sealed. The Quanti-Tray®/2000 consists of 97 wells, 49 larger wells and 48 smaller wells. Colilert® tray were incubated at 35°C for 24 h (Colilert® test kit), and the Enterolert® trays were incubated at 41°C for 24 h (Enterolert® test kit). The number of fluorescing wells was counted on each tray after exposure to short-wave ultra-violet light. The numbers of large and small wells which fluoresced were recorded and a chart provided by IDEXX with the most probable number (MPN) *E. coli* or enterococci was used to quantify the MPN/100 mL of *E. coli* and enterococci for each sample. The MPN/100 mL data collected was multiplied by the dilution factor to ascertain the actual MPN/100 mL. The FIB concentration were multiplied by stream discharge for each site to determine the watershed export of FIB. The watershed exports (MPN/s) were divided by the drainage area (hectares) for each site to normalize the data to address any potential differences related to contributing watershed size (MPN/s/ha).

Microbial Source Tracking (MST) was used in this project; specifically Real Time Quantitative Polymerase Chain Reaction (RTQ-PCR). For each sampling location, 100 mL of water was collected and filtered. The filter was placed in a buffer solution containing a primer, buffer, and probe. The primer split the DNA to allow the acceptance of the probe, which fluoresces when a specific targeted strand of DNA matched. The buffer was used to stop side reactions. Each sample was mixed with ethanol, centrifuged, and the solution was read to

determine if the sample was positive for human DNA. These analyses provided more information with regard to the potential for human sources of bacteria in surface waters.

Concentrations of FIB can vary greatly over time in relation to temperature, masking seasonal differences in concentrations for watershed comparisons. The data were separated by warm and cold months for each watershed and then compared during these periods to help control for variability related to temperature. The monthly data were separated into these two temperature-based categories (warm and cold) using the State Climate Office of North Carolina (2017) 30-year normal mean monthly temperatures for Durham County, NC. The warm months included June (24.0 °C), July (26.3 °C), August (25.2 °C), and September (21.5 °C); and cold months included December (5.3 °C), January (3.6 °C), February (5.3 °C), and March (9.8 °C). Water temperatures for each stream were measured using YSI meters in the field.

### C. Statistical Analyses

Water quality data from watersheds with a high-density of OWS were compared to watersheds with a low-density of OWS and control watersheds with no or very minimal wastewater inputs (Sewer and Natural). Two watersheds were considered to have a high-density of systems (1.86 – 1.88 systems/ ha) and six had a low-density systems (0.09 – 0.37 systems/ha) (Table 1). Mann-Whitney non-parametric tests were used to determine if differences in FIB concentrations and watershed exports of FIB between comparison groups were statistically significant ( $p < 0.05$ ). Environmental parameters such as SC, pH, temperature, DO, and turbidity were summarized for each watershed. Spearman's Rank Correlation analyses were conducted to determine if statistically significant correlations ( $p < 0.05$ ) were observed between FIB concentrations and temperature, turbidity, DO and other physical/chemical characteristics of the

stream water. Boxplots with the monthly FIB concentrations and watershed exports for each site were created to help identify spatial and temporal patterns with regards to FIB concentrations and watershed exports. The non-parametric tests, Mann-Whitney and Spearman's Rank Correlation, and boxplots were created using Minitab version 17.

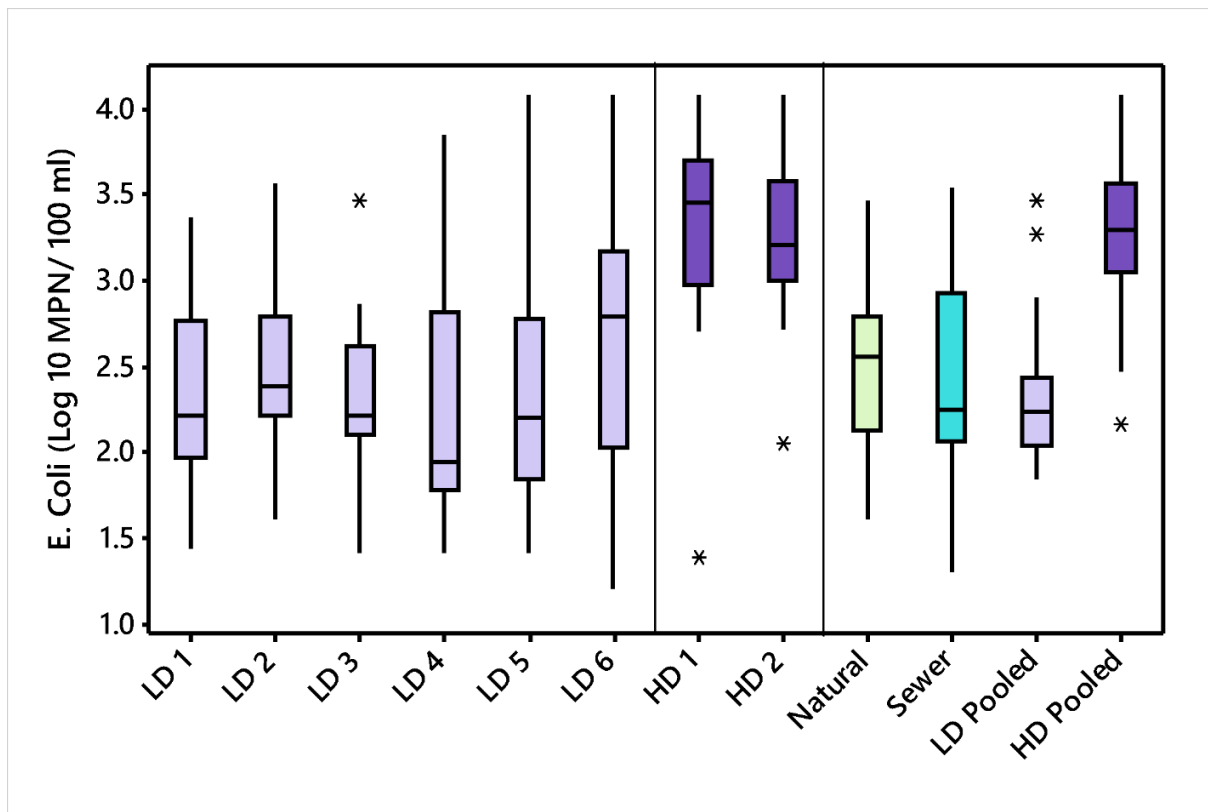
The FIB concentrations observed for each watershed were compared to USEPA standards for *E. coli* and enterococci and the frequency of exceedance of the standards was reported for each watershed. Concentrations and exports of FIB during warm and cold periods were compared to determine statistically significant differences based on temperature.

The FIB concentrations for three watersheds served by conventional OWS, three watersheds served by sand filter OWS, a Sewer watershed and a Natural watershed were compared for the period (April 2015- March 2016). These comparisons were made to determine if statistically significant differences ( $p < 0.05$ ) were observed based on wastewater treatment technologies (or lack thereof).

### **III. Chapter 3: Results and Discussion**

#### **A. Septic Density and Fecal Indicator Bacteria**

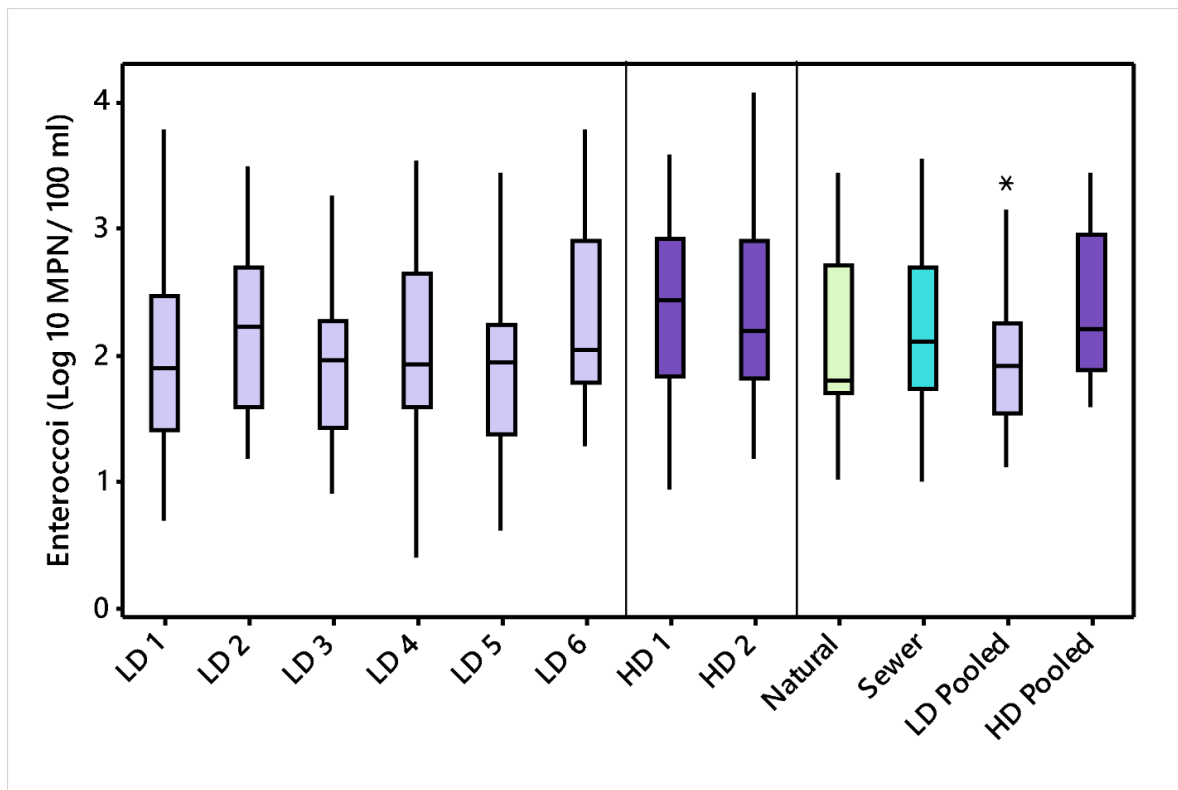
Pooling the watershed data by comparison groups, the median concentration of *E. coli* for the watersheds with a high-density (HD) of systems (2315 MPN/100 mL) was elevated relative to watersheds with a low-density (LD) of systems (183 MPN/100 mL) and the control watersheds (Natural-355 MPN/100 mL and Sewer-177 MPN/100 mL) (Figure 4, Table 2). Watersheds with a high-density of septic systems had concentrations of *E. coli* that were significantly higher than watersheds with a low-density of systems ( $p = 0.0004$ ), and the Natural and Sewer watersheds. Comparisons among individual watersheds show that the watersheds with a high-density of systems (HD 1, HD 2, HD Pool) had *E. coli* concentrations that were significantly greater relative to all watersheds with a low-density of systems (LD 1 – LD6), the Natural, and the Sewer watersheds ( $p \leq 0.007$ ). Differences in concentrations of *E. coli* between the watersheds with a low density systems and control groups were not statistically significant ( $p = 0.28$  and  $p = 0.92$ , respectively).



**Figure 4:** Concentrations of *E. coli* for watersheds, the high-density watersheds (HD 1 and HD 2), the low-density watersheds (LD1-LD 6), the Natural watershed, the Sewer watershed and pooled watersheds.

Concentrations of enterococci for watersheds with high-density of systems including HD 1, HD 2 and HD Pool (medians 289, 152, and 279 MPN/100 mL, respectively) were significantly higher in comparison to LD 5 (median 76 MPN/100 mL,  $p = 0.048$ ,  $p = 0.046$ ,  $p = 0.022$ , respectively), a watershed with a low-density of systems. The pooled data for the watersheds with a high-density of systems (HD pooled 279 MPN/100 mL) was significantly higher in comparison to concentrations from several watersheds with a low-density of systems including LD 1, LD 3, and LD 4 (Medians at 79,  $p = 0.025$ , 91,  $p = 0.022$ , and 86 MPN/100 mL,  $p = 0.047$ , respectively). Median enterococci concentrations for the control watersheds (Natural [62 MPN/100 mL] and Sewer [130 MPN/100 mL]) were lower than medians for all the watersheds with high densities of systems, HD 1 (median 289 MPN/100 mL), HD 2 (152 MPN/100 mL), HD

Pool (279 MPN/100 mL), but differences in concentrations were not statistically significant ( $p > 0.05$ ). While many of the watershed comparisons for enterococci concentrations were not statistically significant, the trends with regards to watersheds with the highest median concentrations were similar for *E. coli* and enterococci. More specifically, the watersheds with a high-density of systems (HD), had median enterococci concentrations that were elevated in comparison to the watersheds with a low-density, of systems (LD) and the Sewer and the Natural watersheds.



**Figure 5:** Concentrations of enterococci for watersheds, the high-density watersheds (HD 1 and HD 2), the low-density watersheds (LD1-LD 6), the Natural watershed, the Sewer watershed and pooled watersheds.

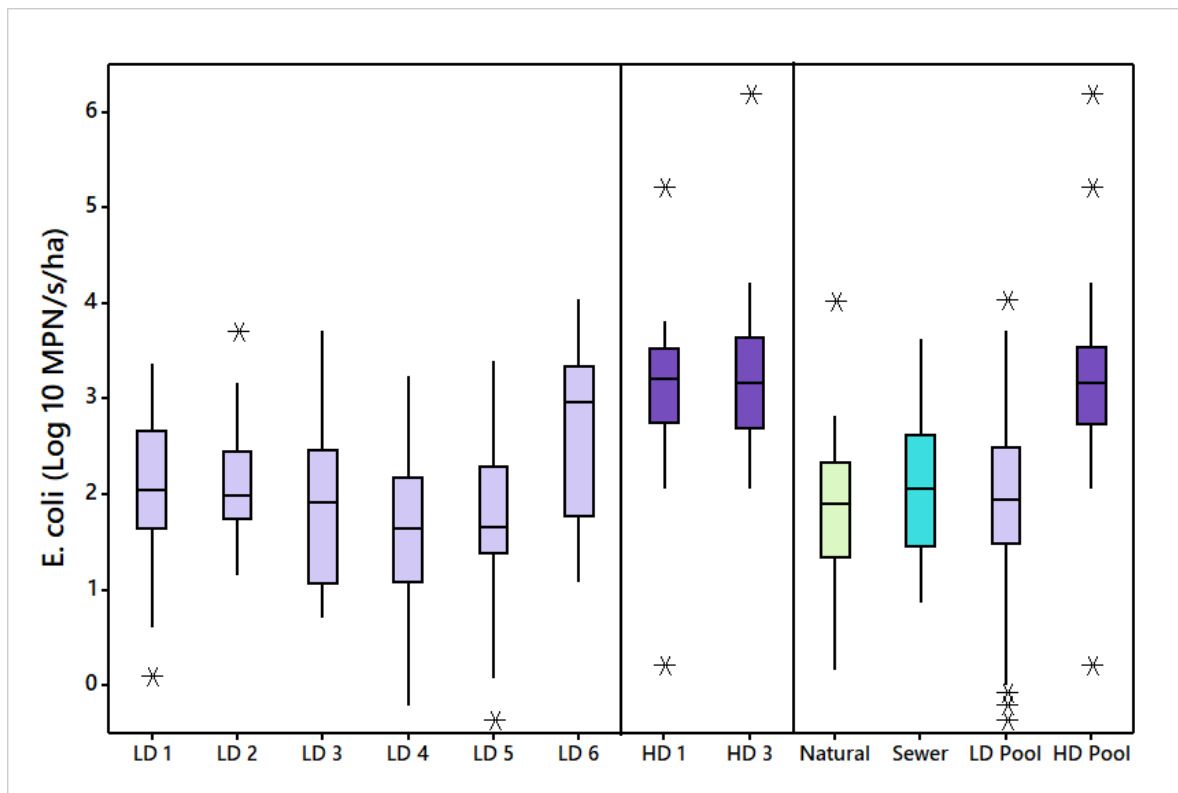
Spearman's Rank Correlations were performed on all *E. coli* and all enterococci data to determine if changes in the concentrations of FIB over the study period were similar. Significant positive correlations were observed between *E. coli* and enterococci concentrations ( $r_s = 0.593$ ,  $p$



= 0.010). This indicates that when concentrations of *E.coli* increased or decreased, there were similar increases or decreases in enterococci concentrations. Statistically significant correlations were also found between concentrations of *E. coli* and enterococci for watersheds with a high-density of systems watersheds (HD Pool:  $r_s = 0.392$ ,  $p = 0.01$ ). and for watersheds with a low-density of OWS (LD Pool:  $r_s = 0.658$ ,  $p = 0.001$ ). These correlations suggest that changes in the concentrations of *E.coli* and enterococci are related and influenced by similar factors.

Exports of *E. coli* and enterococci were also calculated for each watershed. Exports are based on FIB concentration and stream discharge from the 22 sampling events. The watersheds with the highest median exports of *E.coli* were the watersheds with the high-density of systems (HD 1 median: 1081 MPN/s/ha and HD 2 median: 1219 MPN/s/ha). Watersheds with a low-density of systems had median exports ranging from 157 MPN/s/ha at watershed LD 6, to 39 MPN/s/ha at LD 4. The Natural and the Sewer watersheds had exports similar to the LD watersheds with medians of 58 MPN/s/ha and 113 MPN/s/ha, respectively. The pooled watershed export of *E. coli* for the HD watersheds was 1116 MPN/s/ha, which were elevated relative to the pooled export of *E. coli* for the LD watersheds (84 MPN/s/ha). Watersheds with high-densities of systems had the highest median exports of *E. coli* in comparison to watersheds with low-densities of systems, the Natural and the Sewer watershed.

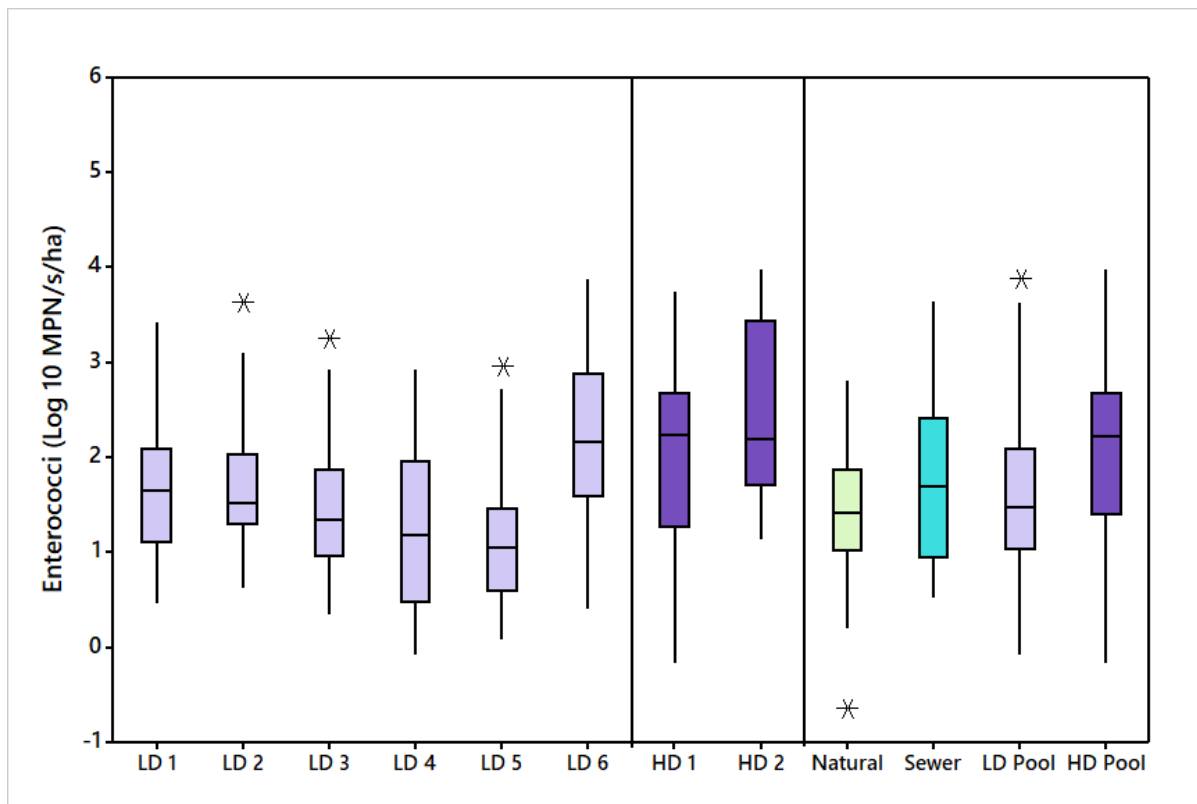
The watersheds with a high-density of systems HD 1, HD 2, and HD Pool had exports of *E. coli* that were significantly greater in comparison to most of the LD watersheds including LD 1, LD 2, LD 3, LD 4, LD5, and LD Pooled, the Natural and the Sewer watersheds at  $p \leq 0.0007$ . This suggests that a watersheds with a high-density of systems may export more *E.coli* in comparison to watersheds with low-density, natural and sewer watersheds.



**Figure 6:** Watershed exports of *E. coli* for the high-density onsite wastewater system watersheds in comparison to the low-density onsite wastewater system watersheds, the Natural and Sewer watershed and, pooled watersheds.

The watershed with the highest median export of enterococci was HD 1 (135 MPN/s/ha). The other high-density watershed (HD 2) had a median export of 53 MPN/s/ha, which was the third highest. The watersheds with a low-density of systems had median exports of enterococci ranging from 96 to 11 MPN/s/ha; (45 MPN/s/ha at LD 1, 33 MPN/s/ha at LD 2, 22 MPN/s/ha LD 3, 16 MPN/s/ha at LD 4, 11 MPN/s/ha at LD 5, and 96 MPN/s/ha at LD 6). The Natural and the Sewer watersheds had median exports at 25 and 50 MPN/s/ha, respectively. Pooling the data by density of OWS, the median enterococci exports for the HD watersheds (101 MPN/s/ha) were elevated relative to each low density watershed (LD1-6), LD Pool (24 MPN/s/ha), Natural (25 MPN/s/ha), and Sewer (50 MPN/s/ha). Therefore, similar trends were found with regards to the influence of OWS density on the watershed exports of enterococci and *E. coli*.

Differences in watershed exports of enterococci were statistically significant when comparing HD 1 to LD 4 ( $p = 0.018$ ), LD 5 ( $p = 0.015$ ) and the Natural watershed ( $p = 0.033$ ). Watershed exports of enterococci were significantly greater for HD 2 in comparison to most of the the low-density watersheds (LD 1, LD 2, LD 3, LD 4, LD 5), the Natural watershed, and the pooled low-density watershed LD Pool ( $p \leq 0.044$ ). Watershed exports of enterococci for HD Pool was significantly greater compared to the low-density watersheds, LD 3, LD 4, LD 5, the Natural watershed, and LD Pool. The watershed exports for both of the FIB tend to be higher in watersheds that have a high-densities of OWS in comparison to watershed exports with low-density of OWS.



**Figure 7:** Watershed exports of enterococci for the high-density onsite wastewater system watersheds in comparison to the low-density onsite wastewater system watersheds, the Natural and Sewer watershed, and pooled watersheds.

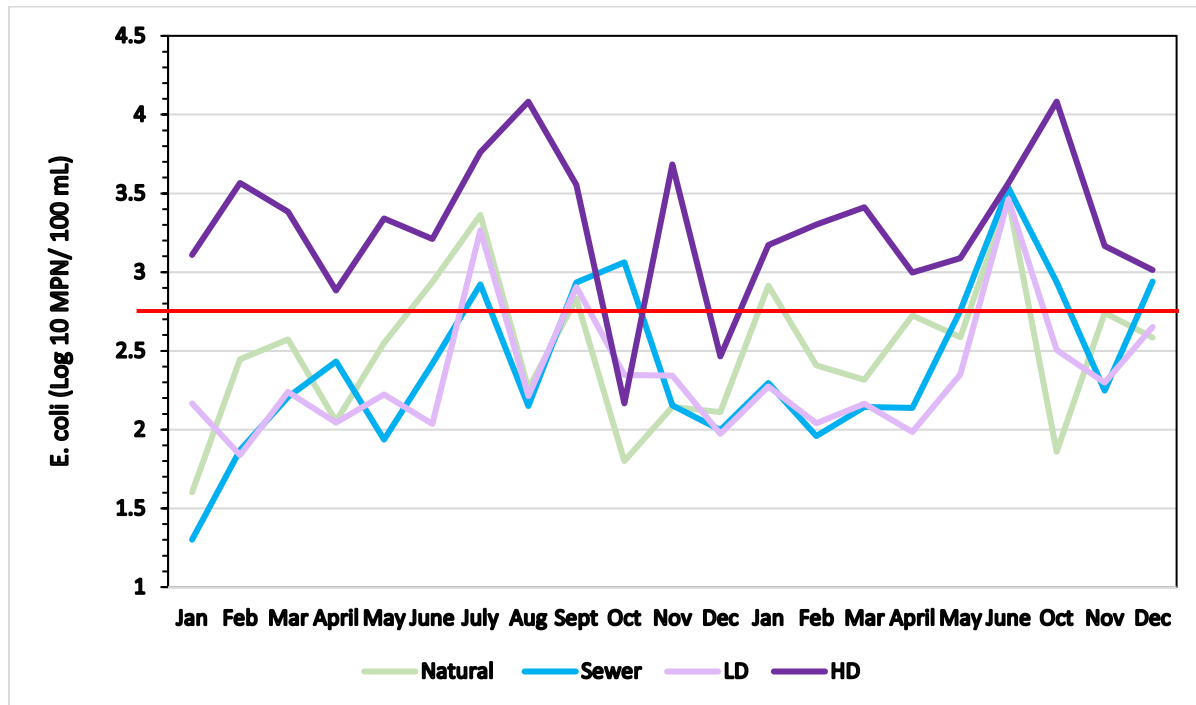
Prior paired-watershed studies by Iverson et al. (2017) and Sowah et al. (2014) evaluated *E. coli* exports in septic watersheds with similar densities to watersheds tested in this study. Sowah et al. (2014) found that the watersheds with a high density of systems (2.2 systems/ha) had exports at 191 MPN/s/ha (range of 1.3 - 1643 MPN/s/ha). Iverson et al. (2017) reported higher *E.coli* exports for its higher density watershed area, 1.3-2.0 systems/ha, at a mean of 690 MPN/s/ha with a range of 64 - 15,995 MPN/s/ha. In this study, the high-density watersheds of systems that ranged from 1.86 – 1.88 systems/ ha had a mean export of 1526 MPN/s/ha with a range of 0 - 1,525,363 MPN/s/ha. There is overlap with the studies in that the data in this study has more range and higher means than the Iverson et al., 2017, and the Sowah et al., 2014 studies showed. The study conducted by Iverson et al. (2017) was set in the coastal plain of North Carolina while Sowah et al. (2014) was set in the piedmont of Georgia. Differences in soils, geology, and system types may have contributed to differences in FIB concentrations reported in these studies. Another possible reason for the difference in exports could be associated with the number of failing systems and gray water pipes that were discharging wastewater to the creeks in the HD watersheds of this study. Wastewater from a malfunctioning septic system and from two gray water pipes were sampled and analyzed for *E.coli* concentrations. The surfacing effluent from a malfunctioning OWS was sampled just prior to the effluent reaching a creek. The effluent near the creek edge had an *E. coli* concentration of 290,900 MPN/100 mL. The discharge from the two gray water pipes had *E. coli* concentrations of 4082 and 1538 MPN/100mL, respectively. The malfunctioning OWS and grey water pipes were contributing to elevated concentrations of *E. coli* to the creeks and to the elevated watershed exports of FIB. Prior work has shown that failing OWS systems and gray water pipes may cause elevated FIB concentrations in surface water (Ahmed et al., 2006,

Harris et al., 2013). Studies by Conn et al., (2012), Habteselassie et al., (2011), and Harris et al., (2013) reported higher concentrations of FIB in groundwater and/or surface waters down-gradient from malfunctioning OWS. This study confirmed that high density septic areas are capable of significant stream water quality impacts.

## B. Fecal Indicator Bacteria Concentrations and Water Quality Standards

Concentrations of *E. coli* and enterococci for each watershed were compared to USEPA standards to determine which watersheds posed the greatest threat to environmental and public health. The watersheds evaluated in this study are not used for full body contact recreation, but all are tributaries to Falls Lake, a recreational water body. Therefore, the single sample standard for infrequently used fresh water of 575/100 mL for *E.coli* and 151/100 mL for enterococci (USEPA, 1986) and the more stringent designated beach area standards of 236/100 mL for *E.coli* and 61/100 mL for enterococci were both used as a comparison metric. The threshold values of 410/100 mL for *E.coli* and 130/100 mL of enterococci were also used as a comparison metric, the samples should not exceed these values more than 10% of the time (USEPA, 2012). Figure 8 shows the pooled *E.coli* concentration data for the high-density and low-density watersheds, along with the control Natural and Sewer watersheds. The highest frequency of *E. coli* concentration standard violations for the 575/100 mL was observed in the HD Pooled watersheds (90%), or 19 of 21 of the times sampled. Other sites also had months that exceed the standard, however with less frequency than the HD sites. The LD Pooled concentrations were above the standard during 3 of 21, or 14% of the times sampled, and the Sewer for 6 of 21 (29%), and Natural during 5 of 21 (24%). The HD watersheds had concentrations of *E. coli* that were the highest for 20 of 21 (95%) sampling events and the Sewer location was highest for 1 out of 21

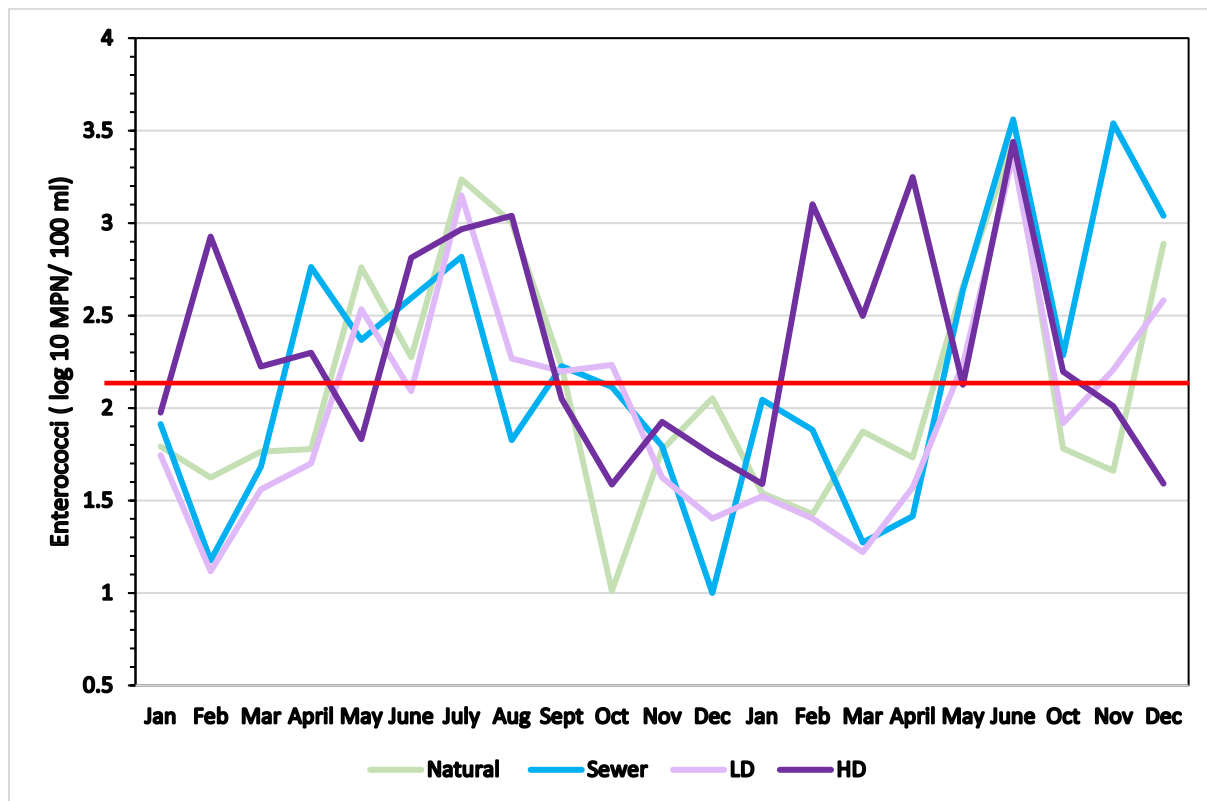
(5%) sampling events. The high density watersheds consistently had the most violations in comparison to all others (Table 2).



**Figure 8:** Pooled watershed data for *E. coli* comparisons of HD, LD, Natural, and Sewer watersheds for USEPA standard of 575 *E. coli* /100 mL, indicated by the red line.

Evaluating individual watersheds revealed that the most exceedances of *E. coli* standards occurred at the two high-density watersheds. The HD watersheds exceeded the 236/100 mL standard 94% and 95 % of the events sampled; for the 575/100 mL standard, USEPA violations occurred on 83% and 90 % of the events sampled (Table 2). For comparison the highest number of exceedances for the low density watershed occurred at LD 6 (67% of the sampling event for 236/100 mL or 52 % of the sampling events for 575/100 mL).

Concentrations of enterococci for the HD Pooled watersheds exceeded the standard of 151/100 mL for the infrequently used water during 62% of sampling events, followed by Sewer (48%), LD (43%), and Natural (38%) (Figure 9). The HD watersheds had the greatest median enterococci concentrations for 9 of the sampling events, followed by the sewer (6 events), Natural (4 events), and LD at 1 event. The Natural and Sewer sites were tied in September for the highest concentration of enterococci.



**Figure 9:** Pooled watershed data for enterococci comparisons of HD, LD, Natural, and Sewer watersheds for the USEPA standard of 151 enterococci/100 mL or 2.17 log transformed, indicated by the red line.

The Enterococci concentrations showed the same trend as *E.coli* with the highest occurrence of exceedance of EPA standards occurring at the HD OWS watersheds. The HD watersheds had violations occurring between 78-80% of the sampling events for 61/100 mL and

between 50-56% of the sampling events for 151/100 mL (Table 2). The Natural watershed exceeded the 61/100 mL 52% of the sampling events, and exceeded the 151/100 mL 38% of the sampling events. The Sewer watershed exceeded the 61/100 mL 76% , and 151/100 mL 48% of the sampling events. The pooled HD watersheds showed exceedences for the 61/100 mL 95% versus the 62% of the sampling events for the pooled LD watershed. The frequency for the number of violations that occurred during the sampling events is highest for the high density watershed indicating these watersheds may be contributing more FIB concentrations than other watersheds sampled.

**Table 2:** Summary of *E. coli* and enterococci for median values, ranges and frequencies at which the sampled watershed exceeded the USEPA standards for infrequently used waters and designated beach areas.

	<i>E. COLI</i>				<i>ENTEROCOCCI</i>			
<i>WATERSHED</i>	Median	Range	Frequency > 236	Frequency > 575	Median	Range	Frequency > 61	Frequency > 151
<b>LD 1</b>	162.5	27 - 2306	0.43	0.24	79.0	5 - 6050	0.57	0.29
<b>LD 2</b>	240.0	40 - 3635	0.52	0.24	165.0	15 - 3066	0.67	0.52
<b>LD 3</b>	161.0	26 - 2897	0.29	0.14	91.4	10 - 1827	0.62	0.29
<b>LD 4</b>	71.0	0 - 7068	0.38	0.24	85.5	3 - 3434	0.62	0.33
<b>LD 5</b>	153.3	0 - 12098	0.38	0.24	76.3	0 - 2747	0.57	0.29
<b>LD 6</b>	620.3	16 - 12100	0.67	0.52	110.3	19 - 6017	0.71	0.43
<b>HD 1</b>	2813.4	24 - 12100	0.94	0.83	288.8	9 - 3883	0.78	0.56
<b>HD 2</b>	1622.0	114 - 12100	0.95	0.90	152.3	15 - 12100	0.80	0.50
<b>NATURAL</b>	355.3	40 - 2897	0.62	0.24	62.0	10 - 2738	0.52	0.38
<b>SEWER</b>	177.2	20 - 3434	0.43	0.29	130.0	10 - 3635	0.76	0.48
<b>LD POOLED</b>	183.4	40 - 3071	0.33	0.14	82.3	24-2906	0.62	0.38
<b>HD POOLED</b>	2315.1	505 - 12100	1.00	0.95	279.0	49-2799	0.95	0.76



One sample sign analyses were run to determine if concentrations of *E. coli* and enterococci were significantly greater than the USEPA standards. For *E. coli*, each watershed was compared to the USEPA standard of 575/100 mL. The watersheds with the high densities of systems (HD 1 and HD 2) had concentrations of *E. coli* that were significantly higher in relation to the standard at  $p = 0.008$ , and  $p = 0.001$ , respectively. The concentrations of *E. coli* for HD Pool was also significantly greater ( $p = 0.001$ ) relative to the standard. No other watersheds had concentrations that were significantly higher than the USEPA standard for *E. coli*. Concentrations of enterococci for each watershed were also analyzed using one sample sign and the USEPA standard of 151/100 mL. The only watershed with concentrations of enterococci that were significantly higher than the standard was the high-density watershed HD 2 at  $p = 0.001$ .

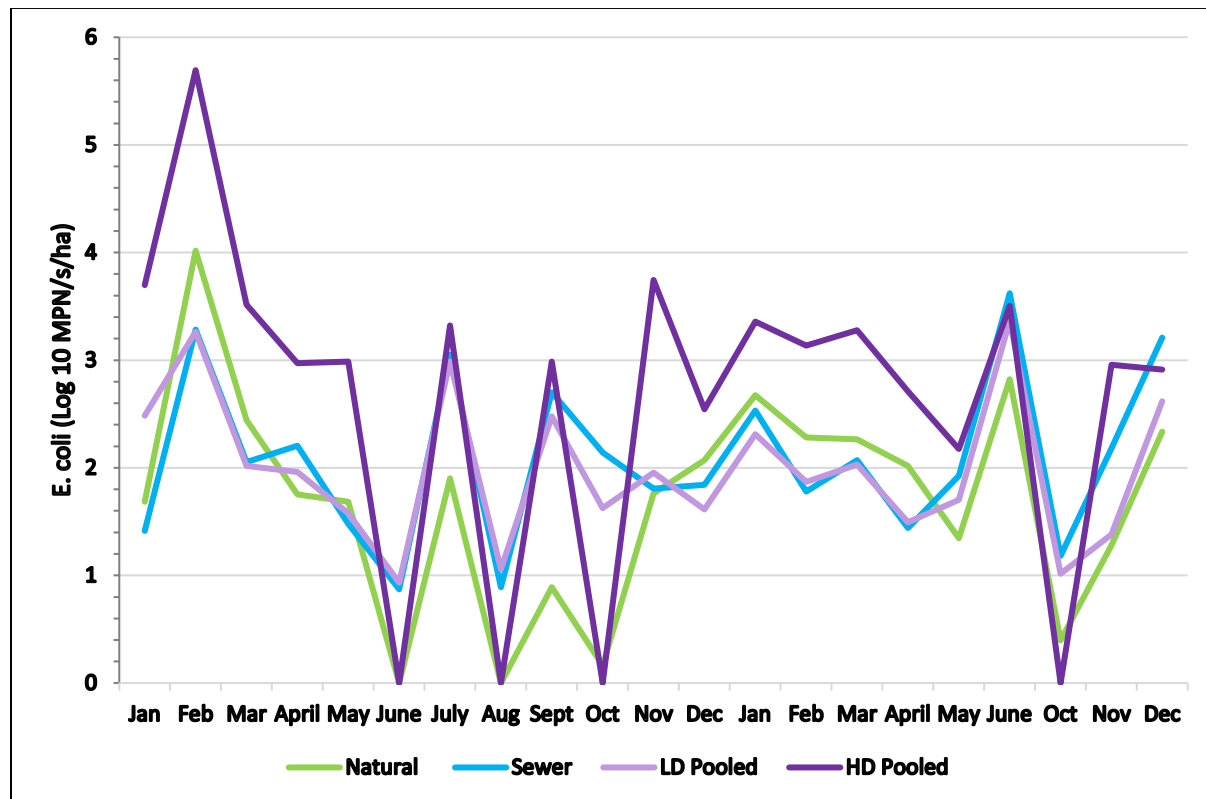
#### C. Variability in Concentrations and Watershed Exports of Fecal Indicator Bacteria

There were several orders of magnitude difference in concentrations of FIB for each watershed throughout the sampling period (Figures 8 and 9). Concentrations of FIB differed with season. The warm weather months tended to have higher concentrations of FIB in comparison to the cooler months. Prior research has also shown these trends (Hathaway et al., 2010; Jokinen et al., 2012). Differences in FIB concentrations may be because bacteria can grow more readily in the warmer weather than in the cooler months, and wildlife activity is higher during warmer periods. Warmer months are when most people will potentially come into contact with surface waters, thus public health may be threatened due to the increased likelihood of full body with recreational waters during periods when FIB concentrations are greatest.

Watershed exports of FIB were dynamic during the study (Figures 10 and 11). There were several sampling events where the watershed export was zero, and during these months

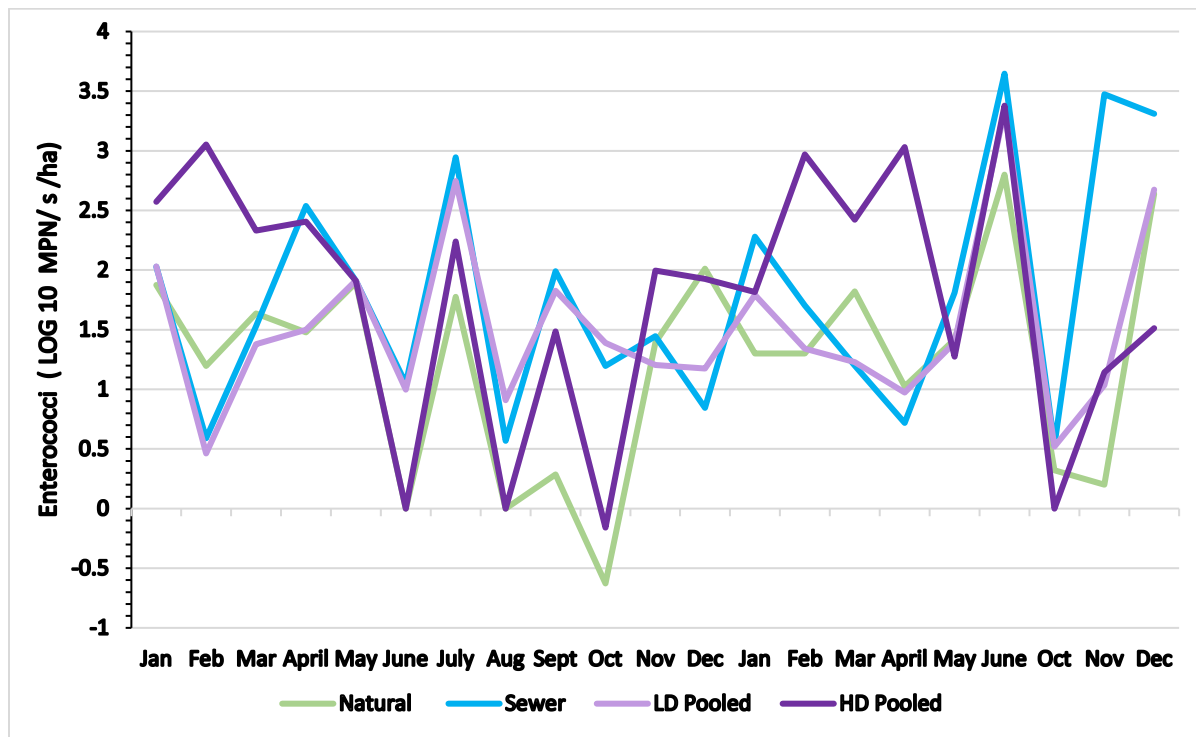
there was little to no flow. Evaporation and transpiration are greatest in warmer months, so stream flow slows or stops during extended dry periods. During and after rain events in warmer months are the periods when high concentrations of FIB can be exported to Falls Lake from the watersheds.

Watershed exports of *E. coli* varied greatly during the study period for each watershed. However, the HD Pool watershed had the highest exports on 15 of the 21 (71%) sampling events. The next highest exports occurred at the Sewer watershed 4 of the 21 (19%) sampling events, the pooled low density watersheds were the highest 2 times (10%), and the Natural watershed never had the highest exports during the months sampled. The data show that the high density OWS watersheds were exporting the most FIB.



**Figure 10:** Exports of *E. coli* for the pooled watersheds for HD, LD, Natural, and Sewer watersheds.

The Sewer watershed had the highest exports of enterococci for 10 of the 21 events (48%). The second highest counts occurred at the HD pooled watershed (7 of 21 or 33% sampling events), the LD pooled watershed was highest 3 events (14%) and the Natural watershed was the highest once (5%).



**Figure 11:** Exports of Enterococci for the pooled watersheds for HD, LD, Natural, and Sewer watersheds.

#### D. Fecal Indicator Bacteria and Stream Property Correlations

Spearman's Rank Correlation analyses were performed to determine if there were significant associations between FIB concentrations and physical and chemical water quality parameters. Physical and chemical characteristics of the water for each watershed are shown in Table 3. Concentrations of *E.coli* and DO and pH showed no significant correlations. Temperature showed a positive moderate correlation with concentrations of *E. coli* ( $r_s = 0.581$  p

= 0.006 at LD 4). There was an inverse association between SC at LD 6 and *E. coli* concentrations ( $r_s = -0.577$  at  $p = 0.006$ ). Turbidity had positive moderate and strong associations with concentrations of *E. coli* for LD 1, LD 6, and LD Pool ( $r_s = 0.640$  at  $p = 0.003$ ,  $r_s = 0.510$  at  $p = 0.026$ , and  $r_s = 0.583$  at  $p = 0.007$ , respectively). The ORP had a positive moderate association with *E. coli* concentrations at LD 3 ( $r_s = 0.435$   $p = 0.05$ ).

Spearman's Rank Correlations were also performed using enterococci concentrations with SC and pH, and correlations were not statistically significant. DO concentrations showed a negative moderate association with LD 1 ( $r_s = -0.522$   $p = 0.015$ ), LD 2 ( $r_s = -0.451$   $p = 0.040$ ), LD 3 ( $r_s = -0.553$   $p = 0.009$ ), LD 4 ( $r_s = -0.516$   $p = 0.017$ ), Natural ( $r_s = -0.426$   $p = 0.054$ ), and LD Pool ( $r_s = -0.374$   $p = 0.001$ ). Temperature and enterococci concentrations showed positive moderate and strong associations at LD 1 ( $r_s = 0.625$   $p = 0.002$ ), LD 2 ( $r_s = 0.458$   $p = 0.037$ ), LD 3 ( $r_s = 0.544$   $p = 0.011$ ), LD 4 ( $r_s = 0.678$   $p = 0.001$ ), LD 5 ( $r_s = 0.568$   $p = 0.009$ ), Natural ( $r_s = 0.688$   $p = 0.001$ ), and LD Pool ( $r_s = 0.542$   $p = 0.011$ ). Turbidity showed positive strong correlations at LD 1 ( $r_s = 0.763$   $p = 0.001$ ) and LD 2 ( $r_s = 0.686$   $p = 0.002$ ); positive moderate associations at LD 5 ( $r_s = 0.580$   $p = 0.012$ ), and Natural ( $r_s = 0.586$   $p = 0.008$ ); and positive weak associations at LD Pool ( $r_s = 0.305$   $p = 0.070$ ). ORP showed positive strong associations at LD 4 ( $r_s = 0.63$   $p = 0.003$ ); positive moderate associations at LD 2 ( $r_s = 0.465$   $p = 0.039$ ), Natural ( $r_s = 0.499$   $p = 0.025$ ), Sewer ( $r_s = 0.441$   $p = 0.050$ ); positive weak associations at LD Pool ( $r_s = 0.314$   $p = 0.001$ ), and negative moderate and weak associations at LD 1 ( $r_s = -0.475$   $p = 0.05$ ), LD Pool ( $r_s = -0.319$   $p = 0.050$ ).

FIB concentrations were correlated with temperature and/or turbidity for many of the watersheds. These correlations (FIB concentrations with temperature and turbidity) have been shown in other studies (Hathaway et al., 2010, Jokinen et al., 2012). Temperature and FIB

concentrations were significantly directly correlated. High turbidity has been connected with improve survivability of FIB in streams (Pachepsky, 2011). FIB may attach to sediment that settles to the bottom and/or sides of a stream. When rain events increase stream flow, the sediment and attached FIB are resuspended thus increasing concentrations in the water. Concentrations of FIB also increase as runoff flushes waste material into drainageways.

**Table 3:** Medians (standard deviations) for sampled parameters for each watershed including DO= Dissolved Oxygen, Temp.= temperature, SC = Specific Conductance.

<i>Watershed</i>	<i>pH</i>	<i>DO</i> ( <i>mg L<sup>-1</sup></i> )	<i>Turbidity</i> ( <i>NTU</i> )	<i>Temp.</i> ( <i>°C</i> )	<i>SC</i> ( <i>μS cm<sup>-1</sup></i> )
<i>HD1</i>	7.2 (0.6)	8.2 (3.4)	13.2 (21.1)	11.3 (6.2)	292 (117.2)
<i>HD2</i>	7.0 (0.5)	7.5 (3.4)	40.9 (65.6)	12.8 (6.4)	232 (261.7)
<i>LD1</i>	7.2 (0.5)	6.6 (3.3)	214 (270.6)	11.4 (7.4)	106 (48.1)
<i>LD2</i>	7.3 (0.5)	9.5 (2.8)	32.3 (38.8)	12.7 (7.3)	198 (94.4)
<i>LD3</i>	7.3 (0.5)	8.9 (3.0)	41.3 (48.9)	12.7 (7.6)	156 (56.5)
<i>LD4</i>	7.4 (0.5)	8.8 (3.1)	15.8 (21.3)	11.9 (6.9)	106 (212.3)
<i>LD5</i>	7.5 (0.5)	10.4 (2.9)	13.5 (15.3)	12.7 (7.2)	102 (35.1)
<i>LD6</i>	7.3 (0.6)	6.5 (4.0)	13.7 (14.7)	11.8 (6.8)	403 (136.5)
<i>HD Pooled</i>	7.1 (0.5)	7.6 (3.6)	30.0 (38.1)	12.3 (6.4)	267 (144.7)
<i>LD Pooled</i>	7.4 (0.5)	8.4 (3.0)	27.0 (28.3)	12.3 (7.2)	159 (58.8)
<i>Natural</i>	7.2 (0.5)	7.9 (3.5)	16.0 (15.4)	13.8 (7.0)	108 (33.3)
<i>Sewer</i>	7.5 (0.5)	9.2 (3.7)	31.7 (35.5)	11.7 (7.8)	207 (81.8)

The USEPA National Recommended Water Quality Criteria (NRWQC) classifies waters exceeding a pH of 9 or a pH of below 5 as impaired waters. During this study there were no observed violations in this water quality criteria (Table 4). The NCDNR (2007) threshold for turbidity in freshwater is 50 NTU, and turbidity was recorded above the threshold at a variety of

the times sampled for each watershed (5% to 100% of the times sampled)(Table 4). LD 1 experienced the highest stream turbidity, with a median of 214 NTU and standard deviation of 271 NTU, with violations occurring during every sampling event (Table 4). Turbidity is a concern because suspended sediment provides protection for pathogens, increasing their life span in the water, and elevated turbidity can also be detrimental for aquatic habitat (USGS, 2016). DO concentrations for freshwaters should not fall below 4.0 mg/L (NC DENR, 2007). The DO concentrations for the watersheds in this study were below the standard between 5% and 24% of the times sampled. Low DO in waters could indicate nutrient or organic matter enrichment and associated algal blooms (some potentially toxic) are present, which may cause water use impairment and/or threaten public health either through dermal contact or through ingestion of toxins (USEPA, 2015).

**Table 4:** Frequency of USEPA and NCDENR violations for pH, temperature, Dissolved Oxygen = DO, and turbidity standards for the studied watersheds.

<i>Watershed</i>	<i>pH</i> <i>6.0-9.0</i>	<i>DO</i> <i>&lt;4.0 mg/L</i>	<i>Turbidity</i> <i>≥ 50 NTU</i>	<i>Temperature</i> <i>&gt;32°C</i>
<i>LD 1</i>	0.0	0.19	1.00	0.0
<i>LD 2</i>	0.0	0.05	0.25	0.0
<i>LD 3</i>	0.0	0.05	0.35	0.0
<i>LD 4</i>	0.0	0.05	0.15	0.0
<i>LD 5</i>	0.0	0.05	0.05	0.0
<i>LD 6</i>	0.0	0.24	0.05	0.0
<i>HD 1</i>	0.0	0.11	0.11	0.0
<i>HD 2</i>	0.0	0.19	0.37	0.0
<i>Natural</i>	0.0	0.14	0.05	0.0
<i>Sewer</i>	0.0	0.05	0.20	0.0
<i>LD Pooled</i>	0.0	0.05	0.15	0.0
<i>HD Pooled</i>	0.0	0.14	0.24	0.0

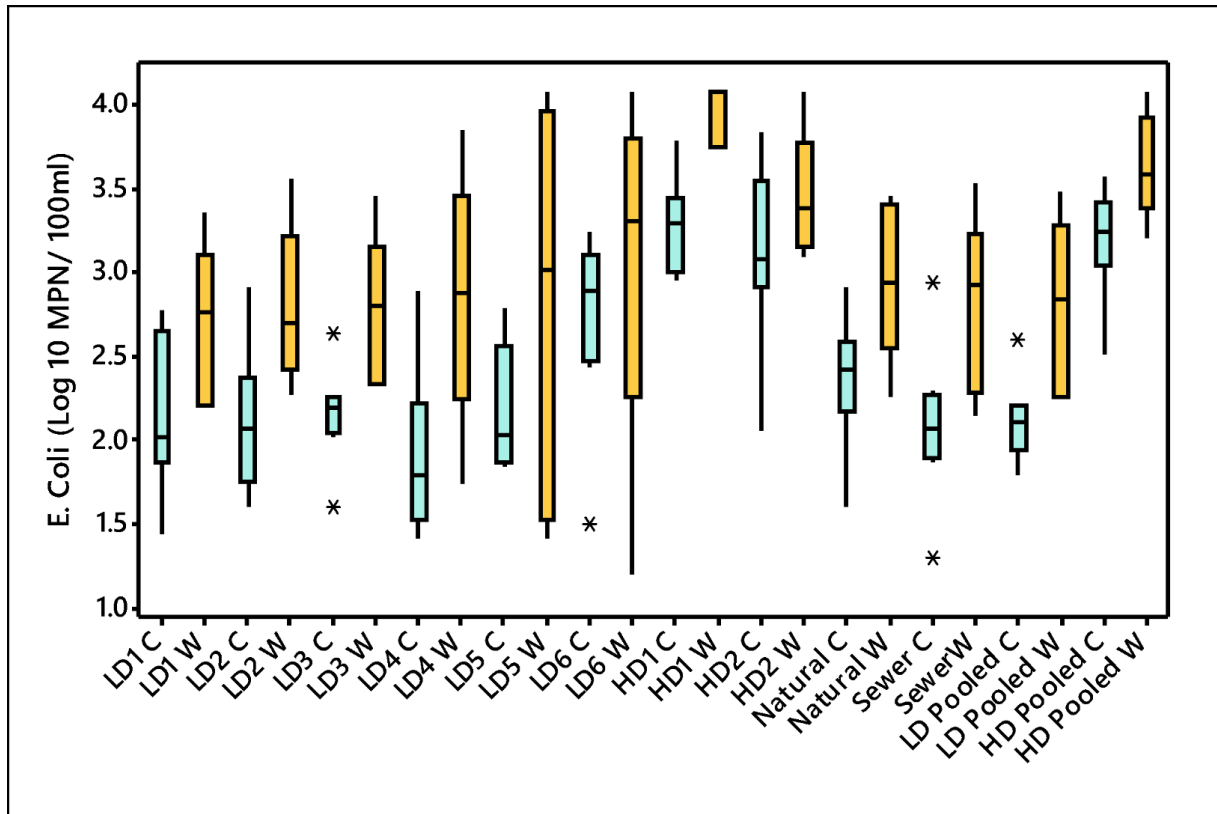
#### C. Seasonality of Bacterial Data

Concentrations of FIB have been shown to be affected by season with higher concentrations generally in the warmer months and lower concentrations in the cooler months.

Every individual watershed sampled showed medians elevated in the warm months in comparison to the cold months (Figure 12). The median concentration of *E.coli* for the warm months for HD Pool (2676 MPN/100 mL) was significantly ( $p = 0.037$ ) elevated in comparison to the warm months for LD Pool (707 MPN/100 mL), the Natural watershed (862 MPN/100 mL,  $p = 0.037$ ) and the Sewer watershed (835 MPN/100 mL,  $p = 0.022$ ).

The median concentration of *E. coli* for the cooler months for HD Pool (1290 MPN/100 mL) was significantly elevated in comparison to LD Pool (124 MPN/100 mL,  $p = 0.001$ ), the Natural watershed (267 MPN/100 mL,  $p = 0.003$ ), and the Sewer watershed (119 MPN/100 mL,  $p = 0.001$ ).

Comparisons were made to determine if statistically significant differences were observed when comparing concentrations of *E.coli* during warm relative to cold periods for each watershed. The concentrations of *E.coli* for the HD Pool watersheds (2676 MPN/100 mL) for the warm months was significantly greater than the cold months for LD Pool (median 124 MPN/100 mL,  $p = 0.004$ ), the Natural watershed (median 267 MPN/100 mL,  $p = 0.004$ ), the Sewer watershed (median 119 MPN/100 mL,  $p = 0.004$ ), and from HD Pool (median 1290 MPN/100 mL,  $p = 0.034$ ) concentrations during cold months. Other p-values for the watersheds can be found in the appendix.



**Figure 12:** Concentrations of *E. coli* for the warm in comparison to cold months at each watershed and for pooled data. Cold data (represented in blue) warm data (represented in yellow).

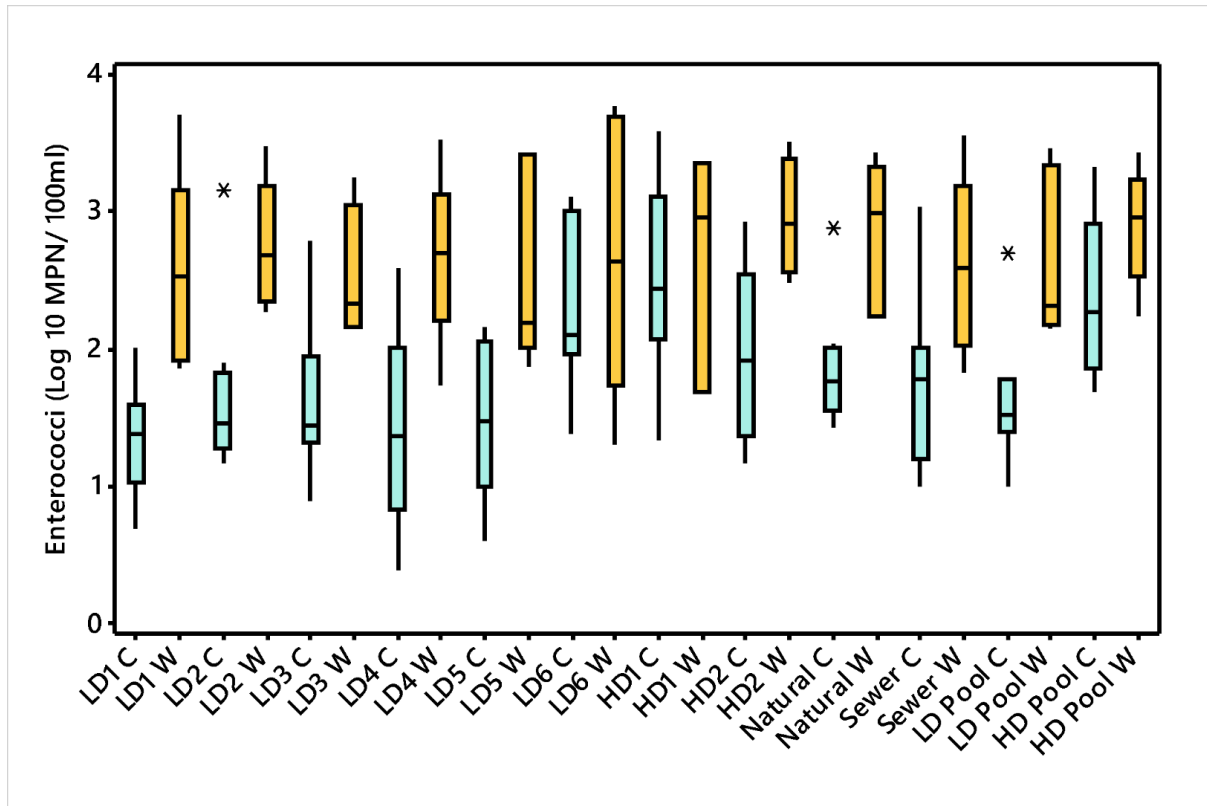
Concentrations of enterococci show a similar trend as *E. coli* in that the medians for the warm months for HD Pool 925 MPN/100 mL were elevated in comparison to the warm months for LD Pool (420 MPN/100 mL), and the Sewer watershed (394 MPN/100 mL). The Natural watershed had a median concentration of enterococci (1007 MPN/100 mL) that was similar to the HD Pool. Differences in concentrations of enterococci for HD, LD, Natural, and Sewer during warmer months were non-significant ( $p > 0.05$ ).

The median concentration of enterococci for the cold months for HD Pool (149 MPN/100 mL) was significantly elevated in comparison to (LD Pool 30 MPN/100 mL,  $p = 0.010$ ), and the Natural watershed (60 MPN/100 mL,  $p = 0.041$ ). Similar trends were found during the cold months between the HD Pool in comparison to the Sewer watershed (62 MPN/100 mL) but



differences in concentrations were non-significant ( $p > 0.05$ ). Overall, concentrations of enterococci for HD Pool were elevated in comparison to the LD Pool watershed, the Sewer watershed and the Natural watershed.

Comparisons were made to determine if statistically significant differences were observed when comparing concentrations of enterococci during warm relative to cold months for each watershed. The median concentration of enterococci for each individual watershed during the warm months was elevated in comparison to the cold months (Figure 13). The HD Pool watersheds (median 925 MPN/100 mL) for the warm months was significantly elevated in comparison to the cold months for LD Pool (median 30 MPN/100 mL,  $p = 0.007$ ), the Natural watershed (median 60 MPN/100 mL,  $p = 0.010$ ), and the Sewer watershed (median 62 MPN/100 mL,  $p = 0.023$ ). These comparisons suggest that temperature and wastewater treatment approach can influence FIB variability and concentrations, with elevated concentrations in the warm months in comparison to cold months and elevated concentrations in HD watersheds relative to LD. Animals may be more active in warm months and less active in cooler months, thus influencing FIB concentrations in summer and spring. Separating the data for comparisons allow understanding FIB concentration comparison by taking into consideration the temperature influence. The HD watersheds trends show elevated concentrations of *E. coli* and enterococci for both the warm and cold months.



**Figure 13:** Concentrations of enterococci for the warm in comparison to cold months at each watershed and for pooled data. Cold data (represented in blue) warm data (represented in yellow).

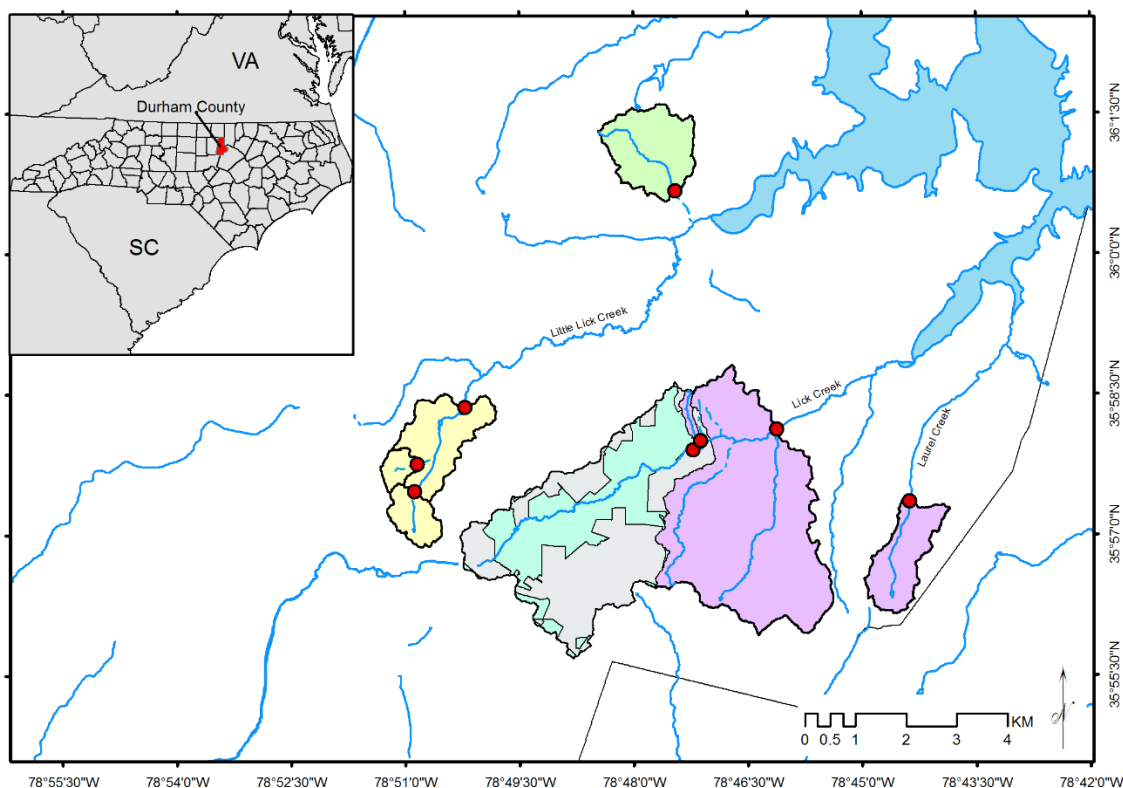
Mean stream flow for the warm months (5.4 L/s) was much lower relative to mean flow during cold months (21.8 L/s); stream flow influences watershed exports of FIB. When streams were dry, with no measurable flow during many summer sampling periods there were often discontinuous pools of water with elevated concentrations of FIB. Concentrations of FIB were often higher during summer, but flow and watershed exports of FIB were low. Concentrations of FIB during winter months were often low, but flow was high and thus watershed exports of FIB were moderate to high also.

## E. OWS Technologies and Fecal Bacteria Concentrations

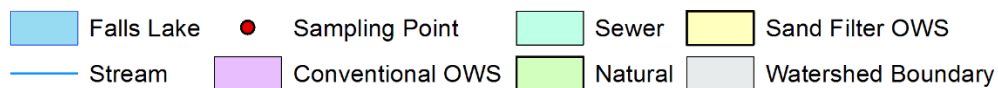
Three watersheds predominately served by sand filter systems (SF1-SF3), were monitored for approximately one year (April 2015 – March 2016). Concentrations and watershed exports of FIB from the three sub-watersheds (SF1-SF3) had a relatively low density of systems, (mean: 0.34 systems/ha) that was comparable to three watersheds served by predominately conventional-style OWS (LD 3, LD 4 and LD 6) with a mean density of 0.25 systems/ha (Figure 14). Therefore FIB concentrations and exports for these watersheds with similar densities of OWS, but with different wastewater system technologies were compared to each other and to the control watersheds (Sewer, and Natural) to determine if OWS technology influenced FIB concentrations and exports. Characteristics of these watersheds are shown in Table 5.

**Table 5:** Watershed characteristics for low-density septic watersheds (LD), sand filter watersheds (SF), a Sewer watershed, and a Natural watershed. <sup>a</sup>= LD 6 contains residences served by sewer, thus increasing impervious cover.

<i>Watershed</i>	<i>Stream</i>	<i>Impervious Cover</i>	<i>Area</i>	<i># of Septic</i>	<i>Septic System Density</i>
	Gradient	(%)	(ha)	Systems	(systems/ha)
<i>LD3</i>	0.002	4%	2283	280	0.12
<i>LD4</i>	0.0047	1%	184	48	0.26
<i>LD6</i>	0.0078	13% <sup>a</sup>	19	7	0.37
<i>SF 1</i>	0.0045	7%	83	35	0.42
<i>SF 2</i>	0.0067	6%	40	15	0.37
<i>SF 3</i>	0.0028	9%	335	75	0.22
<i>Natural</i>	0.0036	2%	246	-----	-----
<i>Sewer</i>	0.002	7%	1128	-----	-----



### Legend

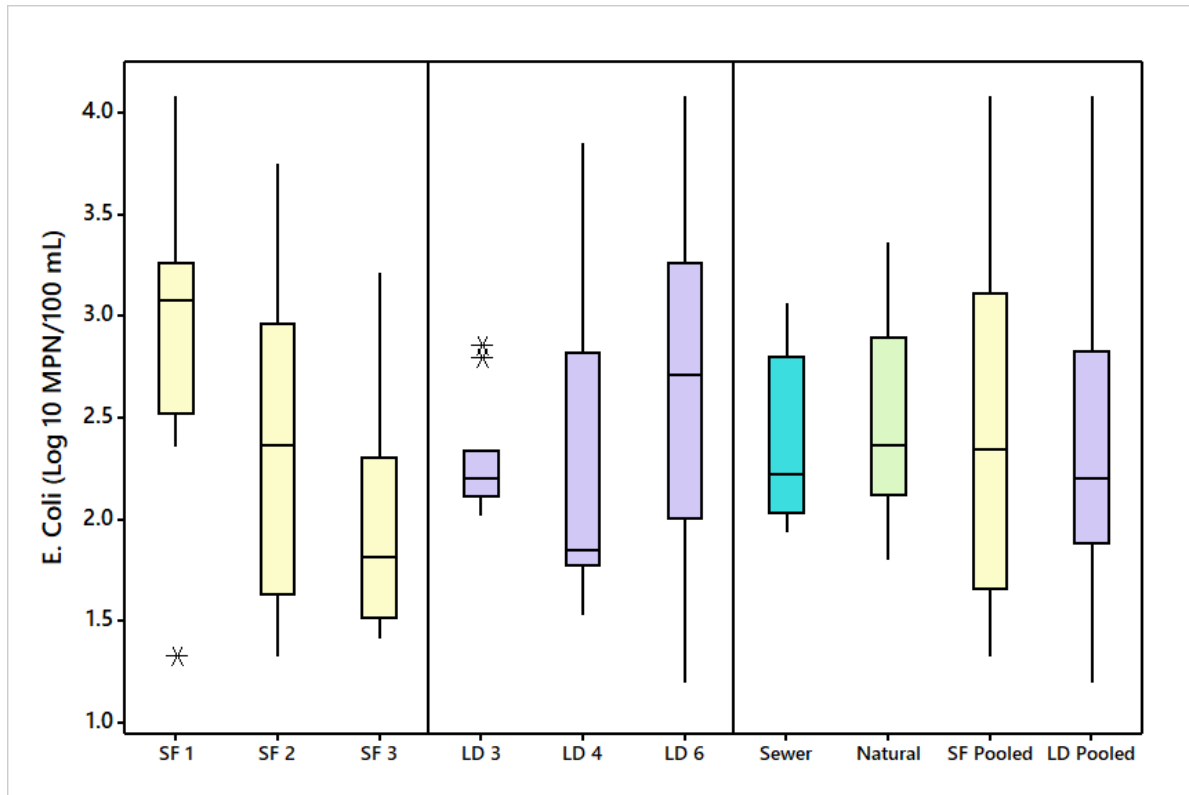


**Figure 14:** Watershed sampling points for conventional onsite wastewater system watersheds (LD 3, LD 4, and LD 6), Sand filter onsite wastewater system watersheds (SF1, SF2 and SF3), a MSW watershed (Sewer), and a mostly forested watershed (Natural).

#### IV. Chapter 4: Results and Discussion

##### A. Fecal Indicator Bacteria Differences for Wastewater Technologies

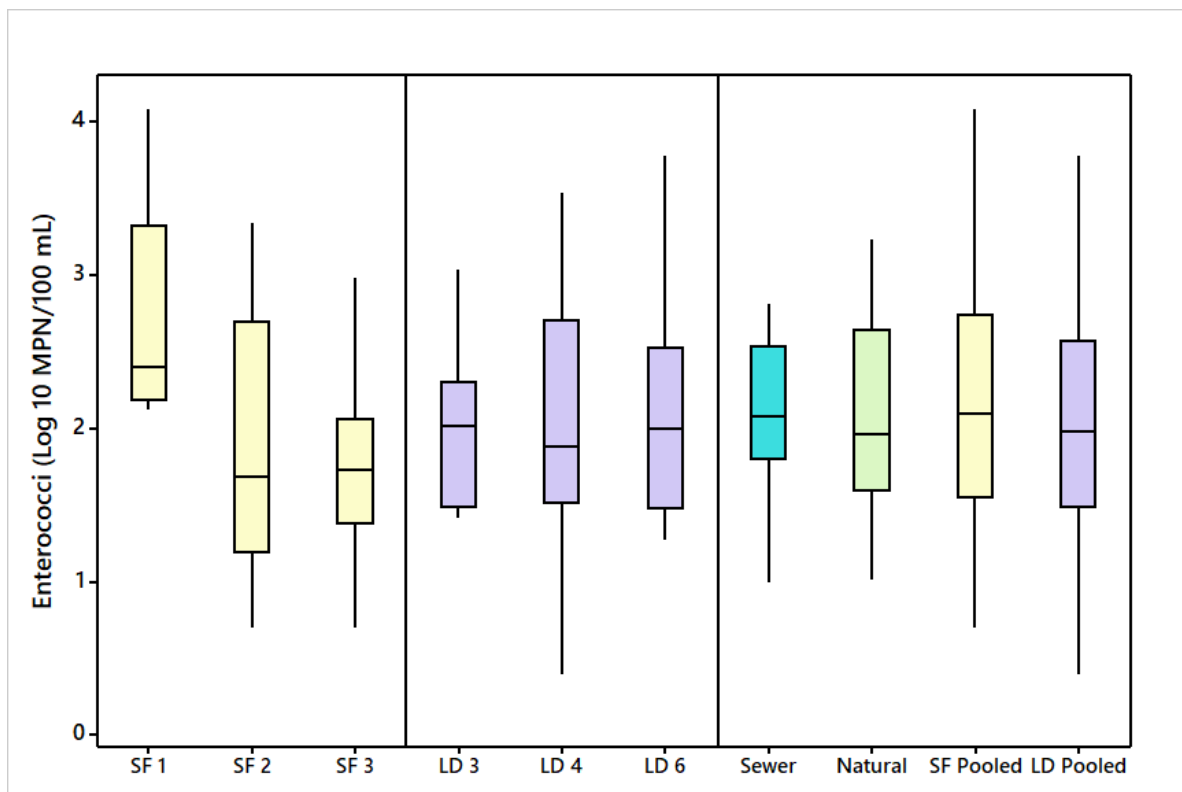
The Sand Filter systems watershed with the highest density of systems (SF1), had the highest median concentration of *E. coli* (1203 MPN/100 mL) (Figure 15). Concentrations of *E. coli* for SF1 were significantly greater relative to the Natural watershed (232 MPN/100 mL,  $p = 0.049$ ), the Sewer watershed (170 MPN/100 mL,  $p = 0.011$ ), the pooled conventional style system watershed LD Pool (159 MPN/100 mL,  $p = 0.018$ ), the conventional style watersheds LD 3 (159 MPN/100 mL,  $p = 0.004$ ) and LD 4 (70 MPN/100 mL,  $p = 0.029$ ). Concentrations of *E. coli* for SF 3 were significantly lower relative to LD 6 (526 MPN/100 mL,  $p = 0.046$ ), LD Pool ( $p = 0.040$ ), the Sewer watershed ( $p = 0.035$ ), and the Natural watershed ( $p = 0.017$ ). This indicates that higher densities of OWS may be related to elevated concentrations of *E. coli* in watersheds.



**Figure 15:** Comparison of *E. coli* concentrations for each watershed and the pooled data for the watersheds.

The watersheds with the highest density of systems, SF 1, had the highest median concentration of enterococci (253 MPN/100 mL). Pooling the watershed data, median concentrations of enterococci were highest for SF Pool (126 MPN/100 mL), followed by Sewer (120 MPN/100 mL), LD pool (95 MPN/100 mL), and Natural (94 MPN/100 mL). Differences in concentrations of enterococci were statistically significant when comparing SF 1 (median 253 MPN/100 mL) to SF 2 (48 MPN/100 mL,  $p = 0.013$ ), and SF 3 (55 MPN/100 mL,  $p = 0.001$ ), LD 3 (105 MPN/100 mL,  $p = 0.006$ ), LD 4 (79 MPN/100 mL,  $p = 0.021$ ), LD 6 (101 MPN/100 mL,  $p = 0.015$ ), the Sewer watershed (120 MPN/100 mL,  $p = 0.015$ ), the Natural watershed (94 MPN/100 mL,  $p = 0.015$ ), and LD Pool (95 MPN/100 mL,  $p = 0.002$ ) (Figure 16). No other comparisons with regards to concentrations of enterococci in watersheds were statistically

significant. These analyses show that higher system densities may contribute to elevated enterococci and *E.coli* concentrations in comparison to lower system densities. The setback distance may also influence the concentrations and exports, typical setback distances are 15 meters or more for conventional style systems, while sand filter systems directly outlet to the stream. Many systems were within 30 meters of the streams in the watersheds. Future research is suggested to better determine the mean distance of systems form the stream in the watersheds. Sand filter watersheds may contribute to elevated concentrations of FIB in comparison to the other different treatment technologies such as conventional style OWS. The density of systems may be more important to watershed management of FIB concentrations than the system type.



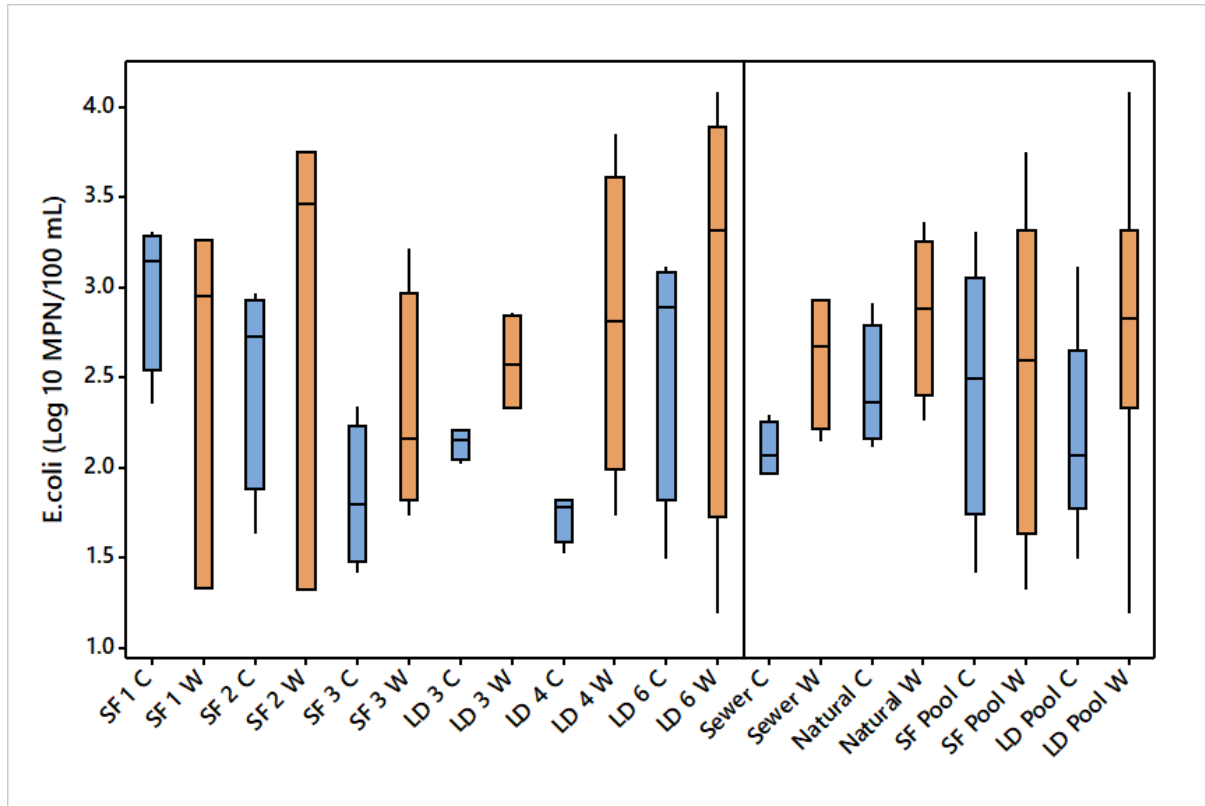
**Figure 16:** Comparison of enterococci concentration data for each watershed and for the pooled watersheds.

## B. Seasonal Related Differences

Concentrations of *E. coli* and enterococci showed seasonal trends, with warmer months showing higher concentrations and cooler months showing lower concentrations, this can be seen at each individual watershed except for SF 1 (Figures 17 and 18). Concentrations of *E. coli* for the warm months in comparison to the warm months for the year sampled showed that the Natural watershed has the highest median (770 MPN/100 mL) in relation to the LD Pool watershed (677 MPN/100 mL), the Sewer (548 MPN/100 mL), and the SF Pool watershed (532 MPN/100 mL). The highest median concentration of *E. coli* during colder months was from SF Pool (329 MPN/100 mL), followed by the Natural watershed (232 MPN/100 mL), the Sewer watershed (119 MPN/100 mL), and LD Pool (117 MPN/100 mL) (Figure 17).

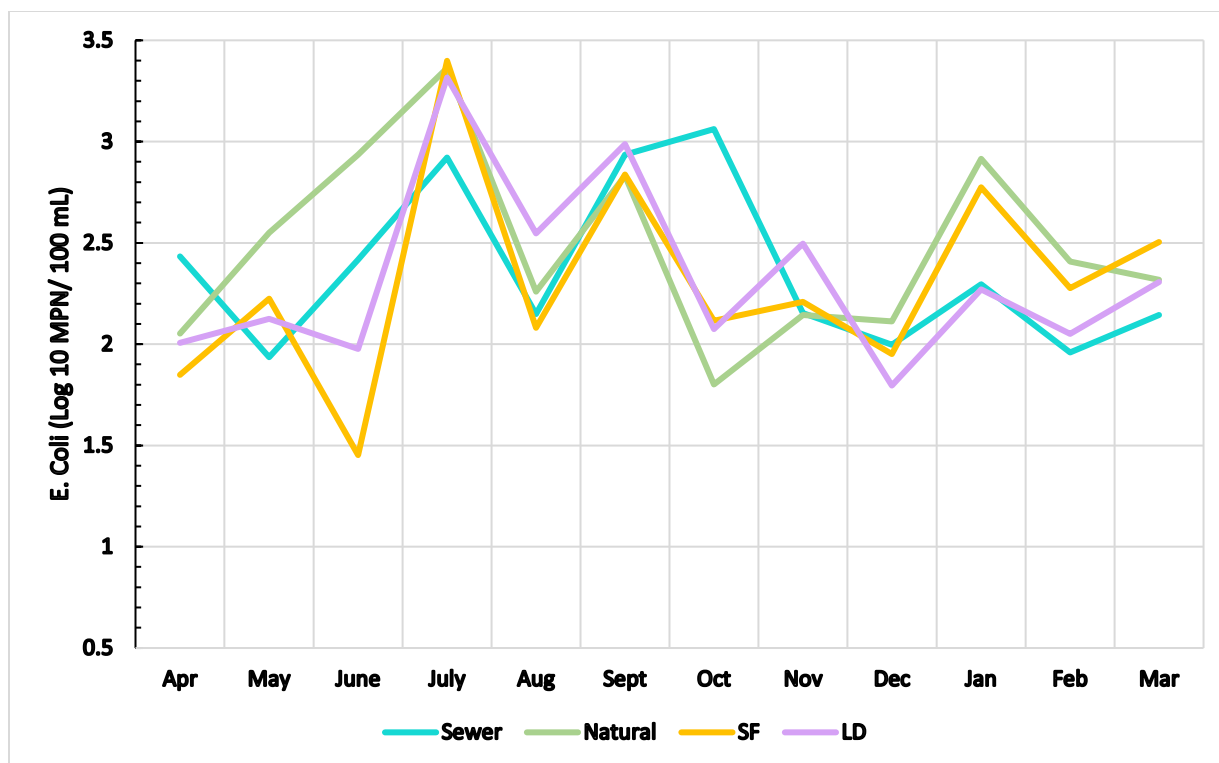
Mann-Whitney tests on *E. coli* concentrations for warm months compared to cold months showed statistical significance between warm months for the Natural watershed and the cold months for the sand filter watershed ( $p = 0.05$ ), the conventional style watershed ( $p = 0.03$ ) and the Sewer watershed ( $p = 0.02$ ). The warm months for the Sewer watershed was significant in comparison to the cold months for the pooled conventional style watersheds ( $p = 0.03$ ) and the cold months for the Sewer watershed ( $p = 0.02$ ).





**Figure 17:** Concentrations of *E. coli* for the warm months, represented in orange, in comparison to the cold months, represented in blue.

Concentrations of *E. coli* for watersheds with different wastewater technologies were compared each month. The natural watershed was the highest during 5 of 12 (42%) sampling events followed by LD with 3 or 25% of the events, the Sewer watershed with 2 or 17% of the events, and the SF Pool with 2 of the 12 (17%) of events. Correlation analyses were performed on concentrations of *E. coli* for the pooled watershed data. Statistically significant correlations for *E. coli* concentrations were only found for SF Pool with LD Pool ( $r_s = 0.727$ ,  $p = 0.007$ ).

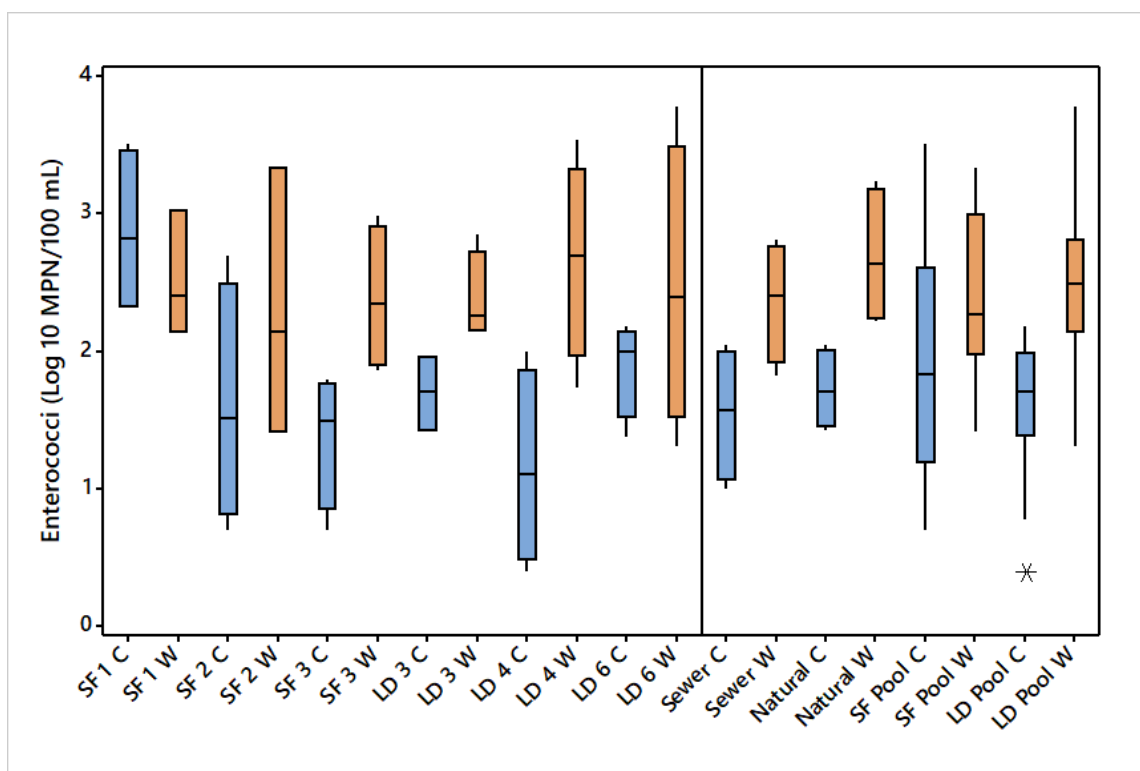


**Figure 18:** Temporal comparison of *E. coli* concentrations for the year sampled.

The Natural watershed had the highest median concentration of enterococci (598 MPN/100 mL) during warmer periods followed by LD Pool (331 MPN/100 mL), the Sewer watershed (281 MPN/100 mL), and SF Pool (197 MPN/100 mL). During the colder months, the SF Pool watershed had the highest median (69 MPN/100 mL), followed by the LD Pool (59 MPN/100 mL), the Natural (55 MPN/100 mL), and the Sewer watershed (48 MPN/100 mL). The median *E. coli* and enterococci concentrations were typically higher during the warm months relative to the cold months for most of the watersheds. Concentrations of FIB in the Natural watershed were highest during warm months, and this may have been associated with animal activity and waste. The SF Pool watershed had the highest FIB concentrations during the cold months which could be due to being a constant source of FIB from the OWS. Animal related waste contributions are expected to decline in colder months, and thus watersheds with mostly

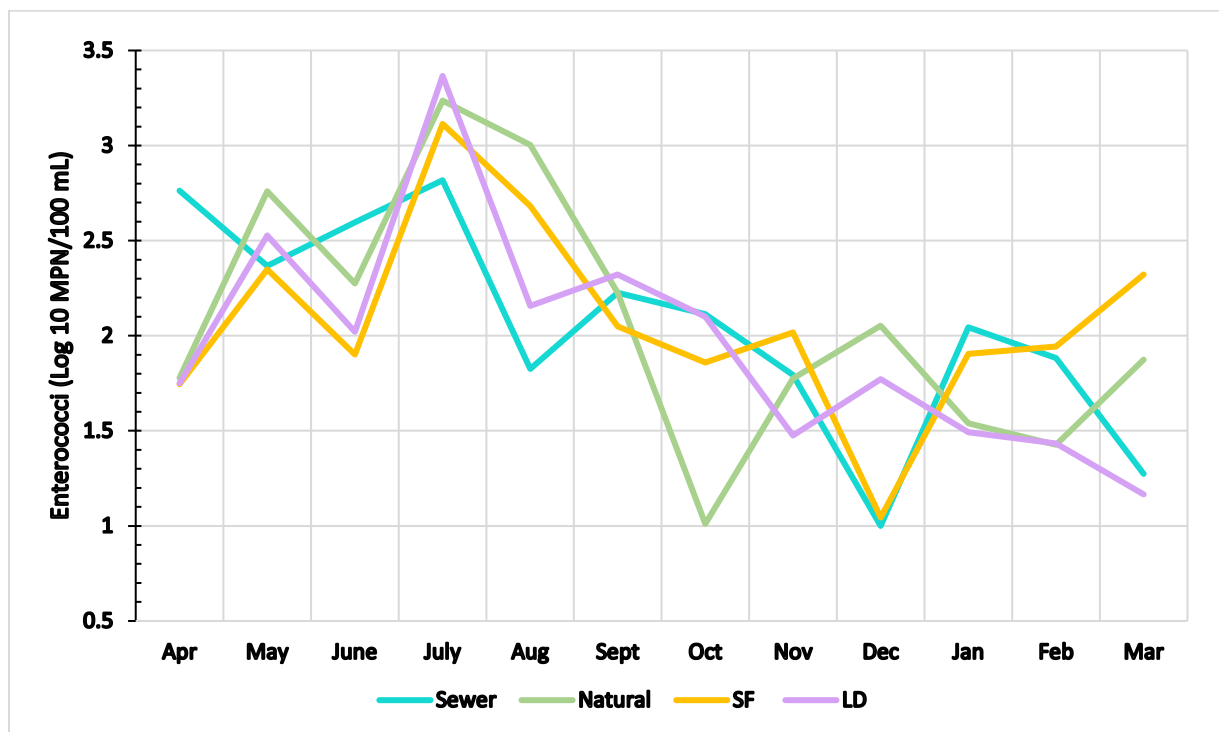
animal related FIB in their streams may see larger changes in concentrations between warm and cold periods.

Mann-Whitney tests on enterococci concentrations showed statistical significance between the warm months for the Natural watershed and the cold months for all other watersheds ( $p < 0.05$ ). Concentrations of enterococci during the warm months for the Sewer watershed were statistically significant in comparison to the cold months for LD Pool ( $p = 0.01$ ), the Natural watershed ( $p = 0.05$ ), and the Sewer watershed ( $p = 0.03$ ). The LD Pool watershed during the warm months was statistically significant in comparison to the cold months for the SF Pool ( $p = 0.05$ ), LD Pool cold ( $p = 0.01$ ), and the Sewer watershed ( $p = 0.03$ ). Statistically significant differences were also found between the warm months for the SF Pool in comparison to the cold months for the LD Pool ( $p = 0.01$ ), and the Sewer watershed ( $p = 0.03$ ).



**Figure 19:** Concentrations of enterococci for each watershed separated into warm months, represented in orange, in comparison to the cold months, represented in blue.

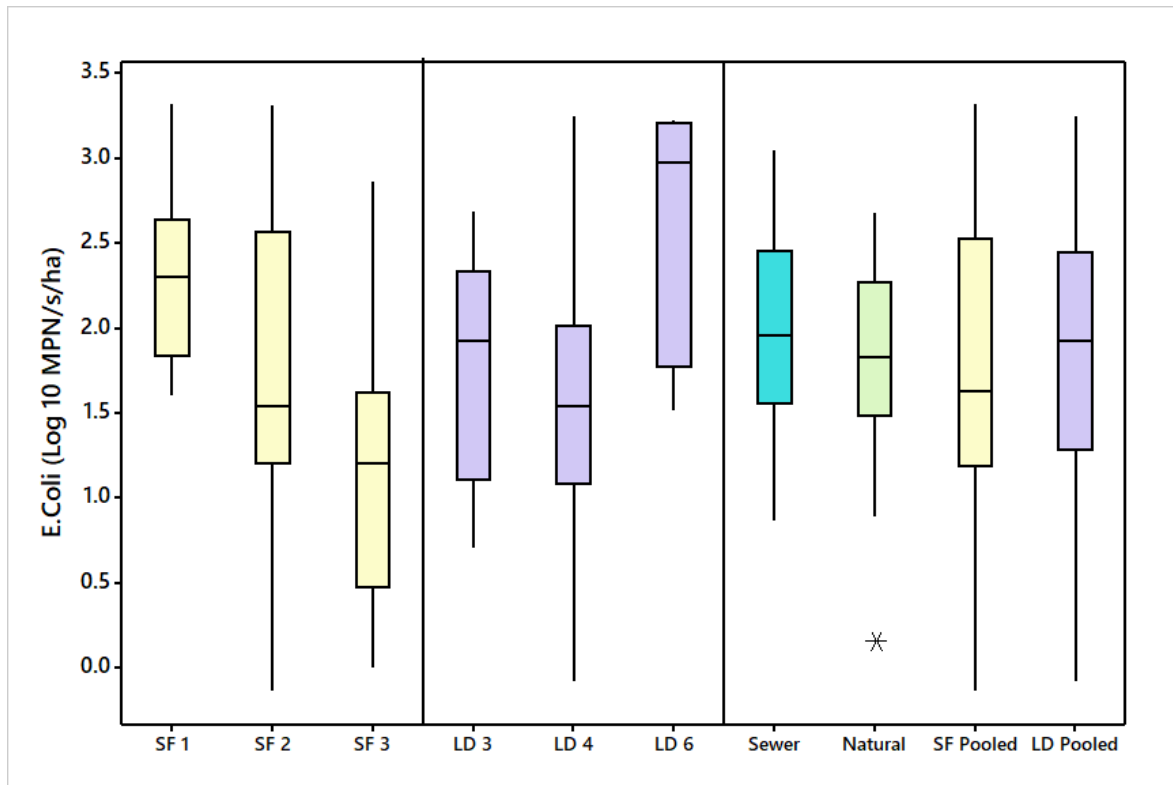
The Sewer watershed had the highest concentration of enterococci during 4 of the 12 (33%) sampling events, the Natural watershed during 3 (25%) events, SF pool 3 (25%) events, and LD pool, 2 of the 12 (17%) events sampled. Concentrations of enterococci for the watersheds were evaluated using correlation analyses (Figure 20). Statistically significant correlations were observed between The Natural watershed and LD pool ( $r_s = 0.685$ ,  $p = 0.014$ ), and SF pool ( $r_s = 0.608$ ,  $p = 0.036$ ). There was also a positive moderate correlation between the Sewer watershed and LD pool ( $r_s = 0.587$ ,  $p = 0.045$ ). Higher concentrations of enterococci were found in the warmer months and lower concentrations found in the cooler months during the one year study period (Figures 19 and 20).



**Figure 20:** Temporal comparison of enterococci concentrations for the Sewer watershed the Natural watershed, and the pooled sand filter watersheds, and the pooled conventional style watersheds for the year sampled.

### C. Watershed Exports of Fecal Indicator Bacteria

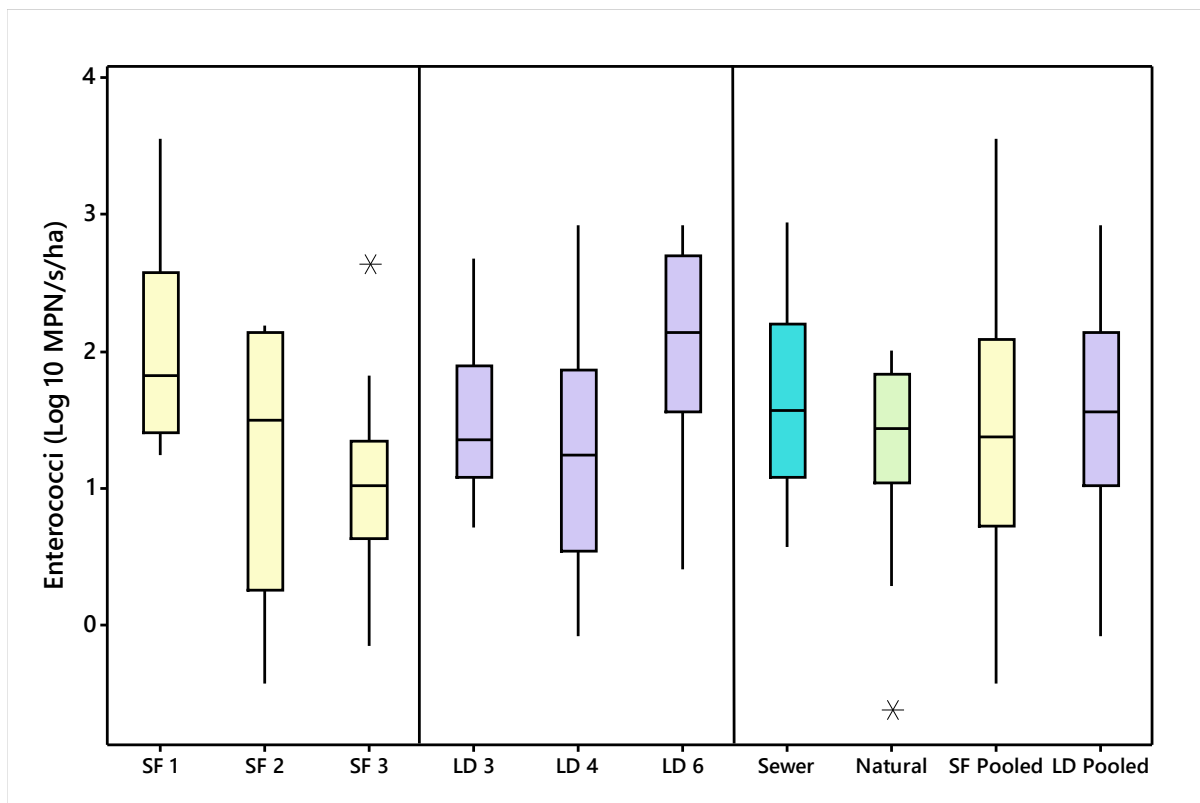
Exports of *E. coli* were the greatest for SF 1 (111 MPN/s/ha), the sand filter watershed with the highest densities of OWS systems. Exports of *E. coli* for SF 1 were significantly elevated relative to SF 3 (18 MPN/s/ha,  $p = 0.003$ ) and LD 4 (35 MPN/s/ha,  $p = 0.008$ ) (Figure 21). Exports of *E. coli* for SF 3 (18 MPN/s/ha) were significantly lower than LD Pool (47 MPN/s/ha) ( $p = 0.026$ ), the Sewer watershed (94 MPN/s/ha) ( $p = 0.023$ ), and LD 6 (46 MPN/s/ha) ( $p = 0.005$ ). Exports of *E. coli* for LD 6 were also significantly greater compared to LD 4 ( $p = 0.026$ ) and SF Pool (38 MPN/s/ha;  $p = 0.038$ ).



**Figure 21:** Comparison of *E. coli* exports for each watershed and the pooled watershed exports.

Watershed exports of enterococci were also greatest for SF 1 (50 MPN/s/ha) the sand filter watershed with the highest densities of OWS systems. Significant differences in exports

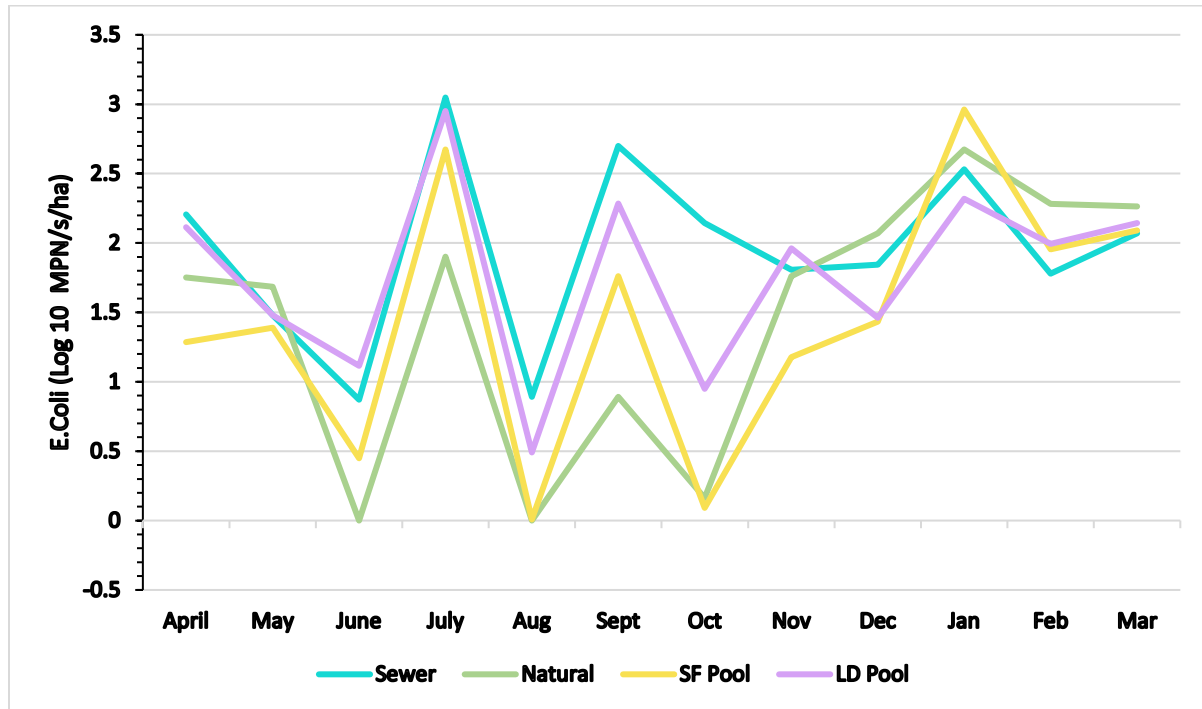
were observed when comparing SF 1 to SF 3 (11 MPN/s/ha,  $p = 0.003$ ), and LD 4 (18 MPN/s/ha,  $p = 0.044$ ) (Figure 22). Exports of enterococci for SF 3 were significantly lower relative to LD 3 (23 MPN/s/ha,  $p = 0.053$ ), LD 6 (20 MPN/s/ha,  $p = 0.025$ ), and LD Pool (21 MPN/s/ha,  $p = 0.050$ ). Watershed exports of enterococci were significantly greater for LD 6 relative to the Natural watershed ( $p = 0.036$ ).



**Figure 22:** Comparison of enterococci exports for each watershed and the pooled data.

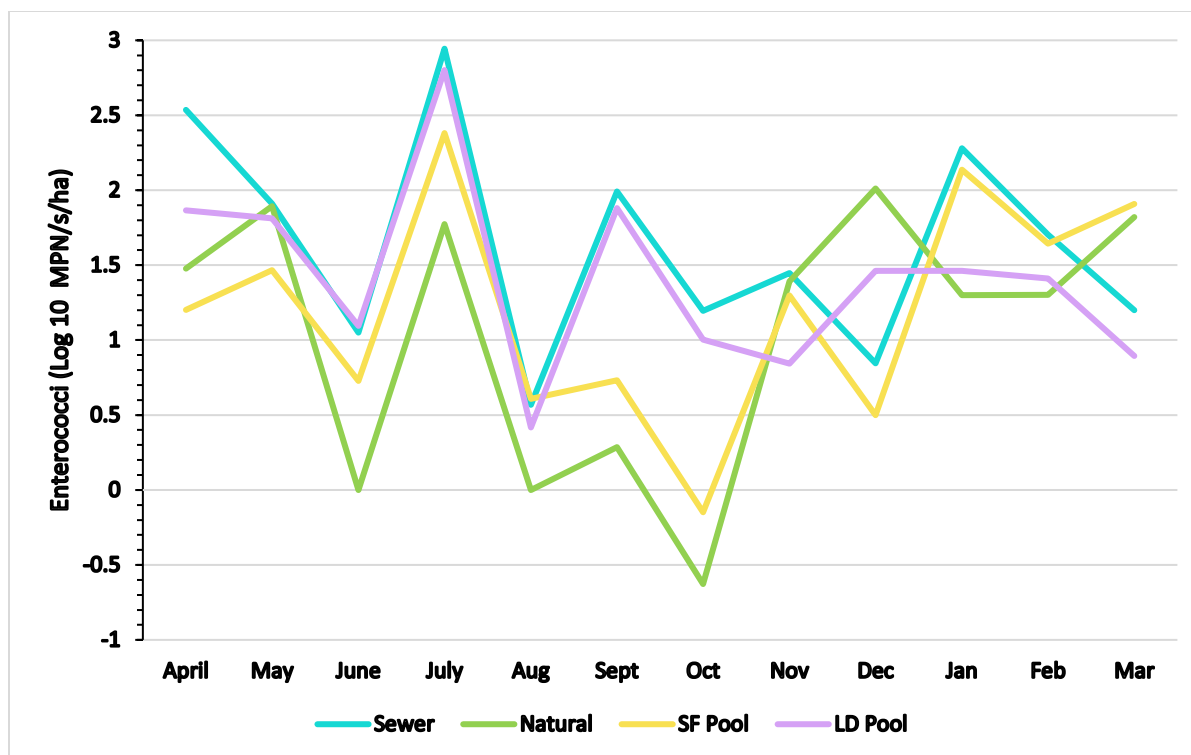
Monthly exports of *E. coli* exports for the pooled watersheds, the Natural, and Sewer watersheds are shown in Figure 23. The Sewer watershed had the highest exports for 5 of the 12 (41%) sampling events. The Natural watershed had the highest exports 4 (33%) events, the pooled LD 2 (17%) of events, and the pooled SF 1 of the 12 (8%) sampling events. Significant correlations were found between the exports of *E. coli* for the Sewer watershed and SF pool ( $r_s = 0.594$ ,  $p = 0.042$ ); the Sewer watershed and LD pool ( $r_s = 0.769$ ,  $p = 0.003$ ); The Natural

watershed and SF Pool ( $r_s = 0.834$ ,  $p = 0.001$ ) the Natural and LD ( $r_s = 0.637$ ,  $p = 0.026$ ); SF Pool and LD Pool ( $r_s = 0.881$ ,  $p = 0.001$ ).



**Figure 23:** Temporal comparison of *E. coli* exports for the pooled sand filter watersheds, the pooled conventional style watersheds, the natural watershed and the sewer watershed.

Watershed exports of enterococci were greatest during 8 of 12 (66%) sampling events for the Sewer watershed, during 2 (17%) events for SF 2, and 1 (8%) event each for The Natural watershed and the LD Pool (Figure 24). Moderate positive correlations were observed between watershed exports of enterococci for the Sewer watershed and SF Pool ( $r_s = 0.699$ ,  $p = 0.002$ ) and the Sewer watershed and LD Pool ( $r_s = 0.790$ ,  $p = 0.002$ ).



**Figure 24:** Temporal comparison of the enterococci exports for the pooled sand filter watersheds, the conventional style watersheds, the natural watershed, and the sewer watershed.

#### D. Fecal Indicator Bacteria Physical and Chemical Correlations

Correlations for *E. coli* and SC show a negative strong association SF 2 ( $r_s = -0.764$ ,  $p = 0.006$ ). A significant inverse correlation was also observed between pH and *E. coli* for LD Pool ( $r_s = -0.383$ ,  $p = 0.021$ ). Turbidity showed a strong positive correlation with *E. coli* for LD 6 ( $r_s = 0.632$ ,  $p = 0.05$ ), the Natural watershed ( $r_s = 0.818$ ,  $p = 0.004$ ), and SF 2 ( $r_s = 0.624$ ,  $p = 0.054$ ). Strong and moderate positive associations for ORP and *E. coli* were observed for LD 4 ( $r_s = 0.755$ ,  $p = 0.005$ ) and the Sewer watershed ( $r_s = 0.559$ ,  $p = 0.059$ ). There were no statistically significant correlations for temperature or DO.

Correlations were also examined for water quality parameters and enterococci concentrations. Positive strong and moderate correlations were found for temperature and



enterococci concentrations at LD 4 ( $r_s = 0.769$ ,  $p = 0.003$ ), the Natural watershed ( $r_s = 0.818$ ,  $p = 0.001$ ), SF 3 ( $r_s = 0.683$ ,  $p = 0.014$ ), and LD Pool ( $r_s = 0.578$ ,  $p = 0.001$ ). DO had negative strong moderate and weak associations for SF 3 ( $r_s = -0.708$ ,  $p = 0.010$ ), LD 4 ( $r_s = -0.573$ ,  $p = 0.050$ ), the Natural watershed ( $r_s = -0.594$ ,  $p = 0.042$ ), and LD Pool ( $r_s = -0.393$ ,  $p = 0.018$ ). Turbidity had positive strong moderate and weak associations with enterococci concentrations SF 2 ( $r_s = 0.842$ ,  $p = 0.002$ ), LD 6 ( $r_s = 0.636$ ,  $p = 0.048$ ), and SF Pool ( $r_s = 0.367$ ,  $p = 0.042$ ). Positive, strong correlations between turbidity and enterococci concentrations at LD 4 ( $r_s = 0.818$ ,  $p = 0.001$ ) and moderate correlations for the Natural watershed were found ( $r_s = 0.573$ ,  $p = 0.051$ ). Significant correlations for ORP, SC and pH with enterococci concentrations were not observed.

**Table 6:** Medians (standard deviation) for environmental parameters including SC= Conductance DO= dissolve oxygen Temp= temperature ORP= oxygen reduction potential.

<i>Watershed</i>	<i>SC</i> ( $\mu S\ cm^{-1}$ )	<i>DO</i> ( $mg\ L^{-1}$ )	<i>pH</i>	<i>Temp.</i> ( $^{\circ}C$ )	<i>ORP</i> ( $mV$ )	<i>Turbidity</i> ( $NTU$ )
<i>SF 1</i>	346 (222)	5.9 (3.9)	7.2 (0.7)	13.0 (17.7)	-27.0 (98.6)	25 (31)
<i>SF 2</i>	174 (134)	6.0 (3.7)	7.2 (0.6)	13.7 (6.6)	-18.4 (81.2)	24 (23)
<i>SF 3</i>	189 (197)	5.9 (3.2)	7.1 (0.5)	13.8 (6.7)	-19.4(100.2)	20 (35)
<i>LD 3</i>	152 (56)	8.9 (3.0)	7.3 (0.6)	13.3 (7.5)	-22.0 (97.9)	39 (38)
<i>LD 4</i>	130 (280)	8.8 (3.1)	7.3 (0.6)	12.8 (6.7)	-24.5 (104)	13 (17)
<i>LD 6</i>	418 (131)	6.5 (4.0)	7.3 (0.6)	12.4 (6.8)	-31.7 (88.3)	12 (16)
<i>Natural</i>	101 (35)	7.9 (3.5)	7.2 (0.5)	14.2 (6.9)	-26.2 (101)	18 (11)
<i>Sewer</i>	204 (86)	9.2 (3.7)	7.4 (0.4)	12.5 (8.0)	-14.7 (81.4)	27 (22)

## **V. Chapter 5: Conclusions**

Watersheds with a high-density of OWS showed elevated concentrations and exports of *E.coli* that were statistically significant in comparison to low-density OWS watersheds, Sewered watersheds and Natural watersheds. Similar trends were found with regard to enterococci median concentrations and exports for watersheds with a high-density of OWS in comparison to the low-density, sewer and natural watersheds; however, the differences in concentration and exports were not always statistically significant. The Natural watershed had FIB concentrations and exports similar to the low-density and Sewer watersheds, indicating that low-density OWS watersheds may not significantly influence FIB concentrations and watershed exports in the NC piedmont region.

This study found that the density of systems in a watershed may also have an impact on the concentrations of FIB found when comparing wastewater technologies. The watersheds with the highest density of systems (SF and conventional-style) typically had the highest median concentrations of FIB.

Watersheds with a high-density of OWS had FIB concentrations that more frequently violated USEPA standards relative to low-density OWS watersheds and control watersheds. It is also important to note that all of the watersheds sampled were higher than the USEPA standards at some point during sampling. Water quality parameters were also compared to standards that were applicable, violations occurred for each watersheds sampled for DO and turbidity. These findings indicate that the water quality in some watersheds sampled may be impaired.

Statistically significant differences were observed when comparing FIB concentrations during warm and cold months for most watersheds. Concentrations were highest during warm periods when water-based recreation most frequently occurs. This may be a public health concern for the area, and highlights the need to reduce FIB exports from sub-watersheds.

The USEPA standards used as a comparison metric in this study may not be achievable for urbanizing watersheds with OWS. For example, the Natural watershed, with little residential or commercial development also had a high frequency of exceedance of the USEPA standards. A more realistic water quality goal for urbanizing watersheds in this region may be to achieve concentrations and exports observed in the Natural watershed. Results show that certain densities of OWS ( $> 0.8$  OWS/ha) may influence concentrations and exports of FIB. More work is needed to reduce the transport of FIB from existing OWS to surface waters. Furthermore, the Little Lick and Lick Creek watersheds are expected to see growth and expansion of urbanized areas and an increase in the number of OWS, potentially increasing exports of FIB. Current riparian buffers rules in the Falls Lake Watershed and Neuse River Basin protect the first 15 m upslope from surface waters. Increasing the buffer width requirement to 30 m may be a strategy for protecting water quality. The increased buffer width may allow more opportunity for wastewater renovation. Effluent from malfunctioning OWS would have to travel twice as far prior to reaching surface waters. The extra distance would allow more opportunity for effluent to infiltrate and come into contact with soil, and the FIB to have more opportunity for predation, biological processes, and filtration to occur.

Data from this study suggest that watersheds may have elevated FIB concentrations and exports due to many factors such as the prevalence of wildlife, density of OWS, failing OWS, or gray water pipes. Efforts are underway to improve the performance of OWS in the watersheds

and better manage storm water runoff. Continued work and commitment to improving water quality in the area is needed.

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## VI. References

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## VII. APPENDIX A: Supplemental Materials

**Table 7:** *E. coli* concentrations for each sampling event.

E.coli MPN/100 ml	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	SF 1	SF 2	SF 3
Jan-15	598	40	40	0	0	1226	1354	1218	40	20			
Feb-15	27	52	187	26	75	407	2827	4839	279	74			
Mar-15	74	212	155	167	69	1741	953	6932	373	161			
Apr-15	167	182	134	71	63	113	519	1159	113	271	334	46	32
May-15	306	209	158	70	153	233	3008	1622	355	86	355	72	210
Jun-15	156	371	219	571	43	16		1622	862	262	22	21	54
Jul-15	688	734	725	7068	12098	2053	12098	2909	2306	835	1827	5600	1628
Aug-15	163	187	214	55	26	12100		12098	182	141			121
Sep-15	575	509	630	750	1049	2053	12100	1241	677	861	891	2897	173
Oct-15	769	418	161	108	303	97	24	2451	63	1153	12098	31	37
Nov-15	120	210	120	690	159	431	4840	4840	139	142	1633	232	26
Dec-15	92	73	130	68	618	32	897	114	130	100	1628	43	26
Jan-16	496	143	163	59	108	977	2827	821	821	198	1203	922	217
Feb-16	118	93	105	34	112	620	2800	1449	256	91	228	431	80
Mar-16	75	246	158	62	74	1293	6050	1197	208	139	2041	651	49
Apr-16	95	240	48	283	61	60	509	2080	532	138			
May-16	2176	509	26	359	538	78	2738	594	386	569			
Jun-16	2306	3635	2897	1200	7068	3244	5600	2442	2897	3434			
Oct-16	107	1032	397	67	1094	637		12100	73	863			
Nov-16	54	821	437	41	167	1226	4840	523	551	177			
Dec-16	321	821	437	775	368	271	1226	870	384	870			

**Table 8:** Enterococci concentration for each sampling event.

Enterococci MPN/100ml	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	SF 1	SF 2	SF 3
Jan-15	20	20	20	104	150	844	150	62	62	82			
Feb-15	10	15	8	10	4	1095	821	872	42	15			
Mar-15	38	43	25	20	0	92	192	147	58	48			
Apr-15	23	63	32	99	69	61	462	96	60	580	133	34	43
May-15	2010	165	118	514	175	747	9	4041	575	233	153	727	118
Jun-15	100	497	221	484	76	21		651	189	394	253	27	103
Jul-15	397	777	715	3434	2586	6017	925		1724	657	1072	2176	968
Aug-15	346	265	153	55	159	442		1094	1007	67			480
Sep-15	73	187	141	510	136	143	49	307	168	168	141	140	74
Oct-15	6050	243	1088	54	30	58	10	309	10	130	12100	16	21
Nov-15	116	224	26	60	15	19	141	53	60	62	1298	48	37
Dec-15	5	21	26	101	32	92	395	15	113	10	214	5	5
Jan-16	29	81	91	6	22	152	111	17	35	111	2092	14	63
Feb-16	40	40	30	28	10	24	3883	480	27	76	215	76	47
Mar-16	13	19	89	3	30	110	1533	91	75	19	3249	497	21
Apr-16	82	37	10	86	21	94	386	12100	54	26			
May-16	241	492	92	372	101	87	606	42	455	435			
Jun-16	5231	3066	1827	556	2747	4082	2306	3293	2738	3635			
Oct-16	79	86	127	74	279	26		158	61	193			
Nov-16	34.6	1095	90	79	213	736	74	146	46	3466			
Dec-16	102	1454	626	398	115	1298	22	76	775	1095			

**Table 9:** P-values for the *E. coli* concentrations (MPN/100 mL). These were calculated using Mann-Whitney.

E. coli p values for Concentration	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	LD Pool	HD Pool	Natural
LD 1											
LD 2	0.3786										
LD 3	0.9599	0.1445									
LD 4	0.213	0.102	0.4281								
LD 5	0.5376	0.2472	0.6597	0.4733							
LD 6	0.1312	0.1824	0.0626	<b>0.0236</b>	0.0701						
HD 1	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.0001</b>	<b>0.0004</b>	<b>0.0044</b>					
HD 2	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.0068</b>	0.4552				
LD Pool	0.5544	0.4208	0.4428	0.102	0.2472	0.1312	<b>0.00001</b>	<b>0.00001</b>			
HD Pool	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.0017</b>	0.6829	0.5885	<b>0.00001</b>		
Natural	0.3204	0.8405	0.1783	0.0741	0.1824	0.2965	<b>0.0001</b>	<b>0.00001</b>	0.2794	<b>0.00001</b>	
Sewer	0.6061	0.6507	0.5544	0.0969	0.352	0.1665	<b>0.0001</b>	<b>0.00001</b>	0.9198	<b>0.00001</b>	0.6327

**Table 10:** P-values calculated for Enterococci concentrations at the sampling locations and for the pooled data.

Enterococci p values for Concentrations	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	LD Pool	HD Pool	Natural
LD 1											
LD 2	0.3786										
LD 3	0.8602	0.3993									
LD 4	0.7153	0.6149	0.9398								
LD 5	0.7153	0.1589	0.7246	0.5628							
LD 6	0.1824	0.5713	0.2176	0.1824	0.1411						
HD 1	0.1083	0.3902	0.1052	0.1179	<b>0.0486</b>	0.7037					
HD 2	0.0925	0.4339	0.09	0.1709	<b>0.046</b>	0.7842	0.9417				
LD Pool	0.546	0.606	0.5713	0.88	0.4039	0.3585	0.1468	0.1477			
HD Pool	<b>0.0252</b>	0.2524	<b>0.0221</b>	<b>0.0469</b>	<b>0.0217</b>	0.3924	0.9775	0.7053	0.0575		
Natural	0.489	0.8209	0.5544	0.782	0.4652	0.4208	0.2597	0.2058	0.6149	0.0848	
Sewer	0.3023	0.9099	0.3717	0.3854	0.2302	0.6597	0.4553	0.5313	0.4355	0.3455	0.505

**Table 11:** discharge data collected for each watershed, in cubic feet per second.

	2015	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	SF 1	SF 2	SF 3
Jan		1.703	7.436	13.783	1.642	5.520	0.600	0.109	0.219	1.054	5.164			
Feb		0.233	1.170	2.234	0.183	0.899	0.007	0.030	0.095	0.324	1.035			
Mar		0.940	2.314	5.312	0.413	1.738	0.106	0.037	0.070	0.648	2.801			
Apr		0.423	2.209	5.525	0.292	1.275	0.947	0.035	0.066	0.434	2.363	0.058	0.079	0.248
May		0.369	1.195	0.717	0.179	0.458	0.046	0.013	0.023	0.118	1.391	0.034	0.030	0.143
June			0.160	0.190	0.064	0.083					0.113			0.062
July		1.100	3.550	5.400	0.160	0.600		0.010	0.020	0.030	5.330	0.060	0.010	0.530
Aug		0.280	0.510	0.430	0.010	0.050					0.220			0.010
Sept		0.720	2.510	3.850	0.110	0.240		0.010	0.010	0.010	2.320	0.050	0.002	0.290
Oct		0.100	0.420	0.620	0.040	0.500		0.004		0.020	0.480	0.002	0.003	0.040
Nov		0.840	1.910	5.670	0.150	0.710	0.009	0.070	0.030	0.360	1.790	0.060	0.000	0.160
Dec		0.011	2.920	6.530	0.100	1.110	0.071	0.064	0.030	0.790	2.790	0.048	0.054	0.455
2016														
Jan		1.200	4.400	13.690	0.420	3.150	0.116	0.080	0.045	0.500	6.840	0.510	0.310	1.270
Feb		0.290	3.190	6.430	0.470	2.010	0.102	0.076	0.010	0.650	2.630	0.090	0.110	0.600
Mar		1.430	2.610	4.290	0.410	1.170	0.084	0.024	0.033	0.770	3.360	0.100	0.035	0.690
Apr		1.050	0.680	1.890	0.110	0.270	0.028	0.017	0.023	0.170	0.800			
May		0.090	0.730	2.560	0.100	0.610	0.010	0.002	0.010	0.050	0.590			
June		0.590	5.730	7.930	0.110	0.970	0.045	0.030	0.040	0.200	4.850			
Oct		0.030	1.020	0.210	0.010	0.100				0.030	0.070			
Nov		0.160	0.790	0.970	0.010	0.150	0.040	0.010		0.030	3.410			
Dec		0.740	3.540	10.740	0.470	2.410	0.040	0.050	0.020	0.490	7.410			

**Table 12:** P-values calculated for *E. coli* exports for the sample sites and pooled data.

E. coli p-values for loadings	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	LD Pooled	HD Pooled	Natural
LD 1											
LD 2	0.7942										
LD 3	0.5399	0.2371									
LD 4	0.0806	<b>0.0286</b>	0.2907								
LD 5	0.1629	0.0663	0.4812	0.7059							
LD 6	<b>0.0290</b>	0.0542	<b>0.0192</b>	<b>0.0023</b>	<b>0.0147</b>						
HD 1	<b>0.0004</b>	<b>0.0003</b>	<b>0.0002</b>	<b>0.00001</b>	<b>0.0002</b>	0.2123					
HD 2	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.00001</b>	<b>0.00001</b>	0.1727	0.7171				
LD Pooled	0.6860	0.4208	0.6327	0.1908	0.4065	<b>0.0209</b>	<b>0.0001</b>	<b>0.00001</b>			
HD Pooled	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	<b>0.00001</b>	0.1623	0.8301	0.9282	<b>0.00001</b>		
Natural	0.5838	0.3861	0.9784	0.1845	0.4011	0.0221	<b>0.0003</b>	<b>0.0001</b>	0.8074	<b>0.00001</b>	
Sewer	0.9066	0.7627	0.4208	0.0701	0.1908	0.0627	<b>0.0007</b>	<b>0.0002</b>	0.7247	<b>0.0001</b>	0.5422

**Table 13:** P-values calculated for Enterococci exports for each sample site and for the pooled data.

Enterococci p-values for loadings	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	LD Pooled	HD Pooled	Natural
LD 1											
LD 2	1.0000										
LD 3	0.3545	0.1665									
LD 4	0.1967	0.1218	0.5377								

<b>LD 5</b>	<b>0.0439</b>	<b>0.0110</b>	0.2405	0.6764							
<b>LD 6</b>	0.0801	<b>0.0291</b>	<b>0.0077</b>	<b>0.0047</b>	<b>0.0015</b>						
<b>HD 1</b>	0.2087	0.1999	<b>0.0736</b>	<b>0.0180</b>	<b>0.0146</b>	0.5752					
<b>HD 2</b>	<b>0.0437</b>	<b>0.0342</b>	<b>0.0047</b>	<b>0.0023</b>	<b>0.0006</b>	0.9010	0.5508				
<b>LD Pooled</b>	0.5399	0.3143	0.6689	0.3266	0.0852	<b>0.0112</b>	0.8830	<b>0.0070</b>			
<b>HD Pooled</b>	0.0698	0.0527	<b>0.0037</b>	<b>0.0023</b>	<b>0.0004</b>	0.7626	0.7289	0.7057	<b>0.0053</b>		
<b>Natural</b>	0.2920	0.2387	0.9784	0.6452	0.3118	<b>0.0040</b>	<b>0.0334</b>	<b>0.0076</b>	0.8496	<b>0.0032</b>	
<b>Sewer</b>	0.8859	0.9398	0.3083	0.1047	<b>0.0446</b>	0.1086	0.3311	0.0724	0.5629	0.0942	0.2610

**Table 14:** P-values calculated for enterococci concentrations for the warm months.

<i>Enterococci p values Warm</i>	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	LD Pool
<i>LD 1</i>											
<i>LD 2</i>	0.5309										
<i>LD 3</i>	0.8345	0.4034									
<i>LD 4</i>	0.5309	1.0000	0.8345								
<i>LD 5</i>	1.0000	0.4034	1.0000	0.8345							
<i>LD 6</i>	0.6761	1.0000	0.8345	1.0000	0.6761						
<i>HD 1</i>	1.0000	1.0000	0.7656	1.0000	0.7656	1.0000					
<i>HD 2</i>	0.3913	0.3913	0.2703	0.5403	0.3913	0.9025	0.8597				
<i>Natural</i>	0.5309	1.0000	0.4034	0.8345	0.5309	1.0000	0.7656	0.7133			
<i>Sewer</i>	1.0000	0.6761	1.0000	1.0000	0.8345	0.8345	1.0000	0.3913	0.6004		
<i>LD Pool</i>	0.8345	0.5309	0.8245	0.8345	0.5309	0.8345	1.0000	0.5403	0.6761	1.0000	
<i>HD POOL</i>	0.2963	0.6761	0.2963	0.4034	0.4034	0.8345	0.8808	0.9017	1.0000	0.4034	0.6761

**Table 15:** P-values calculated for enterococci concentrations for the cold months.

<i>Enterococci p values Cold</i>	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	LD Pool
<i>LD 1</i>											
<i>LD 2</i>	0.3177										
<i>LD 3</i>	0.4619	0.7525									
<i>LD 4</i>	0.9581	0.6742	0.6742								
<i>LD 5</i>	0.6854	0.8622	0.9078	0.7282							
<i>LD 6</i>	<b>0.0074</b>	<b>0.0312</b>	<b>0.0238</b>	<b>0.0404</b>	<b>0.0427</b>						
<i>HD 1</i>	<b>0.0039</b>	<b>0.0181</b>	<b>0.0239</b>	<b>0.0136</b>	<b>0.0175</b>	0.4945					

<i>HD 2</i>	0.0520	0.3442	0.4005	0.2271	0.2243	0.1559	0.1036				
<i>Natural</i>	<b>0.0313</b>	0.1893	0.2271	0.2271	0.1832	0.0829	0.0520	0.5283			
<i>Sewer</i>	0.2072	0.7926	0.9581	0.5283	0.6431	0.0587	<b>0.0273</b>	0.4942	0.7929		
<i>LD Pool</i>	0.3710	0.7927	0.9581	0.4616	0.6428	<b>0.0271</b>	<b>0.0312</b>	0.2069	0.1715	0.6740	
<i>HD POOL</i>	<b>0.0019</b>	<b>0.0181</b>	<b>0.0181</b>	<b>0.0181</b>	<b>0.0128</b>	0.8747	0.7132	0.2271	<b>0.0406</b>	0.0661	<b>0.0101</b>

**Table 16:** P-values calculated for *E. coli* concentrations for the cold months.

<i>E. coli p-values</i> <i>Cold</i>	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	LD Pool
<i>LD 1</i>											
<i>LD 2</i>	0.8748										
<i>LD 3</i>	0.7132	0.7527									
<i>LD 4</i>	0.1278	0.1563	0.1278								
<i>LD 5</i>	0.6365	0.8748	0.3184	0.2072							
<i>LD 6</i>	<b>0.0406</b>	<b>0.0313</b>	<b>0.0239</b>	<b>0.0181</b>	<b>0.0239</b>						
<i>HD 1</i>	<b>0.0009</b>	<b>0.0009</b>	<b>0.0009</b>	<b>0.0009</b>	<b>0.0009</b>	<b>0.0313</b>					
<i>HD 2</i>	<b>0.0039</b>	<b>0.0046</b>	<b>0.0074</b>	<b>0.0019</b>	<b>0.0019</b>	0.3184	0.3720				
<i>Natural</i>	0.3720	0.1886	0.1556	<b>0.0406</b>	0.1278	0.0661	<b>0.0009</b>	<b>0.0117</b>			
<i>Sewer</i>	1.0000	1.0000	0.5635	0.1563	0.5635	<b>0.0239</b>	<b>0.0009</b>	<b>0.0063</b>	0.1278		
<i>LD Pool</i>	0.6365	0.5635	0.6365	<b>0.0406</b>	0.1893	<b>0.0239</b>	<b>0.0009</b>	<b>0.0074</b>	0.1563	0.3720	
<i>HD POOL</i>	<b>0.0028</b>	<b>0.0014</b>	<b>0.0014</b>	<b>0.0014</b>	<b>0.0019</b>	<b>0.0406</b>	0.7132	0.4948	<b>0.0028</b>	<b>0.0014</b>	<b>0.0014</b>

**Table 17:** P-values calculated for *E. coli* concentrations for the warmer months.

<i>E. coli p-values</i> <i>Warm</i>	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	LD Pool
<i>LD 1</i>											
<i>LD 2</i>	0.6761										
<i>LD 3</i>	0.5309	1.0000									
<i>LD 4</i>	0.6761	0.5309	0.6761								
<i>LD 5</i>	0.8345	0.8345	0.8345	1.0000							
<i>LD 6</i>	0.2948	0.4020	0.2948	0.4020	0.8340						

HD 1	<b>0.036</b> 9	<b>0.036</b> 9	<b>0.036</b> 9	0.073 6	0.177 1	0.174 5						
HD 2	0.036 7	0.094 7	0.060 1	0.094 7	0.464 7	1.000 0	0.099 0					
Natural	0.249 2	0.676 1	0.601 5	0.834 5	1.000 0	0.529 6	<b>0.036</b> 9	0.143 7				
Sewer	0.676 1	1.000 0	0.676 1	1.000 0	0.834 5	0.402 0	<b>0.036</b> 9	0.094 7	<b>0.0233</b>			
LD Pool	0.676 1	1.000 0	0.676 1	1.000 0	1.000 0	0.294 8	<b>0.036</b> 9	0.210 1	0.8345	0.834 5		
HD POOL	<b>0.021</b> 6	<b>0.021</b> 6	<b>0.021</b> 6	0.094 7	0.464 7	0.402 0	0.177 1	0.293 3	<b>0.0367</b>	<b>0.021</b> 6	<b>0.0367</b>	

**Table 18:** P-values for enterococci data for warm vs cold months.

Cold Months													
Warm Months	Enterococci	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	LD Pool	HD Pool
	LD 1 W	<b>0.01</b> 04	<b>0.03</b> 38	<b>0.04</b> 81	0.09 23	<b>0.03</b> 48	0.82 60	0.94 17	0.27 23	0.067 3	0.09 23	<b>0.023</b> 1	0.8262
	LD 2 W	<b>0.00</b> 43	<b>0.02</b> 33	<b>0.01</b> 57	<b>0.01</b> 04	<b>0.00</b> 58	0.27 16	0.60 84	<b>0.04</b> 81	<b>0.015</b> 7	<b>0.02</b> 33	<b>0.015</b> 6	0.3413
	LD 3 W	<b>0.00</b> 43	<b>0.02</b> 33	<b>0.01</b> 57	<b>0.01</b> 57	<b>0.00</b> 94	0.34 07	0.94 17	0.09 23	<b>0.023</b> 3	<b>0.02</b> 33	<b>0.015</b> 6	0.7144
	LD 4 W	<b>0.00</b> 68	<b>0.03</b> 38	<b>0.04</b> 81	<b>0.01</b> 57	<b>0.01</b> 49	0.60 79	0.71 44	0.12 43	0.092 3	0.06 73	<b>0.033</b> 5	0.6084
	LD 5 W	<b>0.00</b> 68	<b>0.02</b> 33	<b>0.03</b> 38	<b>0.03</b> 38	<b>0.02</b> 30	0.42 01	1.00 00	0.14 27	<b>0.023</b> 3	<b>0.04</b> 01	<b>0.015</b> 6	0.6084
	LD 6 W	<b>0.02</b> 33	0.05 67	0.09 23	<b>0.03</b> 38	0.07 40	0.51 01	0.71 44	0.21 34	0.164 3	0.06 73	0.123 8	0.6084
	HD 1	<b>0.03</b> 21	0.08 27	0.08 27	0.08 27	0.06 82	0.60 90	0.75 95	0.26 16	0.184 6	0.18 46	0.082 0	0.6098
	HD 2	<b>0.00</b> 85	<b>0.03</b> 38	<b>0.01</b> 38	<b>0.01</b> 38	<b>0.01</b> 07	0.20 19	0.35 02	<b>0.03</b> 38	<b>0.021</b> 9	<b>0.03</b> 38	<b>0.013</b> 6	0.1488
	Natural	<b>0.00</b> 43	<b>0.01</b> 57	<b>0.01</b> 04	<b>0.01</b> 04	<b>0.00</b> 58	0.09 18	0.42 08	<b>0.02</b> 33	<b>0.010</b> 4	<b>0.01</b> 57	<b>0.010</b> 3	0.2134
	Sewer	<b>0.00</b> 68	<b>0.03</b> 38	<b>0.03</b> 38	<b>0.03</b> 38	<b>0.01</b> 49	0.60 79	1.00 00	0.16 43	<b>0.048</b> 1	0.06 73	<b>0.015</b> 6	0.7144
	LD Pool	<b>0.00</b> 43	<b>0.01</b> 57	<b>0.01</b> 57	<b>0.01</b> 57	<b>0.00</b> 94	0.16 37	0.71 44	0.06 73	<b>0.015</b> 7	<b>0.01</b> 57	<b>0.015</b> 6	0.4208
	HD Pool	<b>0.00</b> 43	<b>0.02</b> 33	<b>0.00</b> 68	<b>0.00</b> 68	<b>0.00</b> 58	0.16 37	0.34 13	<b>0.01</b> 57	<b>0.010</b> 4	<b>0.02</b> 33	<b>0.006</b> 7	0.1243

**Table 19:** P-values for *E. coli* data for warm vs cold months.

Cold Months													
Warm	E.coli	LD 1	LD 2	LD 3	LD 4	LD 5	LD 6	HD 1	HD 2	Natural	Sewer	LD Pool	HD Pool

LD 1	0.06 73	0.09 23	0.06 73	<b>0.04</b> <b>81</b>	<b>0.03</b> <b>38</b>	0.71 44	<b>0.02</b> <b>33</b>	0.16 43	0.420 8	0.06 73	0.124 3	0.0673
LD 2	<b>0.04</b> <b>81</b>	<b>0.04</b> <b>81</b>	<b>0.01</b> <b>27</b>	<b>0.02</b> <b>33</b>	<b>0.02</b> <b>33</b>	0.82 62	0.67 30	0.16 43	0.213 4	<b>0.03</b> <b>38</b>	<b>0.023</b> <b>3</b>	0.1643
LD 3	<b>0.04</b> <b>81</b>	<b>0.04</b> <b>81</b>	<b>0.01</b> <b>04</b>	<b>0.02</b> <b>33</b>	<b>0.02</b> <b>33</b>	0.82 62	0.06 73	0.16 43	0.272 3	<b>0.02</b> <b>33</b>	<b>0.010</b> <b>4</b>	0.1243
LD 4	0.09 23	0.09 23	0.06 73	0.06 73	0.09 23	0.94 17	0.16 43	0.42 08	0.124 3	0.12 43	0.092 3	0.2723
LD 5	0.51 01	0.51 01	0.51 01	0.24 09	0.42 08	0.82 62	0.82 62	0.94 17	0.510 1	0.42 08	0.608 4	0.8262
LD 6	0.09 18	0.09 18	0.09 18	0.06 69	0.06 69	0.09 18	0.71 40	0.42 01	0.091 8	0.09 18	0.091 8	0.5095
HD 1	<b>0.01</b> <b>89</b>	<b>0.01</b> <b>89</b>	<b>0.01</b> <b>89</b>	<b>0.01</b> <b>89</b>	<b>0.01</b> <b>89</b>	<b>0.01</b> <b>89</b>	<b>0.03</b> <b>21</b>	<b>0.03</b> <b>21</b>	<b>0.018</b> <b>9</b>	<b>0.01</b> <b>89</b>	<b>0.018</b> <b>9</b>	<b>0.0189</b>
HD 2	<b>0.00</b> <b>43</b>	<b>0.00</b> <b>43</b>	<b>0.00</b> <b>43</b>	<b>0.00</b> <b>43</b>	<b>0.00</b> <b>43</b>	<b>0.01</b> <b>57</b>	0.42 08	0.12 43	<b>0.004</b> <b>3</b>	<b>0.00</b> <b>43</b>	<b>0.004</b> <b>3</b>	0.4208
Natural	<b>0.01</b> <b>57</b>	<b>0.02</b> <b>33</b>	<b>0.01</b> <b>04</b>	<b>0.01</b> <b>04</b>	<b>0.01</b> <b>04</b>	0.51 01	0.21 34	0.60 84	0.067 3	<b>0.02</b> <b>33</b>	<b>0.023</b> <b>3</b>	0.4208
Sewer	<b>0.04</b> <b>81</b>	<b>0.03</b> <b>38</b>	<b>0.04</b> <b>81</b>	<b>0.01</b> <b>57</b>	<b>0.02</b> <b>33</b>	0.82 62	0.06 73	0.27 23	0.164 3	<b>0.04</b> <b>81</b>	<b>0.048</b> <b>1</b>	0.1243
LD Pool	<b>0.04</b> <b>81</b>	0.06 73	<b>0.04</b> <b>81</b>	<b>0.01</b> <b>57</b>	<b>0.02</b> <b>33</b>	0.82 62	0.21 34	0.51 01	0.272 3	<b>0.04</b> <b>81</b>	<b>0.048</b> <b>1</b>	0.2723
HD POOL	<b>0.00</b> 43	<b>0.00</b> 43	<b>0.00</b> 43	<b>0.00</b> 43	<b>0.00</b> 43	<b>0.00</b> 68	0.06 73	0.06 73	<b>0.004</b> 3	<b>0.00</b> 43	<b>0.004</b> 3	<b>0.0338</b>

**Table 20:** P-values for the *E. coli* concentrations for thr sand filter watersheds in comparison to the conventional style watersheds, the Natural watershed, and the Sewer watershed.

<i>E.coli</i> <i>p-values</i> <i>concentration</i> <i>n</i>	SF 1	SF 2	SF3	LD 3	LD 4	LD 6	SF Pool	LD Pool	Sewer
<i>SF 1</i>									
<i>SF 2</i>	0.1679								
<i>SF 3</i>	<b>0.0035</b>	0.2815							
<i>LD 3</i>	<b>0.0035</b>	0.9754	0.0605						
<i>LD 4</i>	<b>0.0289</b>	0.9755	0.2855	0.1409					
<i>LD 6</i>	0.5180	0.4416	<b>0.0463</b>	0.2853	0.1748				
<i>SF Pool</i>	0.0684	0.9368	0.1012	0.6982	0.6616	0.3612			
<i>LD Pool</i>	<b>0.0176</b>	0.7919	<b>0.0394</b>	0.8769	0.2384	0.3113	0.9766		
<i>Sewer</i>	<b>0.0106</b>	0.7350	<b>0.0351</b>	0.8398	0.0885	0.3407	0.9900	0.6944	
<i>Natural</i>	<b>0.0488</b>	0.6444	<b>0.0166</b>	0.2985	0.0885	0.6440	0.6706	0.3469	0.6650

**Table 21:** P-values for the enterococci concentrations for the sand filter watersheds, the conventional style watersheds the Natural watershed, the Sewer watershed, and the pooled watersheds.

<i>Enterococci</i> <i>p-values</i> <i>concentration</i> <i>n</i>	SF 1	SF 2	SF3	LD 3	LD 4	LD 6	SF Pool	LD Pool	Sewer
<i>SF 1</i>									
<i>SF 2</i>	<b>0.0126</b>								
<i>SF 3</i>	<b>0.0010</b>	1.0000							
<i>LD 3</i>	<b>0.0062</b>	0.4791	0.2854						
<i>LD 4</i>	<b>0.0210</b>	0.6444	0.5443	0.8399					
<i>LD 6</i>	<b>0.0151</b>	0.4060	0.4024	0.8852	0.7508				
<i>SF Pool</i>	<b>0.0151</b>	0.3217	0.1764	0.7356	0.6435	0.7928			
<i>LD Pool</i>	<b>0.0021</b>	0.3999	0.2893	0.8770	0.8210	0.9525	0.6092		
<i>Sewer</i>	<b>0.0151</b>	0.4601	0.2144	0.9310	0.5067	0.8173	0.9005	0.6682	
<i>Natural</i>	<b>0.0151</b>	0.4060	0.2854	0.8852	0.6236	0.8399	0.8708	0.7121	0.8625

**Table 22:** P -values for the enterococci exports for the sand filter watersheds, the conventional style watersheds the Natural watershed, the Sewer watershed, and the pooled watersheds.

<i>Enterococci</i> <i>loading p</i> <i>values</i>	<i>SF 1</i>	<i>Sf 2</i>	<i>SF3</i>	<i>LD 3</i>	<i>LD 4</i>	<i>LD 6</i>	<i>SF</i> <i>Pool</i>	<i>LD</i> <i>Pool</i>	<i>Sewer</i>
<i>SF 1</i>									
<i>Sf 2</i>	0.1309								
<i>SF3</i>	<b>0.0033</b>	0.4996							
<i>LD 3</i>	0.0927	0.8036	<b>0.0531</b>						
<i>LD 4</i>	0.0443	0.8036	0.5067	0.4025					
<i>LD 6</i>	0.7237	0.0903	<b>0.0251</b>	0.0993	0.0832				
<i>SF Pool</i>	<b>0.0538</b>	0.7830	0.1399	0.7555	0.5424	0.0768			
<i>LD Pool</i>	0.1110	0.6043	<b>0.0496</b>	0.9030	0.3362	0.1271	0.6123		
<i>Sewer</i>	0.2225	0.5458	0.0606	0.7508	0.2602	0.2908	0.5424	0.7350	
<i>Natural</i>	0.1041	0.9025	0.1563	0.8691	0.7169	<b>0.0359</b>	0.9637	0.5744	0.5752

**Table 23:** P -values for the *E. coli* exports for the sand filter watersheds, the conventional style watersheds the Natural watershed, the Sewer watershed, and the pooled watersheds.

<i>E.coli</i> <i>loading</i> <i>p</i> <i>values</i>	<i>SF 1</i>	<i>Sf 2</i>	<i>SF3</i>	<i>LD 3</i>	<i>LD 4</i>	<i>LD 6</i>	<i>SF</i> <i>Pool</i>	<i>LD</i> <i>Pool</i>	<i>Sewer</i>
<i>SF 1</i>									



<i>Sf 2</i>	0.2057								
<i>SF3</i>	<b>0.0033</b>	0.241							
<i>LD 3</i>	0.1985	0.7491	0.0606						
<i>LD 4</i>	<b>0.0076</b>	0.594	0.3708	0.3708					
<i>LD 6</i>	0.5259	0.1384	<b>0.0046</b>	0.0572	<b>0.0251</b>				
<i>SF</i>	0.0661	0.9871	0.0959	0.8391	0.3789	<b>0.0384</b>			
<i>Pool</i>									
<i>LD</i>	0.1043	0.9742	<b>0.0255</b>	0.8602	0.2496	0.0622	0.6728		
<i>Pool</i>									
<i>Sewer</i>	0.2766	0.7491	<b>0.0226</b>	0.655	0.126	0.1179	0.4565	0.6747	
<i>Natural</i>	0.1212	0.9025	0.0698	0.7667	0.1985	0.071	0.7964	0.9154	0.5752

**Table 24:** P-values for E.coli concentrations for Pooled watersheds for sand filter and conventional style OWS watersheds, and the Natural and Sewer watersheds for the warm and cold months.

<i>E. coli p-values Warm vs. Cold</i>	<i>SF Pool C</i>	<i>LD Pool C</i>	<i>Natural C</i>	<i>Sewer C</i>
<i>SF Pool W</i>	1.000	1.000	0.5284	1.000
<i>LD Pool W</i>	0.2933	0.2073	0.8335	0.4005
<i>Natural W</i>	<b>0.0586</b>	<b>0.0356</b>	0.0927	<b>0.0208</b>
<i>Sewer W</i>	0.2933	<b>0.0356</b>	0.1412	<b>0.0208</b>

**Table 25:** P-values for Enterococci concentrations for Pooled watersheds for sand filter and conventional style OWS watersheds, and the Natural and Sewer watersheds for the warm and cold months.

<i>Enterococci p-values Warm vs. Cold</i>	<i>SF Pool C</i>	<i>LD Pool C</i>	<i>Natural C</i>	<i>Sewer C</i>
<i>SF Pool W</i>	0.2933	<b>0.0117</b>	0.2073	<b>0.0356</b>
<i>LD Pool W</i>	<b>0.0586</b>	<b>0.0117</b>	0.0927	<b>0.0356</b>
<i>Natural W</i>	<b>0.0586</b>	<b>0.0117</b>	<b>0.0117</b>	<b>0.0117</b>
<i>Sewer W</i>	0.0927	<b>0.0117</b>	<b>0.0586</b>	<b>0.0356</b>

