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# Environmental Impacts on Enterococcus in Shem Creek, South Carolina, and Characterization of Changing Land Use

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ENVIRONMENTAL IMPACTS ON *ENTEROCOCCUS* IN SHEM CREEK, SOUTH CAROLINA, AND CHARACTERIZATION OF CHANGING LAND USES

by

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## Abstract

Zoning and land-use practices have a direct influence on hydrologic systems and can impact water resources. Linkages between change in land uses and degradation of water quality in stream and watershed environments have been well established. Shem Creek, located in Mount Pleasant, SC, has a history of fecal indicator bacteria levels that exceed the Environmental Protection Agency's recreational water quality standards. With recent coastal population and development trends, proper management and the sustainability of beach and estuary environments are a rising public health concern. The objective of this study is to determine what climatic and water quality parameters are associated with *Enterococcus* density levels and to characterize the changes in zoning between 2010 and 2017 in the Shem Creek watershed. Public health implications of development and impaired waters are also addressed. Geographic Information Systems allowed for analysis of changes in zoning in the Shem Creek watershed between 2010 and 2017. Multivariate partial least squares regression was used to determine statistically significant correlations between *Enterococcus* density levels and the following predictor variables: water quality monitoring station location; month; water temperature, height, and specific conductance; precipitation collected at two locations for 1, 2, and 3 days leading up to *Enterococcus* sampling; and number of septic tanks located within a 0.5 and 1 mile radius of each water quality monitoring station. Because the amount of impervious surface is directly related to water quality degradation, a change from zoning categories associated with permeable surface to zoning categories associated with impervious surface was calculated. This equated to 3.2% of the total land area in the Shem Creek watershed that changed from agricultural, recreational, vacant, or undevelopable to commercial or residential. Results indicate that *Enterococcus* density levels have increased over time and that precipitation and water height are positively correlated with bacteria levels in Shem Creek. In addition, stations located further inland, where the creek was surrounded by extensive marsh, had higher concentrations of *Enterococcus* compared with stations located near the outflow of the creek into the harbor surrounded by seawalls. Understanding what parameters are associated with increasing *Enterococcus* density levels in Shem Creek will allow for future mitigation procedures to be implemented, protecting ecosystem services and the public's health.

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## List of Abbreviations

CV ................................................................ Coefficient of Variation (SAS code)

DEM ................................................................ Digital Elevation Model

EPA ................................................................ U.S. Environmental Protection Agency

GLM ................................................................ General Linear Model (SAS code)

LIDAR ................................................................ Light Detection and Ranging

MPN ................................................................ Most Probable Number

NOAA ................................................................ National Oceanic and Atmospheric Administration

PLS ................................................................ Partial Least Squares (SAS code)

PRESS ................................................................ Predicted Residual Error Sums of Squares

PROC ................................................................ Procedure (SAS code)

REG ................................................................ Regression (SAS code)

SAS ................................................................ Statistical Analysis Software

SCDHEC ................................................................ South Carolina Department of Health and Environmental Control

SCDNR ................................................................ South Carolina Department of Natural Resources

VIP ................................................................ Variable Importance for Projection

# Chapter 1

## Introduction

Coastal shoreline counties in the United States (U.S.) account for 39% of the total U.S. population and have grown steadily in recent decades (Crossett, Ache, Pacheco, Haber, & National Oceanic and Atmospheric Administration, 2013). Population trends indicate that there has been an increase of 40 million people living in coastline counties in the U.S. between years 1960 and 2008 (Wilson, Fischetti, & U.S. Census Bureau, 2010). With population increases, development and urbanization of coastal areas will play a fundamental role in the changes that occur within these coastal environments (Brown, Johnson, Loveland, & Theobald, 2005). Anthropogenic changes to coastal surroundings in the U.S. are increasing pollution, stimulating biological changes, and compromising the sustainability and function of coastal ecosystems (Mallin, Williams, Esham, & Lowe, 2000). The abundant supply of water in the form of streams, rivers, wetlands, and lakes offers a rich source for outdoor recreational activities and has significantly contributed to the development and growth of coastal state economies (Haley, Parrish, Gaines, & South Carolina Department of Parks, Recreation, and Tourism, 2014). In South Carolina (SC), beaches and coastal towns are the state's greatest attraction for the travel and tourism industry. However, the population in SC coastal shoreline counties continues to increase (Wilson et al., 2010), and such growth will result in transformation of forested, un-developed land to residential areas, shopping establishments, industrial sites, and transportation structures (Holland et al., 2004), which can adversely affect the environmental quality of the state's most precious resource.

Types of land uses and zoning have a direct influence on hydrologic systems and can impact water resources (Lee, Hwang, Lee, Hwang, & Sung, 2009; Tong & Chen, 2002). Linkages between change in land uses and degradation of water quality in stream and watershed environments have been established in many studies (DeFries & Eshleman, 2004; DiDonato et al., 2009; Foley et al., 2005; Kelsey, Porter, Scott, Neet, & White, 2004; Nelson, Scott, & Rust, 2005; Schoonover & Lockaby, 2006; Van Dolah et al., 2008). Impervious surface cover (e.g., parking lots, roads, buildings) can cause surface water to run directly into streams, rather than soaking into vegetation and soil where it would undergo natural filtration (Holland et al., 2004). Urbanization presents a unique threat to estuaries and coastal marshes that tend to be shallow where the rivers or streams do not have adequate volumes of water to flush out pollutants (Vernberg, 1997). Studies by Sanger, Holland, and Scott (1999b) indicate that when impervious cover exceeds 10-20% of the inland region of the watershed near the headwaters, there are changes in hydro-geography, salinity, sediment characteristics, and contaminant levels. Van Dolah et al. (2007) documented that over 77% of the sites they sampled in SC watersheds with >50% urban/suburban land cover had elevated sediment contaminant concentrations; such results compared with only 27% of the sites they sampled with ≤30% urban/suburban land cover. Elevated levels of fecal coliform bacteria have also been associated with urbanization and anthropogenic activity. Bacterial pollution affecting estuaries, inlets, streams, and rivers is a rising environmental and public health concern in coastal zones throughout the U.S., especially in Southern regions with warmer water temperatures. The elements that can contribute to bacterial growth and survival in marine waters include salinity, temperature, predation, sunlight, toxic substances, and nutrients. Estuaries offer ideal ranges for these factors and can additionally allow for stresses in temperature or salinity, which might normally affect bacteria survival negatively; however, such stresses are neutralized by the high nutrient content, enabling persistent bacterial survival and growth (Apple, del Giorgi, & Kemp, 2006; Hendrickson, Wong, Allen, Ford, & Epstein, 2001; Singleton, Attwell, Jangi, & Colwell, 1982).

Fecal bacteria including fecal coliform, *Escherichia coli*, and *Enterococcus* serve as indicator species for health risk assessments and fecal bacteria pollution in water and sediment bodies (Meays, Broersma, Nordin, & Mazumder, 2004). Fecal bacteria growth is shown to increase in warmer temperatures making it a particular concern for the SC coast where average water temperatures stay above 70°F for seven months and above 60°F for nine months out of the year (Howell, Coyne, & Cornelius, 1996; National Oceanic and Atmospheric Administration, 2017). In 1976, the U.S. Public Health Service and the U.S. Environmental Protection Agency (EPA) recommended fecal coliform bacteria as an indicator for fecal bacterial contamination. The EPA later evaluated the use of multiple organisms—including fecal coliform, *E. coli*, and *Enterococcus*—for fecal indicator bacteria in epidemiological studies. These studies revealed that *E. coli* are good predictors for gastrointestinal illness in freshwater and enterococci are good predictors in marine and fresh recreational waters. The genus *Enterococcus* consists of gram-positive, anaerobic organisms that are ovoid in shape and that are the current recommended fecal indicator bacteria for marine and fresh recreational water standards published by the EPA (Environmental Protection Agency, 2012; Murray, 1990). In a study by Noble, Moore, Leecaster, McGee, and Weisberg (2003) that tested three indicator species (*Enterococcus*, total coliform, and fecal coliforms), *Enterococcus* was the indicator that exceeded the recreational bacterial water quality standards the most.

Enterococci are part of the naturally occurring gastrointestinal flora that live in the intestinal tracks of humans and wildlife. The public health concern occurs when these bacteria contaminate recreational waters or waters where filter-feeding shellfish may be harvested for human consumption. Swimmers are exposed to contaminants in water that can easily enter the ears, eyes, nose, mouth, and other bodily openings as well as through cuts or skin abrasions (Hendrickson et al., 2001). High fecal bacteria levels have also been reported in the sand of wave-wash zones at public bathing beaches (Alm, Burke, & Spain, 2003). Gastrointestinal illness and infections of the ear, eye, respiratory tract, urinary tract, or skin among swimmers are directly associated with marine exposure and marine bacterial counts (Balarajan, Raleigh, Yuen, & Machin, 1992; Prieto et al., 2001; Pruss, 1998; Seyfried, Tobin, Brown, & Ness, 1985). Medical costs from these illnesses due to marine exposures and the economic loss from beach closures and advisories because of high bacteria levels in the water contribute substantially to public health burdens in the United States (Given, Pendleton, & Boehm, 2006). Through gene transfer, *Enterococcus* organisms have become inherently resistant to a number of antimicrobial agents (Moellering, 1992). Exposure to antibiotics in the environment from agricultural facilities and improper human disposal has generated the emergence of resistant enterococci. Antibiotic resistant bacteria are of particular concern in hospital settings or among vulnerable populations with weakened immune systems.

Bacterial contamination also poses a threat to marine organisms living in or around coastal environments. Shallow tidal creeks and salt marshes act as nursery habitat for fish and shellfish and provide feeding grounds for birds and predatory fish. Shellfish such as oysters, clams, and mussels absorb nutrients by filtering water, thus absorbing bacteria or other contaminants that may be in the water. These pollutants can become concentrated in the shellfish, making them dangerous for raw human consumption (Nelson et al., 2005). As a result, the South Carolina Department of Health and Environmental Control (SCDHEC) has a shellfish harvesting monitoring program that helps ensure the shellfish that are harvested meet the health and environmental quality standards provided by federal recommendations and state guidelines (SC Department of Health and Environmental Control, 2017d).

In addition to the shellfish monitoring program, SCDHEC has established the ambient surface water quality monitoring program and the beach water quality monitoring program. Each of these programs has its own standards and purpose but all were created to meet the health and environmental quality standards provided by federal guidelines and state regulations. SCDHEC's standardized limit for enterococci in Class SB tidal saltwater is 35 MPN (most probable number) per 100 ml (monthly average) and 501 MPN per 100 ml (daily maximum). In shellfish harvesting areas SCDHEC's standardized limit for fecal coliform is 14 MPN per 100 ml (monthly average) and 43 MPN per 100 ml (daily maximum). For enterococci in Class SA saltwater, the standard is 35 MPN per 100 ml (monthly average) and 104 MPN per 100 ml (daily maximum). Class SA and SB tidal saltwater are suitable for primary and secondary contact recreation, marine habitat and reproduction, crabbing and fishing. These waters are not protected for harvesting of clams, mussels, or oysters for market purposes or human consumption. Class SA waters must maintain a higher dissolved oxygen level than Class B waters and lower levels of single sample *Enterococcus* (SC Department of Health and Environmental Control, 2014). The shellfish harvesting monitoring program provides a database that is used to annually evaluate shellfish growing areas. This program includes 465 sample sites along the coast of South Carolina located in non-prohibited classified shellfish areas (SC Department of Health and Environmental Control, 2017d). Shem Creek is classified as a Class SB water body (SC Department of Health and Environmental Control, 2017c). SCDHEC's beach monitoring program consists of 123 beach-water monitoring stations that test for *Enterococcus* bacteria. The program began monitoring state beaches routinely as a result of the federal Beaches Environmental Assessment and Coastal Health Act of 2000. If high numbers of bacteria are found (>501MPN), a swimming advisory for that portion of the beach is issued. If bacteria levels are above 104 MPN but below 501 MPN, the sample will be re-tested. However, advisories do not mean the beach is closed. Advisories are lifted when sample results fall below 104 MPN per 100 ml. Samples are only taken during the swimming season (May 1 to October 1) (SC Department of Health and Environmental Control 2017a). SCDHEC's ambient surface water quality monitoring program takes a large variety of water quality indicator measurements (including fecal indicator bacteria) and creates a database used to understand the conditions of water bodies, how they can be improved, where closer attention needs to be focused, and how permit limits for water discharge can be framed. This program includes 145 permanent sites and additional sites chosen each year in both fresh and saltwater environments (SC Department of Health and Environmental Control, 2017b). Several ambient surface water quality monitoring sites are located in Shem Creek and will be used in this study (SC Department of Health and Environmental Control, 2017c).

With recent coastal population and development trends, proper management and the sustainability of beach and estuary environments must remain a priority. Public policies for land use and water quality are progressively more interconnected (Abdalla, 2008). For example, wastewater treatment plants are required to meet technology-based standards; farmers are encouraged to use best management practices that emphasize fertilizer use and crop cover; and residential and commercial developers are encouraged to control or manage stormwater runoff and prevent leaky septic systems. Zoning categories—including commercial, industrial, residential, and agricultural sectors—often incorporate policies limiting the number of buildings per acre and could be an approach used for targeting land use in areas with compromised water quality. However, in some areas concentrated development may actually have lower stormwater runoff compared with large areas that are developed and more spread out; thus, policies that target effective water quality improvement are not always clear (Walls & McConnell, 2004). In addition, there are many different potential sources for bacterial contamination, both point and non-point. Sources of human waste include improper disposal from waste water treatment plants, poorly maintained septic systems, malfunctioning or failing sewer infrastructure, and improper disposal of waste from marine boats (Scalf & Dunlap, 1997). The South Carolina Department of Natural Resources implemented the Clean Vessel Act Program in 1992, supporting a portion of the costs associated with the operation and maintenance of shore-side and mobile pump facilities for boats (SC Department of

Natural Resources, 2016). Nevertheless, it is up to the boater to follow recommended guidelines for waste disposal. Agricultural facilities can also be a source for bacterial pollution in water due to storm water runoff. Wildlife and pet waste also contribute substantially to bacterial contamination of waterways (Harwood, Whitlock, & Withington, 2000). Despite the complexity of dealing with such multiple, varied sources of contamination, there are several methods that can be used for microbial source tracking to determine if the bacterial pollution is predominately anthropogenic or from other animals (Scott, Rose, Jenkins, Farrah, & Lukasik, 2002).

Although many studies have looked at the relationship between change in land uses and bacteria levels in marine waters, no studies have been published with a detailed characterization of the bacterial levels and land uses surrounding Shem Creek. Shem Creek, located in Mount Pleasant, SC, has had a history of fecal indicator bacteria levels that exceed the EPA's recreational water standards. Sanger, Holland, and Scott (1999b) documented that in their study of 28 tidal creeks along the SC coast, Shem Creek had the highest population density and largest percent of impervious surface.

## 1.1 Thesis Statement

The objective of this study is to investigate associations with higher *Enterococcus* density levels in Shem Creek and to characterize the changes in zoning between 2010 and 2017 in the Shem Creek watershed. Public health implications of development and impaired waters are also addressed. The null hypothesis is that there will be no associations between *Enterococcus* density levels in Shem Creek and selected water quality parameters, climatic occurrences, or other observations. A corollary of the null hypothesis is that that there were no significant changes in zoning from 2010 to 2017 in the Shem Creek watershed. The alternative hypothesis is that there will be an association between *Enterococcus* density levels in Shem Creek and selected water quality parameters, climatic occurrences, or other observations. The alternative hypothesis also has a corollary that zoning in the Shem Creek Watershed between 2010 and 2017 increased in developed impervious area and decreased in vacant or undeveloped permeable area.

## 1.2 Study Area

Shem Creek is a tidal creek that empties into the Charleston Harbor on the coast of South Carolina. It is known for its beautiful views, boardwalks, recreational use, boating, and restaurants. Shem Creek runs though the town of Mt. Pleasant, which is characterized by residential, commercial, agricultural, recreational, and other specialty types of land use. The Shem Creek watershed is approximately 11.8 $km^2$. Mt. Pleasant had an estimated population of 82,215 in 2016, and population has been growing exponentially since the 1960s. In the year 2016, 1,377 new dwellings were built and 1,622 were permitted (Town of Mount Pleasant Department of Planning and Development, 2017).

Shem Creek was chosen as the study area because it has had persistently high bacterial levels that surpass the recreational water quality standards. In 2016 SCDHEC’s list of impaired waters listed Shem Creek with a priority 1 ranking for TMDL development (SC Department of Health and Environmental Control, 2016). It is also a popular destination for recreation and tourism. No direct sources have been established for being the cause of bacterial contamination. There are no wastewater treatment facility outflows into the creek. Shem Creek is a popular docking site for recreational boating, fisherman, and shrimp boats, so improper marine disposal of human waste and also leaky septic tanks along the creek could be contributing factors towards the elevated fecal bacteria levels. Pet and wildlife waste introduced via stormwater runoff is also a concern.

Shem Creek has been a site for shipbuilding, mill production, and factories, providing varied economic support to the surrounding area throughout history (Moultrie News, 2014). Shrimping became Shem Creek's main industry in the 1930s when Captain C. Magwood became the first fisherman to bring an ocean shrimp trawler into Mount Pleasant. A bridge was built over Shem Creek and docks were constructed, allowing the creek to develop into a major docking site for fisherman and shrimpers. Up to 70 shrimp trawlers operated off these docks. Over time, this number has decreased significantly because of increases in property tax and docking expenses. Today, Shem Creek is known for its restaurants, bars, and recreation. Only ten fish and shrimp companies remain actively working out of Shem Creek (Town of Mount Pleasant, n.d.).

This study first describes the methodology of determining a watershed for Shem Creek and how geospatial zoning data were used to analyze changes in zoning over time. Next, methodology of statistical analyses specifies positive and negative correlations between water quality parameters; climatic factors, such as precipitation; location of *Enterococcus* bacteria sampling; and *Enterococcus* density levels in Shem Creek. Finally, results from statistical analyses performed are presented, concluding that *Enterococcus* density levels in Shem Creek have increased over time. In addition, the research shows that precipitation and water height are drivers for *Enterococcus* bacteria levels in Shem Creek, with more concentrated bacterial pollution towards the headwaters as opposed to the outflow of the creek.

# Chapter 2

# Methods

Methodology used for identifying an appropriate watershed for Shem Creek, determining changes in zoning over time, and all statistical analyses performed in the study are outlined in Chapter two.

## 2.1 Watershed Selection

There were three identified potential watersheds delineated for Shem Creek that were taken into consideration before choosing the appropriate watershed for this study. A watershed was delineated from a digital elevation model (DEM), using ArcGIS Pro's watershed tool. The Light Detection and Ranging (LIDAR) derived DEM was collected from the National Oceanic and Atmospheric Administration's (NOAA) Office for Coastal Management and their Digital Coast Partnership Program. The outflow point designated for the watershed was placed at the mouth of Shem Creek where it begins to flow into the Charleston Harbor. In addition to the watershed delineated using ArcGIS Pro's watershed tool, there was a watershed boundary created by Charleston Waterkeeper, using visual imagery. The selected watershed used for this study was derived from the Town of Mount Pleasant Public Service Department's Stormwater Division (Figure 2.1). This watershed was created by on-the-ground mapping of the hydrologic piping systems throughout the town. Because it takes into account the man-made water pumping systems and water flow direction, this watershed was selected as the best watershed to use for this study.

## 2.2 Land Use and Zoning

Zoning data shapefiles for years 2010 and 2017 were acquired from Berkeley, Charleston, and Dorchester Council of Government's GIS office and clipped to the boundaries of the Shem Creek watershed. Because the town of Mount Pleasant is highly developed, detailed zoning data were used instead of generalized land cover maps. The zoning shapefiles include description of each parcel for Charleston County. In the Shem Creek watershed, 20 different property classifications were listed for year 2010, and 36 property classifications were listed for year 2017. Based on the details of the land use files obtained, a new field or zoning classification system that made the 2010 and 2017 zoning files comparable was created using ArcGIS Pro. This field consisted of seven categories: residential, commercial, recreational, agricultural, vacant, undevelopable, and other (Appendix B). The summarize tool in ArcGIS Pro was used to sum the square area of land in each category. The percent of each category within the Shem Creek watershed was derived by dividing the land area of each category into the total land area of the watershed. In addition, a spatial join between the 2010 and 2017 zoning files was created. Using the summarize tool, the sum of land area within the new land use field classifications that changed from 2010 to 2017 was calculated. This allowed for determination of how much land changed from one category (e.g., vacant) to another (e.g., residential). Final production of color-coded maps to create visual representation of this change was created and exported from ArcGIS Pro.

## 2.3 Variable Selection

Values for *Enterococcus* density were obtained from four water quality monitoring stations in Shem Creek (Figure 2.2). Charleston Waterkeeper has measured

*Enterococcus* density levels in recreational swimming areas around Charleston on a weekly basis during the months of May through October since in 2013. Charleston Waterkeeper has three water quality monitoring stations located in Shem Creek (Shem Creek station 1, 2, and 3). SCDHEC previously measured *Enterococcus* as part of routine surveys for its ambient water quality monitoring program in one location within Shem Creek (RT-10116 station). In 2010, *Enterococcus* density levels were collected monthly from SCDHEC's water quality monitoring station. *Enterococcus* data was not collected for years 2011 and 2012. Observed *Enterococcus* densities were calculated using standardized methods. A total of 372 water sample results were included in the analysis: 13 readings from site RT-10116; 120 readings from Shem Creek 1; 119 readings from Shem Creek 2; and 120 from Shem Creek 3. The station, date, and time were recorded for each sample. SCDHEC's standardized limit for enterococci in Class SB tidal saltwater, which is a monthly average of 35 MPN per 100 ml and a daily maximum of 501 MPN per 100 ml, was used to complete analyses in this study (SC Department of Health and Environmental Control, 2014).

Water temperature (°C) and specific conductance (µS/cm) were collected from a U.S. Geological Survey's water quality monitoring station located in the Cooper River near the U.S. Customs House in downtown Charleston. Values were collected every 15 minutes. The nearest value to the time of *Enterococcus* sample collection was used. Verified water height (ft) was collected by a NOAA water quality monitoring station, also located in the Cooper River near the U.S. Customs House in downtown Charleston. Values were recorded every six minutes. The closest value to the time of each *Enterococcus* sample was used. Daily summaries of rainfall (inches) were collected from two NOAA site locations: one in downtown Charleston at the U.S. Customs House and the other at the Charleston International Airport located in North Charleston. Because most *Enterococcus* density samples were collected in the mornings, rainfall data were summed by the total number of inches of rain during the previous day, two days, or three days leading up to *Enterococcus* sample collection. The number of septic tanks was approximated by those businesses or homes that were not connected to the municipal sewage system but that had running water (Figure 2.3). The number of septic tanks located within a half mile and a mile radius of each *Enterococcus* water quality sampling station was calculated.

## 2.4 Statistical Analysis

Analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC). *Enterococcus* data were ln(x) transformed prior to analysis to obtain normality and homoscedasticity. The natural log was chosen because it best represents the way that bacteria multiply in the water. Helsel's Robust Method was used to assign a value to any *Enterococcus* density measurements that were below the limit of detection (<10MPN/100ml). This method has been frequently used and is well established for dealing with non-detection values in water quality samples (Helsel & Cohn, 1988; Newman, 1995). The methods consisted of a series of steps in order to assign a value based on a normal distribution curve for those values under the limit of detection. First, the data were ranked and transformed to compute normal scores from the ranks. The resulting ranks appear normally distributed (SAS Institute Inc., 2009a). All ties in *Enterococcus* observations were assigned a mean averaged rank score. The PROC UNIVARIATE procedure was used to test and confirm that the ranks generated actually fit a normal curve. PROC REG was used to generate a prediction equation, which could then be used to predict the values below the detection limit (<10MPN/100ml). Results from this method were verified using UnCensor v4.0 (Newman & Dixon, 1990), a program designed specifically for this type of environmental analysis (Newman, 1995).

Multivariate partial least squares (PLS) regression was used to determine statistically significant associations between *Enterococcus* density level and the following input variables: sampling station; month; water temperature; water height; specific conductance; rainfall for 1, 2, and 3 days leading up to sampling at two locations (U.S. Customs House and Charleston International Airport); and number of septic tanks located within a 0.5 and 1 mile buffer of each sampling station. The PLS procedure was used to carry out this analysis. All of the methods executed in PROC PLS work by obtaining consecutive linear combinations of the predictors. These are called factors, which explain the variation in both the response and predictor variables. Factors are extracted from a matrix, which includes both the predictor and response variables. A one-at-a-time cross validation method was used to choose the number of extracted factors to fit the model specified by the CV=ONE option. This option requires a re-calculation of the PLS model for every entered observation. The absolute minimum PRESS (predicted residual error sums of squares) is achieved with the number of extracted factors that have a statistically significant p-value less than 0.05. The PRESS statistic is a form of cross-validation used in regression analysis as a measure of the fit of a model and is based on the residuals generated from calculating the sums of squares of the prediction residuals for each observation in the model (SAS Institute Inc., 2013). The CVTEST option was used, which allowed for statistical model comparison to test whether differences in residuals from different models are significant. This methodology, proposed by Van der Voet (1994), extracts the smallest number of factors that have residuals insignificantly larger ($p > .1$) than the residuals of the model with minimum PRESS.

The PLS procedure outputs a variable importance plot, which based on the Variable Importance for Projection (VIP) statistic of Wold (Wold, 1995), displays the influence of each predictor variable in fitting the PLS model for the predictors and response variables. According to Wold, when a predictor variable has a small coefficient (in absolute value) and a small VIP (less than 0.8) value, it is a suitable candidate for deletion (SAS Institute Inc., 2009b). Predictor variables that fell below 0.8 on the variable importance plot were dropped from the model and the PLS procedure was re-run. This process was repeated until the best model explaining the variance in the predictor and response variables was found. The results from the PLS procedure were confirmed using PROC GLM.

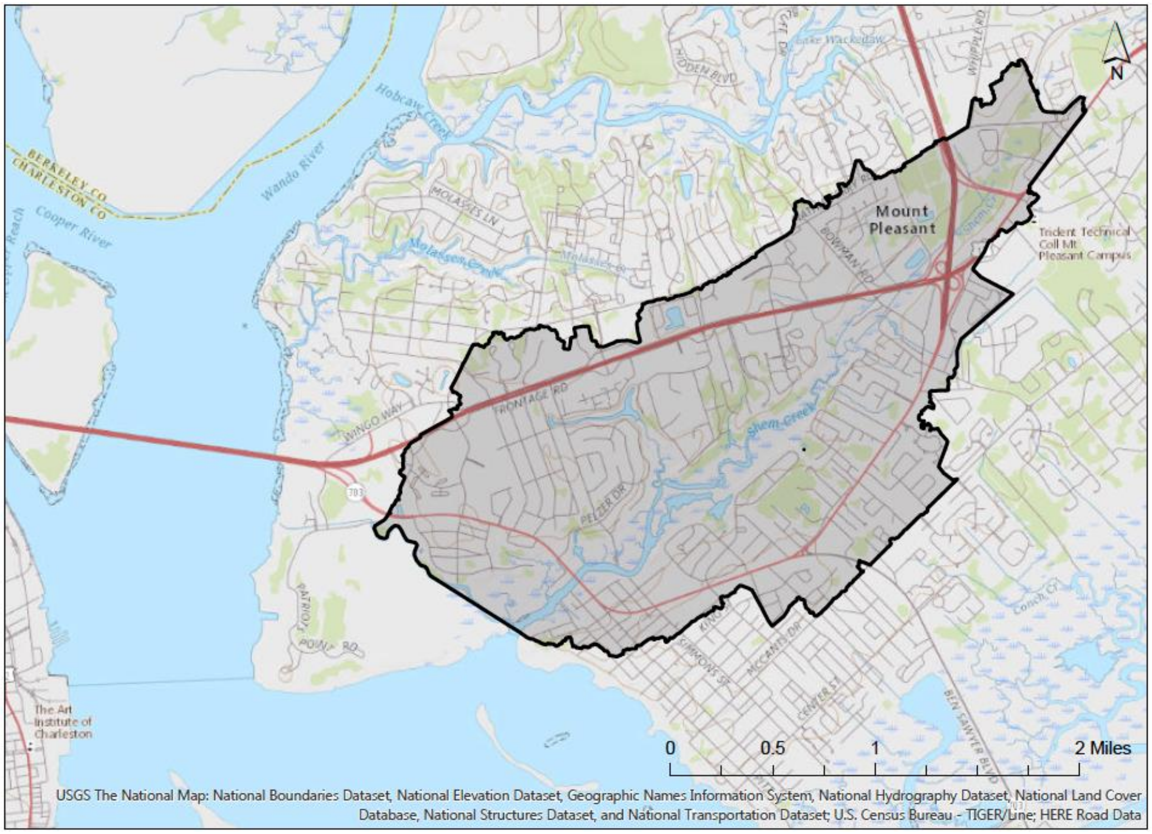


**Figure 2.1:** Selected Shem Creek watershed

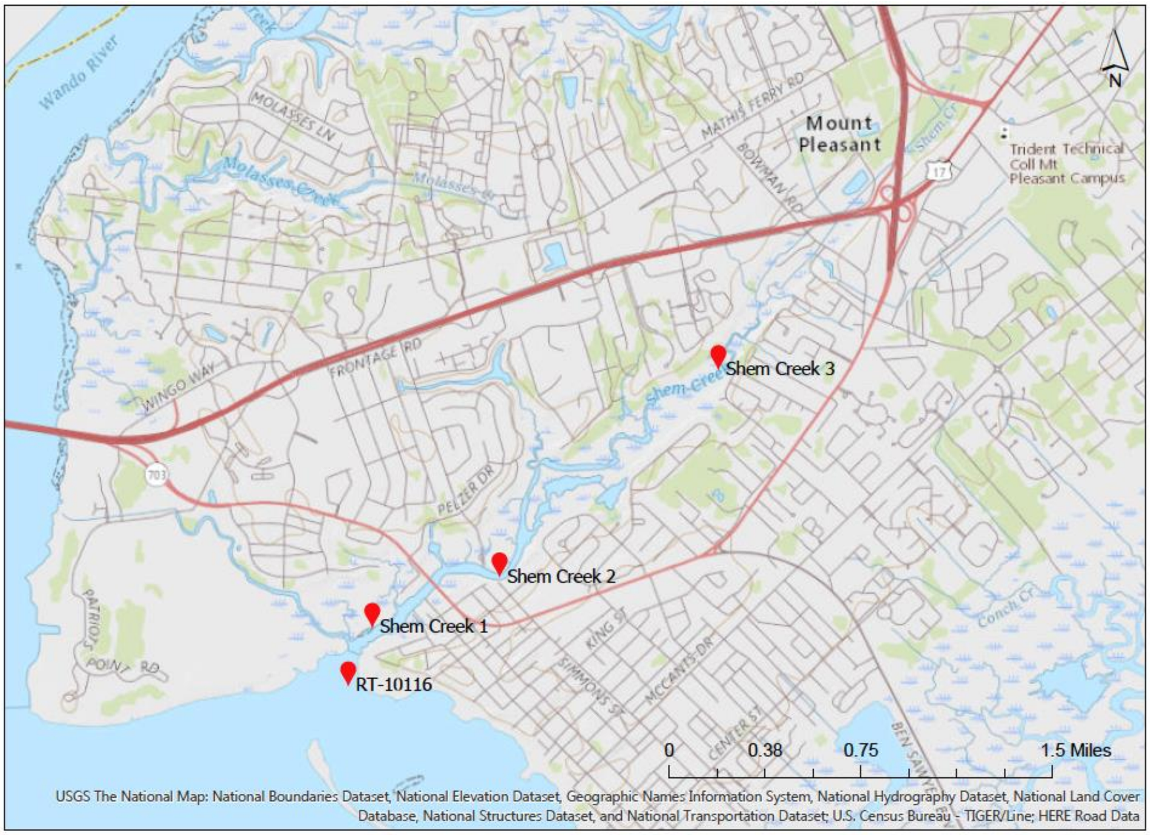


**Figure 2.2:** Water quality monitoring station locations

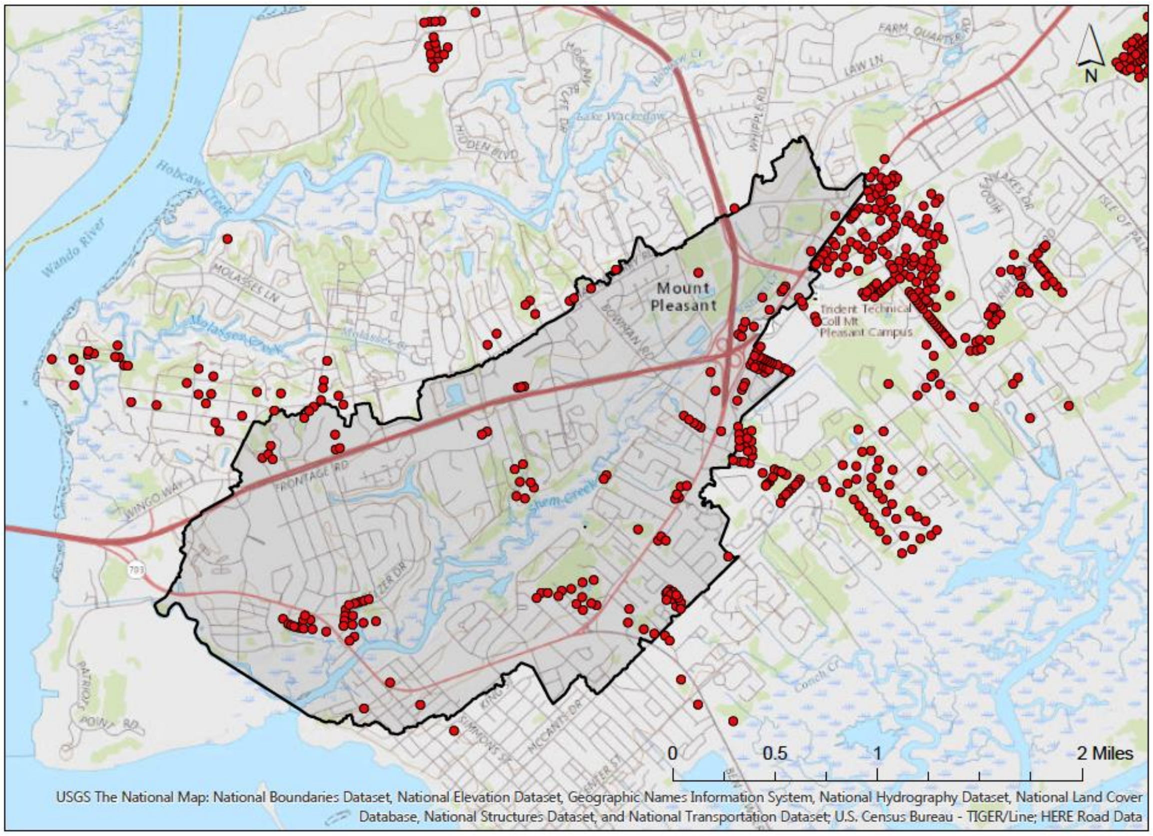


**Figure 2.3:** Septic tank locations, indicated by the red dots in and around the Shem Creek basin

# Chapter 3

# Results

Chapter three describes results for changes in zoning in the Shem Creek watershed between 2010 and 2017, multiple trends associated with *Enterococcus* density levels in Shem Creek, and positive or negative correlations between water quality variables and *Enterococcus*.

## 3.1 Land Use and Zoning

Shem Creek was categorized by seven zoning descriptions. Figures 3.1 and 3.2 show the zoning categories for 2010 and for 2017. The zoning categories that would likely contain the highest amount of impervious surface on the lot would be commercial and residential. In contrast, the zoning categories that would contain the least amount of impervious surface would be agricultural, vacant, recreational, and undevelopable (Arnold & Gibbons, 1996). Table 3.1 represents the percent of land area described by zoning in the Shem Creek watershed in 2010 and 2017. In the Shem Creek watershed, the largest percent of land area consisted of residential zoning areas: 82.2% (2010) and 83.9% (2017). The percent of land area that was zoned as vacant in 2010 equated to 10.3%, which decreased to 8% in 2017. When comparing 2010 to 2017, 69.3% of the land area in the Shem Creek watershed stayed as the same zoning classification. Because the amount of impervious surface is directly related to water quality degradation (Foley et al., 2005), a change from zoning classifications associated with permeable surfaces to zoning classifications associated with impervious surfaces was calculated. This equated to 3.2% of the total land area in the watershed that changed from agricultural, recreational, vacant, or undevelopable in 2010 to commercial or residential in 2017 (Figure 3.3).

For each zoning category, the largest change in square area was calculated as follows:

- $2.36km^2$ of land that was categorized as commercial in 2010 changed to residential zoning in 2017
- $39.48km^2$ of land that was categorized as residential in 2010 stayed as residential zoning areas in 2017
- $5.17km^2$ of land that was categorized as other in 2010 changed to residential zoning areas in 2017
- $0.91km^2$ of land that was categorized as undevelopable in 2010 changed to residential zoning areas in 2017
- $0.51km^2$ of land that was categorized as vacant in 2010 changed to residential zoning areas in 2017
- $0.02km^2$ of land that was categorized as recreational in 2010 stayed as recreational zoning areas in 2017
- $0.05km^2$ of land that was categorized as agricultural in 2010 changed to vacant in 2017

## 3.2 Descriptive Results for Water Quality Analysis

Figure 3.4 displays a plot of the natural log transformed *Enterococcus* density levels (MPN), excluding those that fell below the detection limit, which are later accounted for and included in this study. The highest values of *Enterococcus* density levels obtained in each year increased over time, increasing the yearly variability of the samples taken over time. Shem Creek station 3 (SC3) had the highest amount of septic tanks located within one mile (n=109), but Shem Creek station 1 (SC1) had the highest amount of septic tanks located within a half-mile radius (n=26). All septic tanks located within a one or half-mile buffer of each station can be seen in Figure 3.5. The number of *Enterococcus* density levels that exceeded the state daily maximum for recreationally used Class SB tidal saltwater (<501MPN/100ml) has increased over time with most of these exceedances occurring in September, followed closely by August (Figures 3.6 and 3.7).

## 3.3. Helsel's Robust Method Statistical Results

Of the total of 372 samples of *Enterococcus* analyzed in this study, 23 were below the detection limit (<10MPN/100ml) equating to 6.18% of the total sample size. In the tests for normality of the ranked transformed *Enterococcus* (ln(MPN)) values, the Shapiro-Wilk test statistic had an associated p-value of <0.0001. Statistically significant p-values are defined as those less than $\alpha=0.05$. Because the p-value was statistically significant, the null hypothesis that there was no significant departure from normality was rejected, concluding that the ranks assigned to the transformed *Enterococcus* (ln(MPN)) values fit a normal distribution. The distribution of the ranks was slightly positively skewed because of the number of ties in the data set (Figure 3.8). Figure 3.9 displays where the ties occurred on the normal distribution curve. The F-value in the analysis of variance (Table 3.2) was statistically significant (p-value <0.0001), indicating that the rank variables reliably predict the transformed *Enterococcus* (ln(MPN)) values. The R-Square value, which indicates the proportion of variance in the dependent variable (ln(MPN)) that can be predicted from the independent variable (computed ranked scores), was 0.9791. Figure 3.10 displays how closely the data for transformed *Enterococcus* (ln(MPN)) and the computed ranked scores fit together. Based on the parameter estimates (Table 3.3), a prediction equation was computed to assign values for those *Enterococcus* data points that were under the detection limit (<10MPN/100ml). These assigned values are displayed in Figure 3.11.

## 3.4 Multivariate Partial Least Squares Regression Results

Multivariate partial least squares (PLS) regression was used to determine statistically significant associations between *Enterococcus* density levels and the variables listed in Table 3.4. A total of six different models were run in order to determine the best-fit model. The following paragraphs walk through the model selection process and results of the PLS procedures.

The variable "month" was taken out of the model because there were months in year 2010 that did not have observations for any of the other years. When the PLS procedure was run with "month" in it, there were no significant factors extracted, which prevented the analysis from working appropriately or presenting any results. The PLS procedure was re-run, excluding "month" from the model. In this model (Model 1), there were two statistically significant factors extracted (factor 1: p-value <0.0001, factor 2: p-value 0.008). Appendix A displays the percent variation accounted for by the partial least squares factors for each variable in all the models tested leading up to the final selected model. The following six variables in Model 1 fell below Wold's criteria of 0.8 in the variable importance plot: station SC2, precipitation values from the Charleston International Airport, water temperature, and number of septic tanks located within a half mile of each station.

The PLS procedure was re-run, excluding all precipitation values from the Charleston International Airport, water temperature, and number of septic tanks located within a half mile of each station (Model 2). Station SC2 was not excluded from the model even though it fell below Wold's criteria because this was a categorical variable. Taking out SC2 would exclude 119 observations, equating to almost a third of the total data set used in this study. In Model 2—which included all stations, all precipitation data for the downtown U.S. Charleston Customs House, conductivity, water height, and septic tanks located in a one-mile buffer of each station—there were two statistically significant factors extracted (factor 1: p-value <0.0001, factor 2: p-value 0.014). Station SC2 still fell below Wold's criteria of .8 on the variable importance plot.

Because station RT-10116 contained observations from only the year 2010 and none of the other years, it was taken out of the model to make sure this station was not skewing the results. The PLS procedure was re-run excluding station RT-10116 (Model 3). Model 3 included stations SC1, SC2, and SC3; all precipitation data from the downtown Charleston U.S. Customs House; conductivity; water height; and septic tanks located within a one-mile buffer of each station. Model 3 extracted two statistically significant factors (factor 1: p-value <0.0001, factor 2: p-value 0.044). Because the percent variation accounted for by the partial least squares factors did not change substantially for the predictor values (totals: 36.93 for Model 2 and 41.38 for Model 3), keeping station RT-10116 in the model remains appropriate in order to keep observations from year 2010.

Before adding station RT-10116 back into the model, station SC2 was also excluded from the model (Model 4) in order to see how the omission impacted the results. This was done because station SC2 fell below Wold's criteria of 0.8 in the variable importance plot for all the previous models. In this effort to analyze what results would be produced by excluding these observations, the PLS procedure was re-run excluding both station SC2 and station RT-10116 (Model 4). Model 4 extracted two statistically significant factors (factor 1: p-value $<0.0001$, factor 2: p-value 0.064). Model 4 included station SC1 and SC2, all precipitation data from the downtown Charleston U.S. Customs House, conductivity, water height, and septic tanks located within a one-mile buffer of each station. Precipitation values two and three days before sample collection and conductivity fell below Wold's criteria of 0.8 but only by about a tenth of a decimal. Because taking out station SC2 excluded so many observations in the data set, both station SC2 and station RT-10116 should be added back into the model.

After considering the data further, it was realized that because the number of septic tanks located in a 1-mile buffer around each station was a constant value for each station, this was essentially a weighted numerical value assigned for the variable "station." Therefore, the number of septic tanks was taken out of the model completely. The PLS procedure was re-run (Model 5) and extracted two statistically significant factors (factor 1: p-value $<0.0001$, factor 2: p-value 0.001). Model 5 included stations RT-10116, SC1 and SC2; all precipitation data from the downtown Charleston U.S. Customs House; conductivity; and water height. All variables except for station SC2 remained above Wold's criteria of 0.8 on the variable importance plot. In Model 5, conductivity was just slightly above Wold's criteria of 0.8 on the variable importance plot. In addition, only 15.48% of the variation accounted for by the partial least squares factors for the model explained water specific conductance.

In an effort to obtain a model in which the predictor variables in the model explain the highest percent of variation, conductivity was excluded from the model and the PLS procedure was re-run (Model 6). In Model 6, the variation summary shows that the two factors in the model explain 46.71% of the total predictor variation and 43.16% of the response variation. The percent variation accounted for by the predictor variables increased with Model 6, compared to Model 5, which was 42.44%. Therefore, Model 6 appeared to be the best-fit model. Because there were several missing observations in the precipitation data set, PROC PLS excluded these from the analysis, and no predictions were computed for those missing observations. The final model contained 367 records of observations used in the final analysis. In Model 6, the absolute minimum PRESS was achieved with two extracted factors that have a statistically significant p-value less than 0.05 (factor 1: p-value <0.0001, factor 2: p-value 0.002). The complete factor selection process is shown in Tables 3.5 and 3.6. The percent variation accounted for by the partial least squares factors in the final model is shown in Table 3.7.

The correlation loading plot summarizes the two factors and the features in the PLS model, displaying the primary results (Figure 3.12). This plot is composed of blanketed scatter plots, which include the variation explained by both factors for each variable and the weighted effects of the model (SAS Institute Inc., 2009b). The amount of variation explained by the model for each of the variables is comparable to the distance from the origin of the plot. The transformed *Enterococcus* levels, represented by their observation number in the data set on this plot, are randomly clustered towards the origin, indicating that the data contribute appropriate information about the two factors. Drawing perpendicular lines from the predictor variables on the plot to a line that connects the origin and the response variable produces relative positive and negative correlations between the predictor and response variables. Figure 3.13 displays the drawn lines that were used to interpret the plot. The correlation loading plot indicates that station SC3 is highly positively correlated with the transformed *Enterococcus* density levels (labeled "log_MPN_adj" on the plot). Station SC3 was the most correlated with the transformed *Enterococcus* density levels compared to all other predictor variables in the model. Water height values followed closely by precipitation are also positively correlated with the transformed *Enterococcus* density levels. Station SC2, which is located towards the origin of the plot, has no correlation with the transformed *Enterococcus* density levels. Station RT-10116 is slightly negatively correlated with the transformed *Enterococcus* density levels. Station SC1 is also negatively correlated with the transformed *Enterococcus* density levels.

All variables in the final model, except for station SC2, remained above Wold's criteria of 0.8 on the variable importance plot (Figure 3.14). As stated previously, station SC2 was kept in the model to avoid eliminating almost a third of the data set. The regression coefficients profile in Figure 3.15 signifies the importance each predictor variable has in the prediction of only the response variable. In the regression coefficients profile plot, station RT-10116 and SC1 have negative coefficients. Looking back at the correlations loadings plot, these are the variables that tend to be negatively correlated with the dependent variable. The plot shown in Figure 3.16 gives the distance from each point to the PLS model with regard to the predictors first and then the responses. This allows for identification of potential outliers. Points that are dramatically farther from the model than the rest of the points could be considered outliers. Those points scattered far to the right on the X-axis of this plot are potential outliers. However, because of the reliable methods for reading *Enterococcus* density levels and because of the many factors that can drastically impact *Enterococcus* density levels in water, these were not excluded from the analysis. The parameter estimates that are used to create the prediction equation are displayed in Table 3.8.

In order to confirm that the PLS procedure analysis results were accurate, the GLM procedure was run, using the same data from the final model. The F-value in the analysis of variance (Table 3.9) was statistically significant (p-value $<0.0001$), indicating that the model does explain the variance of the response variables. The $R^2$, which is the total variance explained by the model was 0.462199 (46.22%). This remains very close to the variation summary from the PLS procedure in Model 6 that concluded 46.71% of the predictor variation was explained by the model.

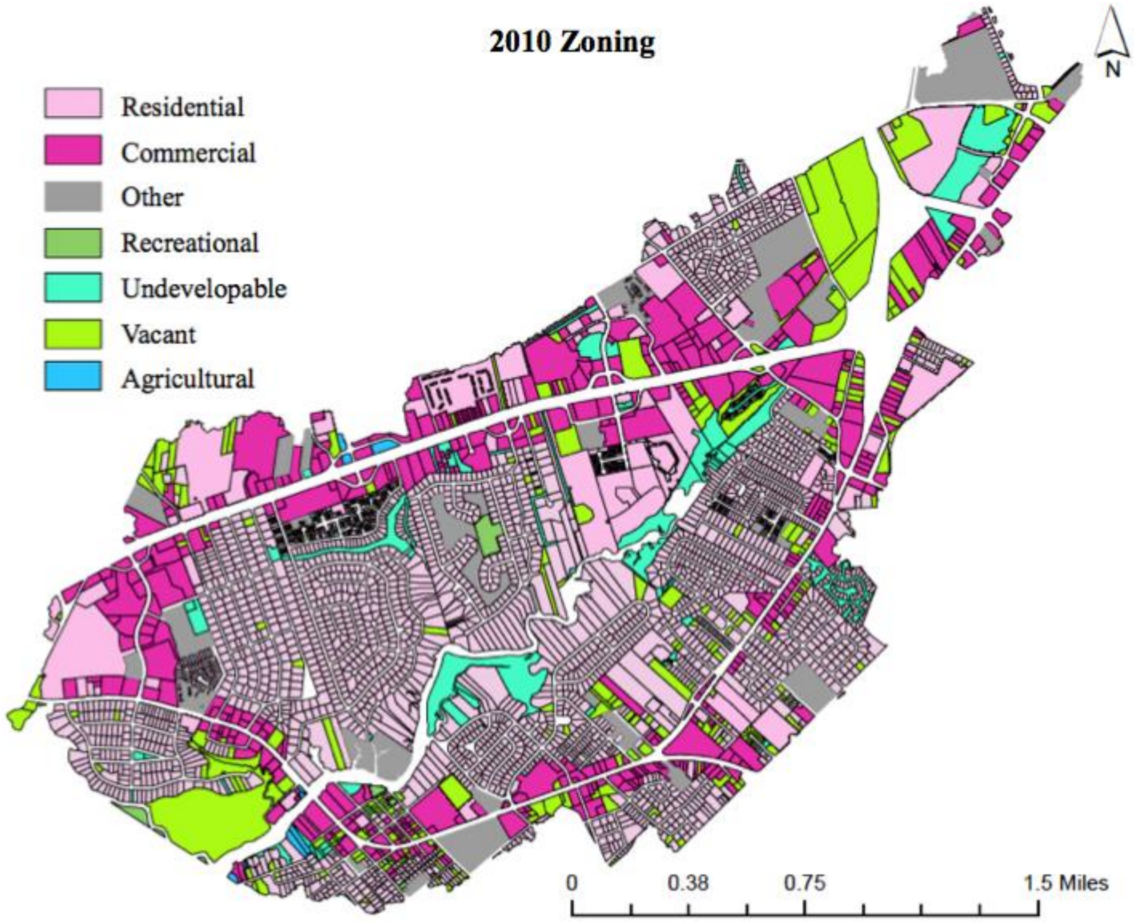


**Figure 3.1:** Map of zoning categories in 2010 for the Shem Creek watershed

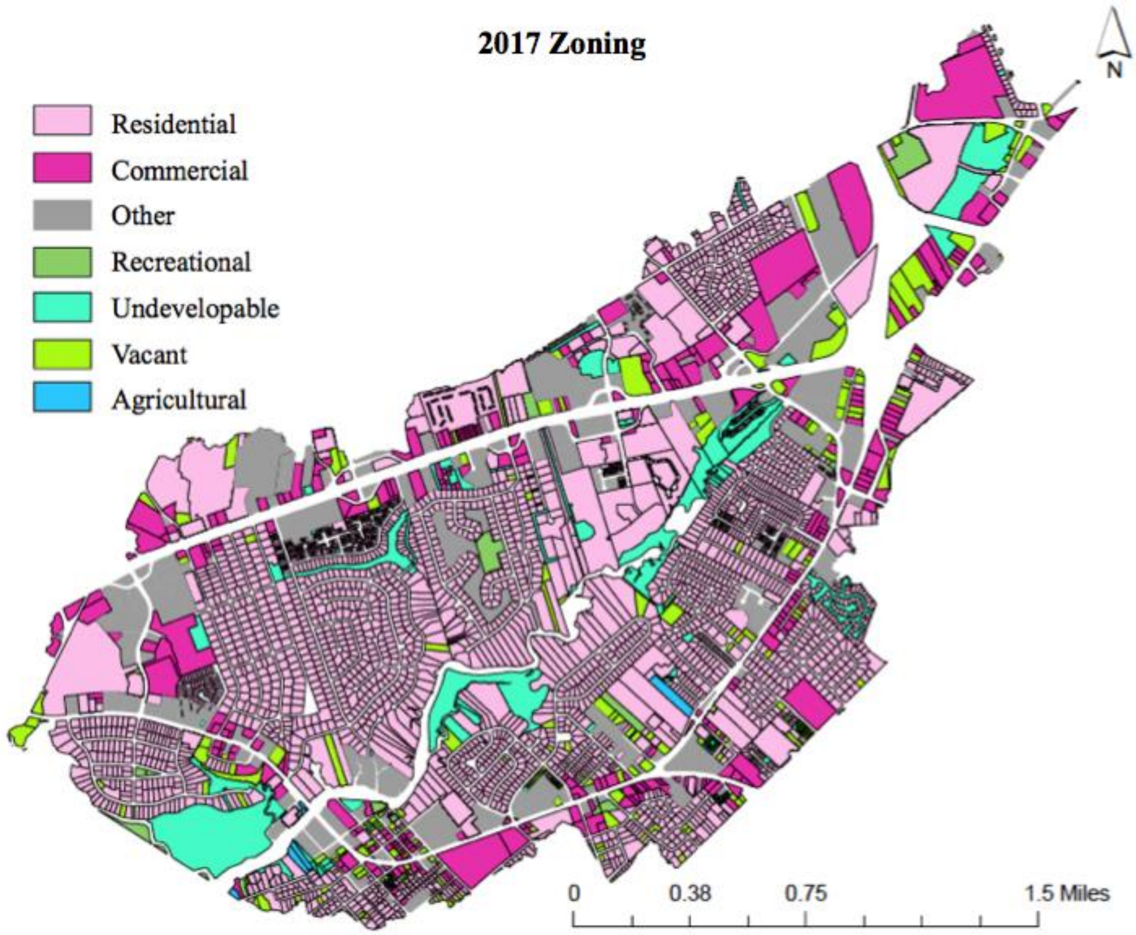


**Figure 3.2:** Map of zoning categories in 2017 for the Shem Creek watershed

**Table 3.1:** Percent land use by zoning category for the Shem Creek watershed in 2010 and 2017

| Zoning Category | 2010 (% cover) | 2017 (% cover) |
|---|---|---|
| Commercial | 3.5 | 2.3 |
| Residential | 82.2 | 83.9 |
| Vacant | 10.3 | 8.0 |
| Recreational | 1.5 | 1.6 |
| Agricultural | 0.0 | 0.0 |
| Undevelopable | 0.9 | 1.3 |
| Other | 1.6 | 2.9 |

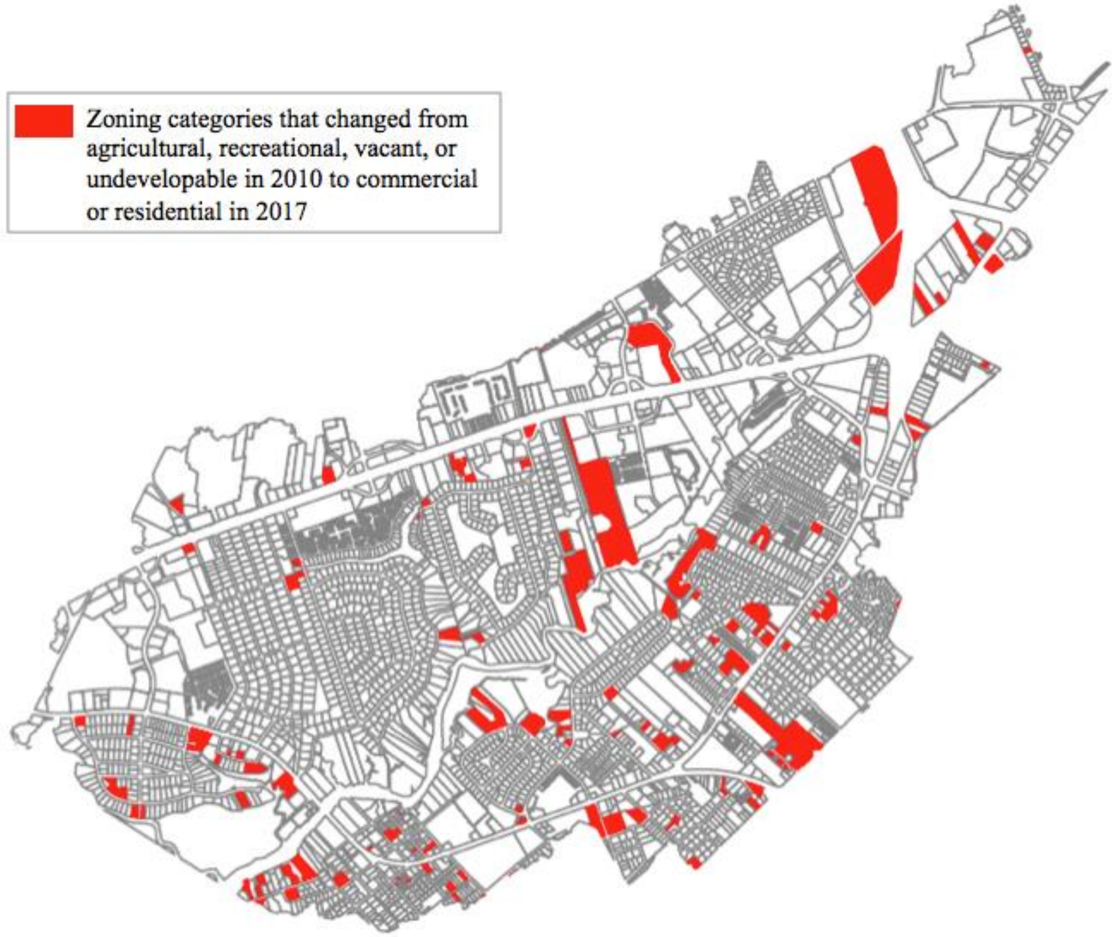


**Figure 3.3:** Zoning categories that changed from agricultural, recreational, vacant, or undevelopable in 2010 to commercial or residential in 2017 in the Shem Creek watershed

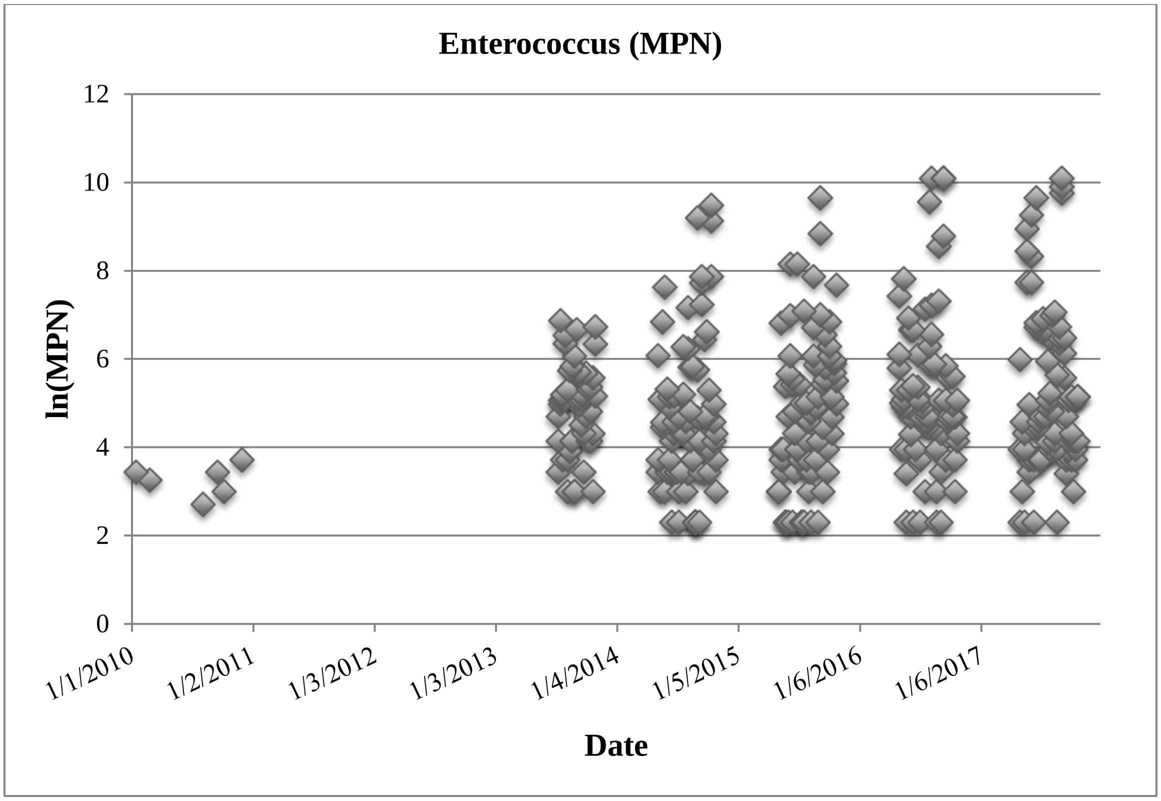


**Figure 3.4:** Natural log of *Enterococcus* density levels (ln(MPN)) included in the analysis graphed over time. This figure excludes *Enterococcus* density levels that fell below the detection limit (<10MPN/100ml).

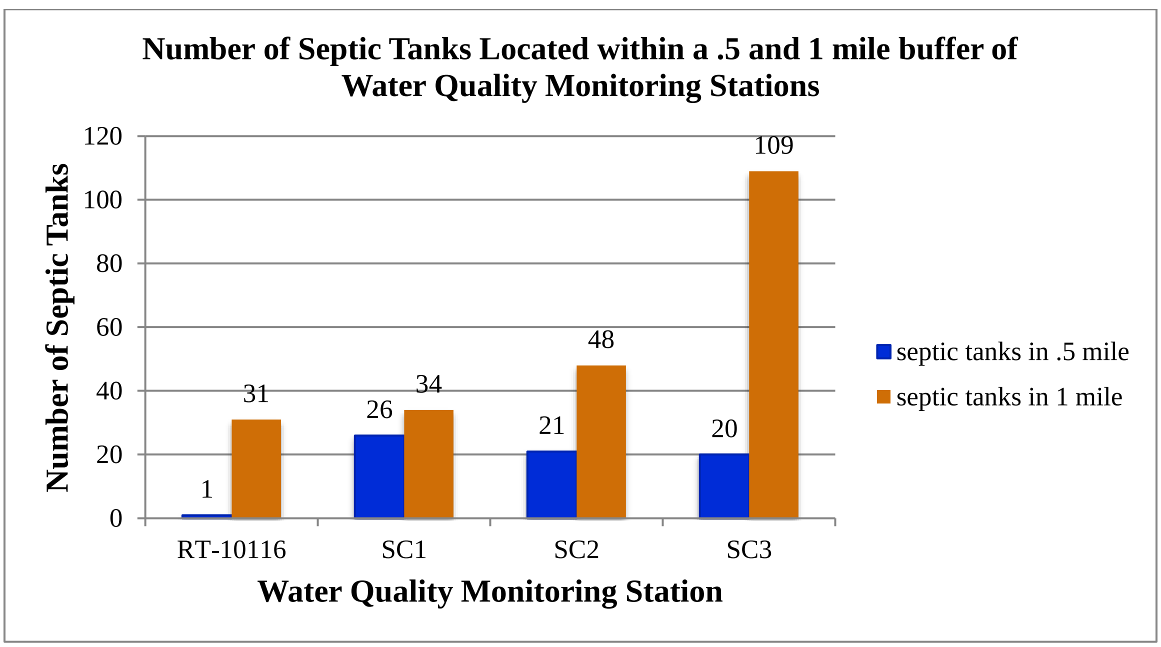


**Figure 3.5:** Number of septic tanks within a half-mile and a mile buffer or radius of each water quality monitoring station

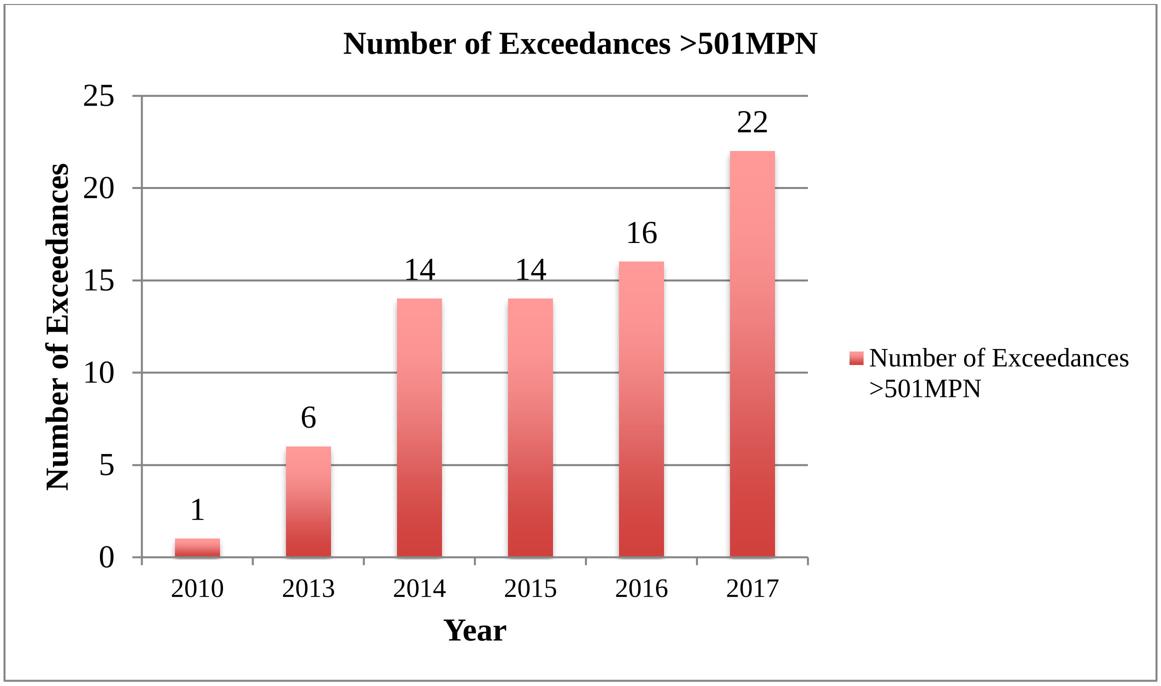


**Figure 3.6:** Number of *Enterococcus* density levels that exceeded the Class SB saltwater recreational limit for a single sample (501MPN/100ml) by year

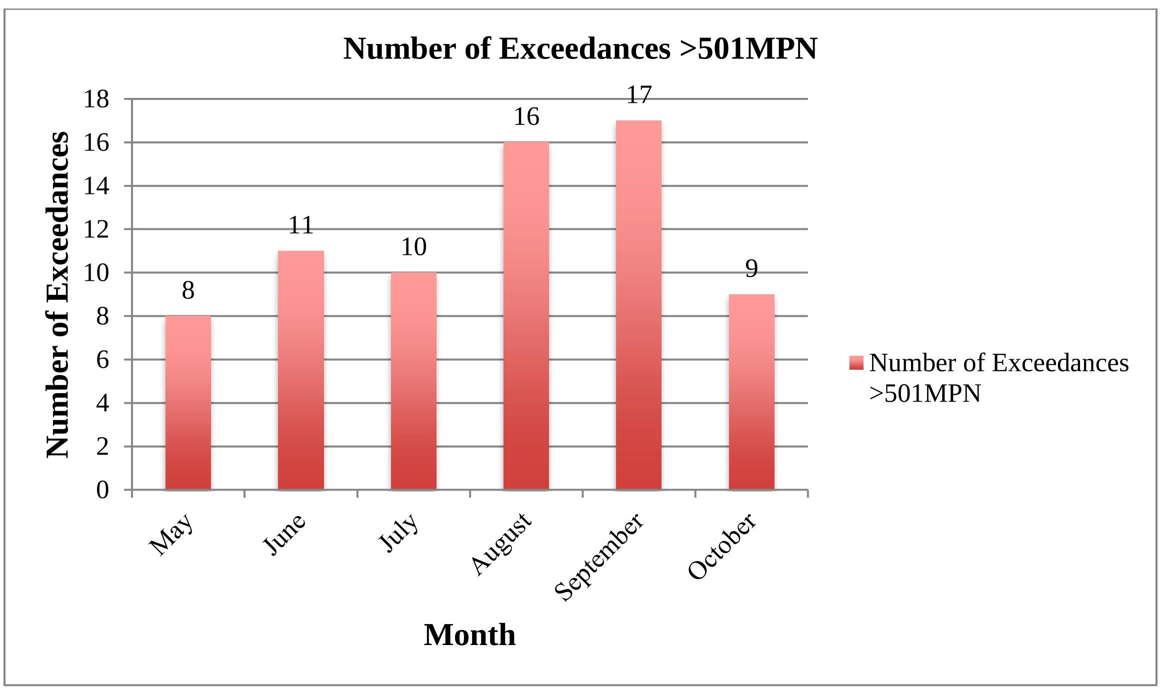


**Figure 3.7:** Number of *Enterococcus* density levels that exceed the Class SB saltwater recreational limit of 501MPN/100ml by month

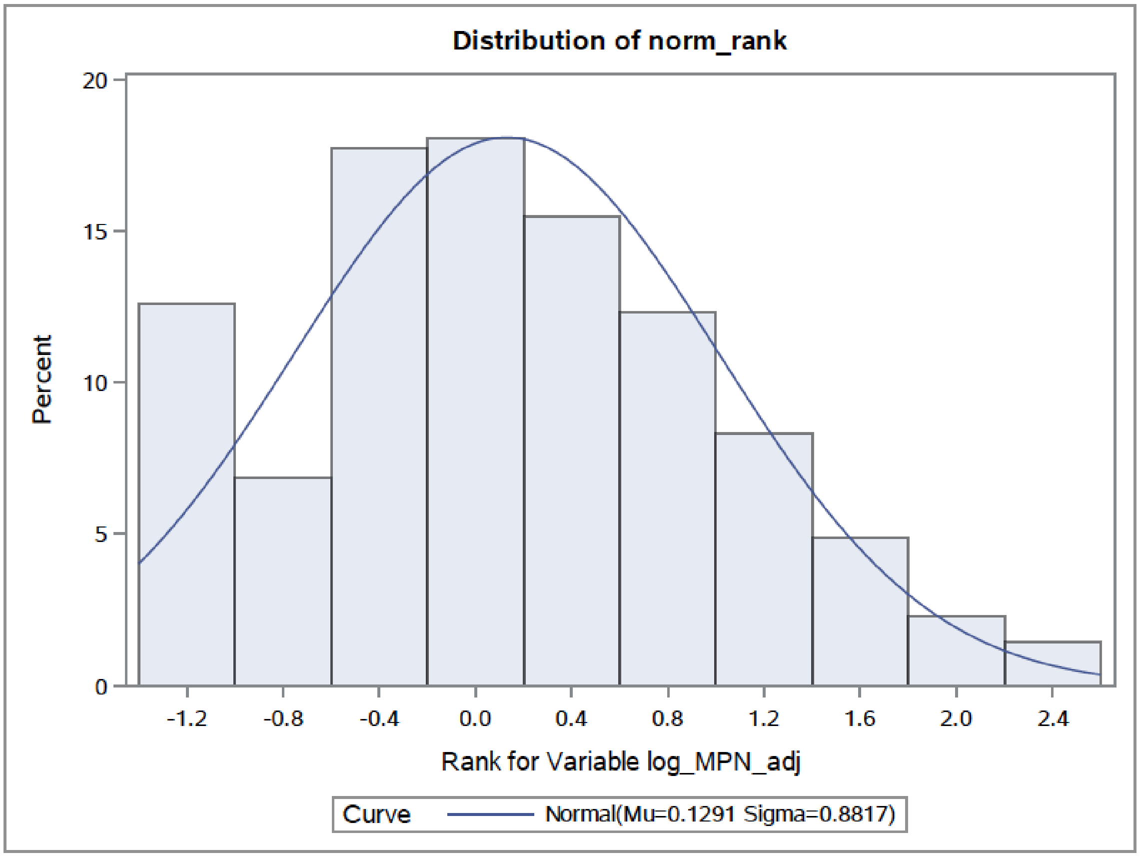


**Figure 3.8:** Distribution of the computed normal scores from the ranks (norm_rank) for natural log transformed *Enterococcus* data (log_MPN_adj). Note that SAS's terminology for the natural log (ln) is "log".

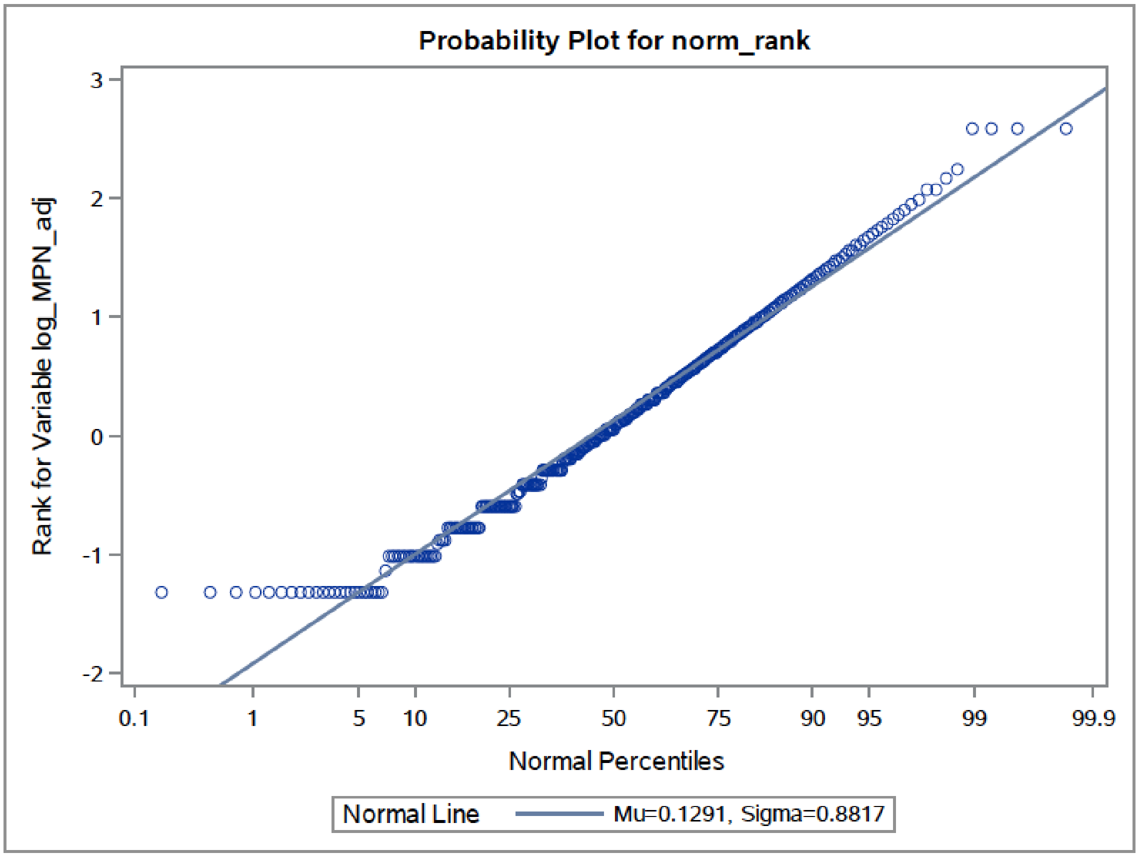


**Figure 3.9:** Probability plot for the computed normal scores from the ranks (norm_rank) of the natural log transformed *Enterococcus* data (log_MPN_adj) against normal percentile values. A perfect normal curve would be on the "normal line" indicated by the figure. The ties can be seen where there are multiple points on the same Y-axis value.

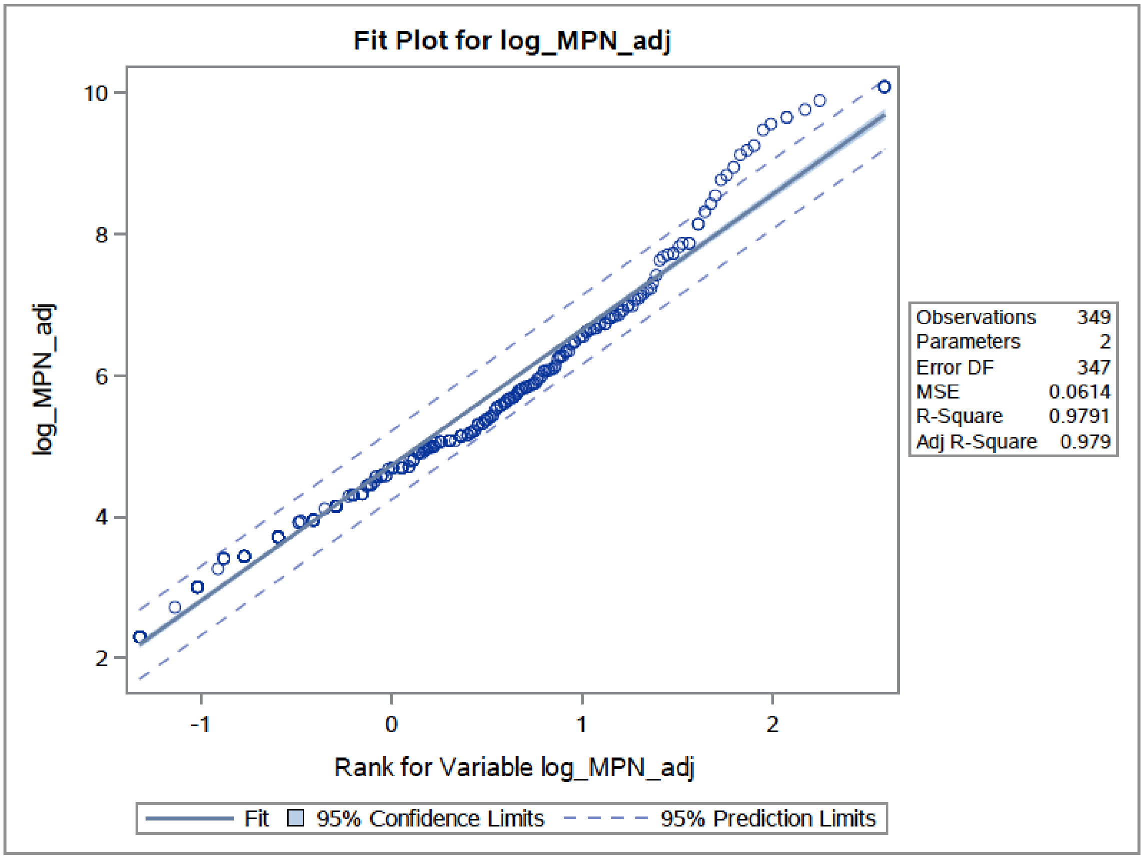


**Figure 3.10:** Fit plot for the computed normal scores from the ranks (Rank for Variable log_MPN_adj) and the natural log transformed *Enterococcus* data (log_MPN_adj)

**Table 3.2:** Analysis of Variance for testing that the Rank Variables Reliably Predict the Transformed *Enterococcus* (ln(MPN)) Values in the Helsel's Robust Method

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 1 | 996.07825 | 996.07825 | 16231.2 | <0.0001 |
| Error | 347 | 21.29479 | 0.06137 | | |
| Corrected Total | 348 | 1017.37304 | | | |

**Table 3.3:** Parameter Estimates for the Helsel's Robust Method for Predicting Values of *Enterococcus* that Fell Below the Detection Limit (<10MPN/100ml)

| Variable | Label | DF | Parameter Estimates | Standard Error | t value | Pr > \|t\| |
|---|---|---|---|---|---|---|
| Intercept | Intercept | 1 | 4.72752 | 0.01340 | 352.74 | <0.0001 |
| norm_rank | Rank for Variable log_MPN_adj | 1 | 1.91890 | 0.01506 | 127.40 | <0.0001 |

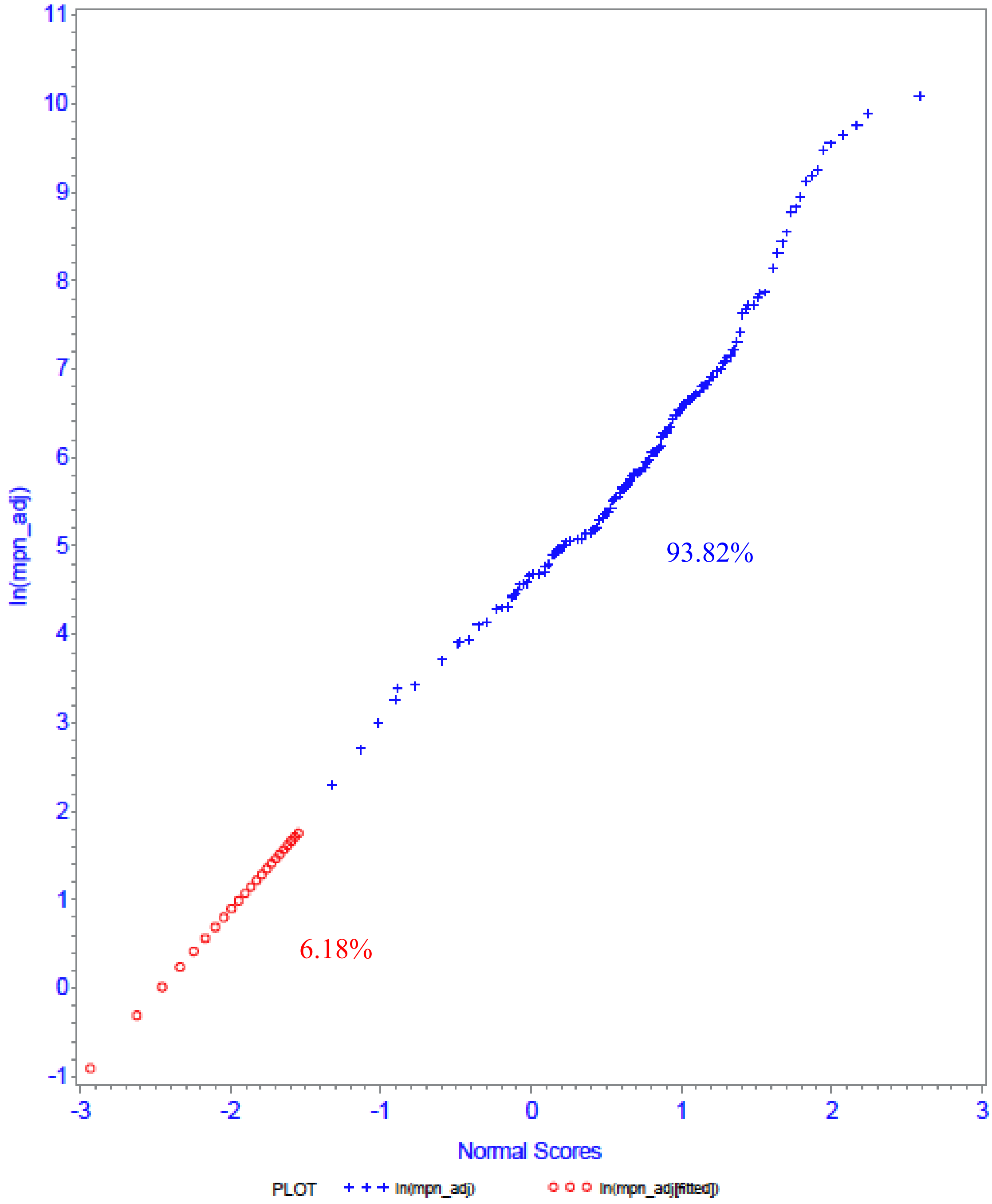


**Figure 3.11:** Data points with uncensored (<10MPN/100ml) fitted values computed by Helsel's Robust Method

**Table 3.4:** All Variable Names Included in the Analysis and Their Variable Description

| Variable Name | Variable Description |
|---|---|
| RT-10116 | Water quality monitoring station |
| SC1 | Water quality monitoring station |
| SC2 | Water quality monitoring station |
| SC3 | Water quality monitoring station |
| Month | Month |
| Rain_1d_airport | Total precipitation on the day prior to water sample collection at the Charleston International Airport |
| Rain_2d_airport | Total sum of precipitation on the 2 days prior to water sample collection at the Charleston International Airport |
| Rain_3d_airport | Total sum of precipitation on the 3 days prior to water sample collection at the Charleston International Airport |
| Rain_1d_dt | Total precipitation on the day prior to water sample collection at the Charleston Clearing House, located Downtown |
| Rain_2d_dt | Total sum of precipitation on the 2 days prior to water sample collection at the Charleston Clearing House, located Downtown |
| Rain_3d_dt | Total sum of precipitation on the 3 day prior to water sample collection at the Charleston Customs House, located Downtown |
| Cond_bottom | Specific conductance of the water |
| Temp | Water temperature |
| Height | Water height |
| Sep_pt5 | Number of septic tanks located within a half-mile radius of each water quality monitoring station |
| Sep_1 | Number of septic tanks located within a one-mile radius of each water quality monitoring station |

**Table 3.5:** Cross Validation for the Number of Extracted Factors

| Number of Extracted Factors | Root Mean PRESS | $T^2$ | Prob > $T^2$ |
|---|---|---|---|
| 1 | 1.002732 | 50.70773 | <0.0001 |
| 2 | 0.796101 | 8.496466 | 0.0020 |
| 3 | 0.764914 | 0.42245 | 0.5420 |
| 4 | 0.763042 | 0 | 1.0 |
| 5 | 0.763379 | 0.196669 | 0.6380 |
| 6 | 0.763596 | 0.46835 | 0.4800 |
| 7 | 0.763807 | 0.74074 | 0.3740 |
| 8 | 0.763798 | 0.717576 | 0.3720 |
| 9 | 0.763798 | 0.717576 | 0.3720 |

**Table 3.6:** Descriptive Results of the Cross Validation for the Number of Extracted Factors Process

| | |
|---|---|
| Minimum root mean PRESS | 0.7630 |
| Minimizing number of factors | 3 |
| Smallest number of factors with $p > 0.1$ | 2 |

**Table 3.7:** Percent Variation Accounted for by Partial Least Squares Factors

| | **Variable** | **Percent Variation Accounted for by the 2 PLS factors** |
|---|---|---|
| Model Effects | Station RT-10116 | 17.5537 |
| | Station SC1 | 28.9036 |
| | Station SC2 | 0.3066 |
| | Station SC3 | 57.5639 |
| | Rain_1d_dt | 76.6546 |
| | Rain_2d_dt | 91.6853 |
| | Rain_3d_dt | 77.9720 |
| | Height | 23.0367 |
| | **Current** | **20.8880** |
| | **Total** | **46.7095** |
| Dependent Variables | log_MPN_adj | 43.1631 |
| | **Current** | **4.9529** |
| | **Total** | **43.1631** |

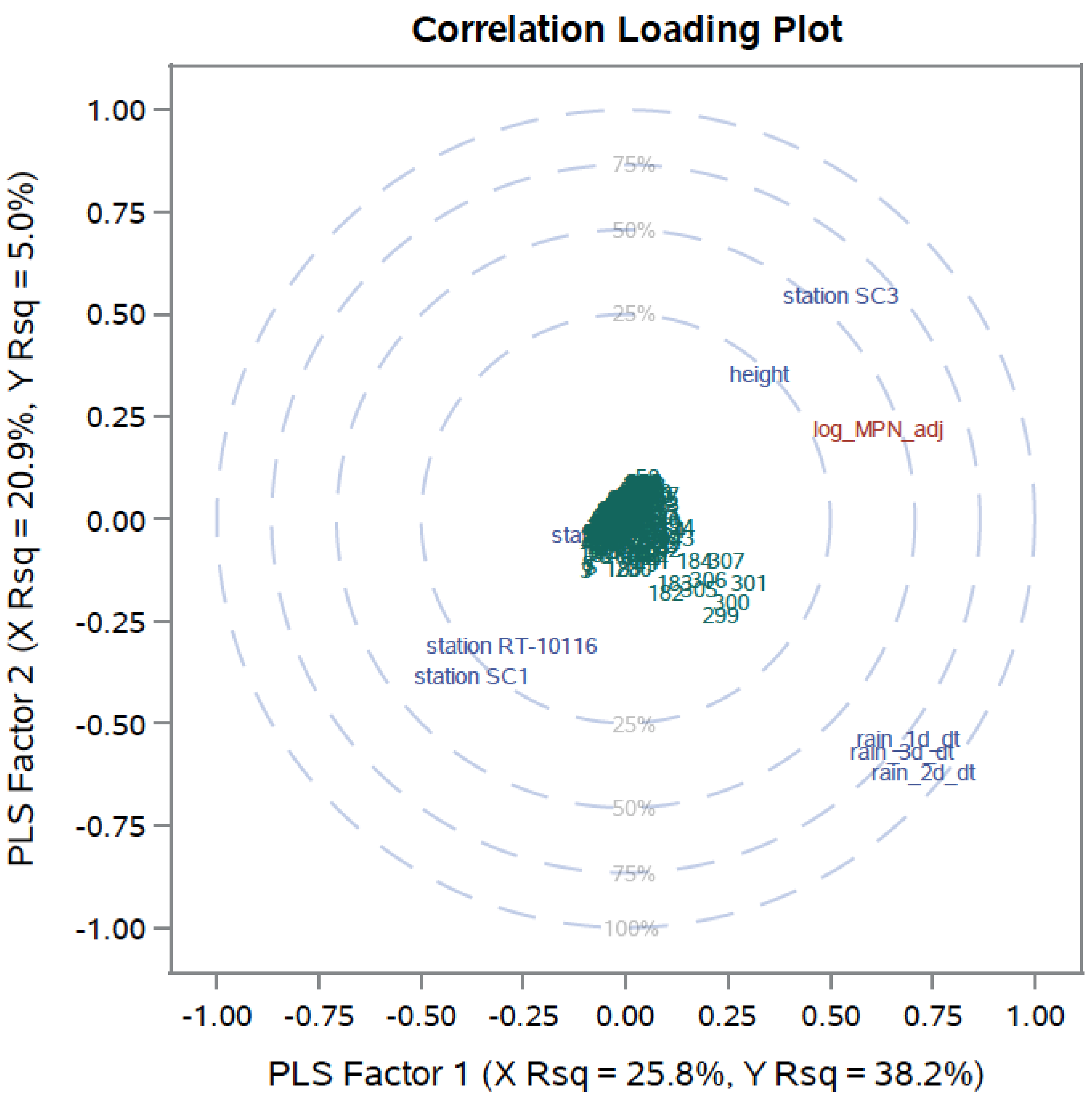


**Figure 3.12:** Correlation loading plot from Model 6, the final model

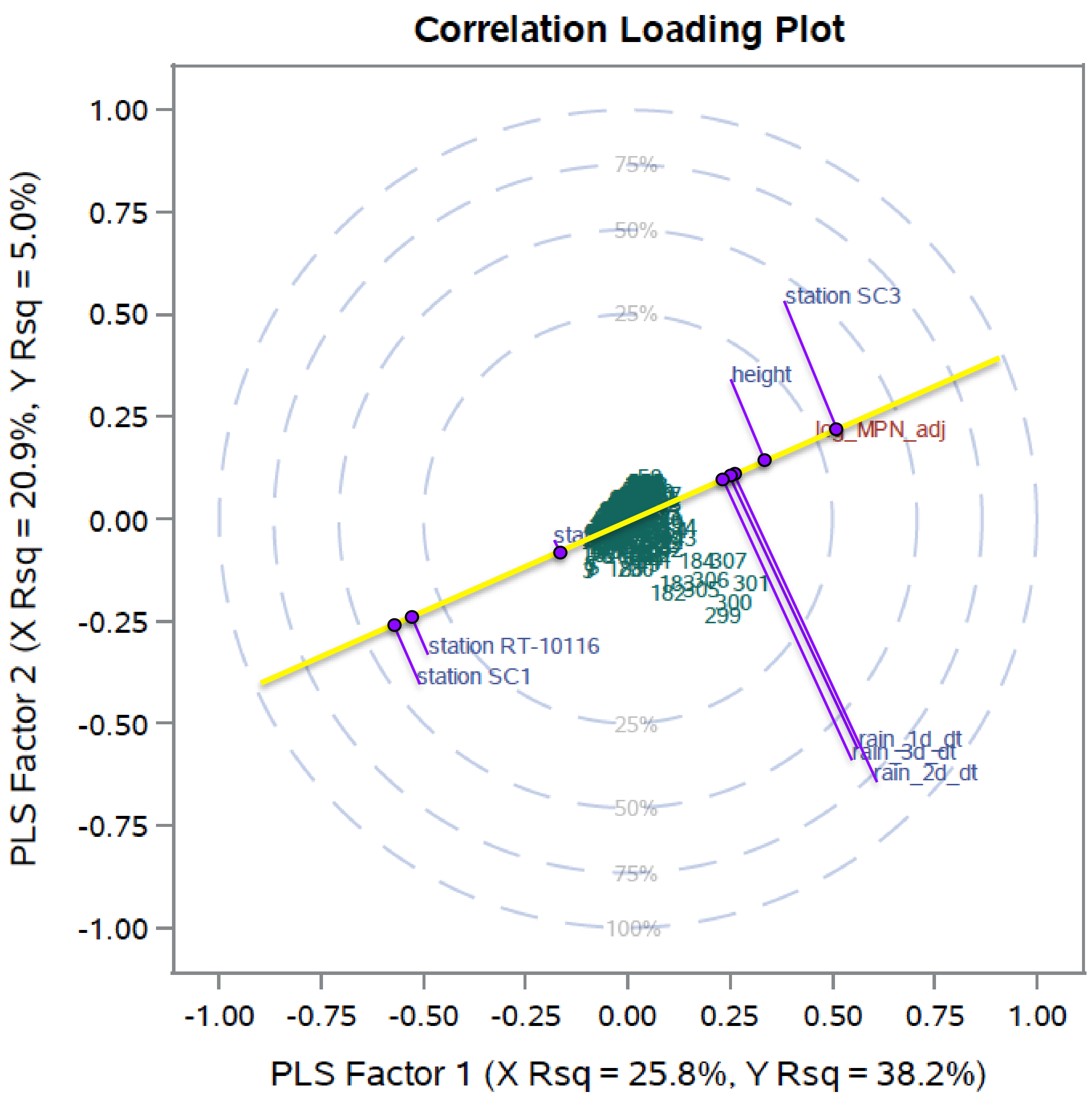


**Figure 3.13:** Correlation loading plot from Model 6 with lines drawn in for reading and analyzing the plot. The closer the purple dots are towards log_MPN_adj, the more correlated the predictor variable at the end of the purple lines is with the transformed *Enterococcus* density levels.

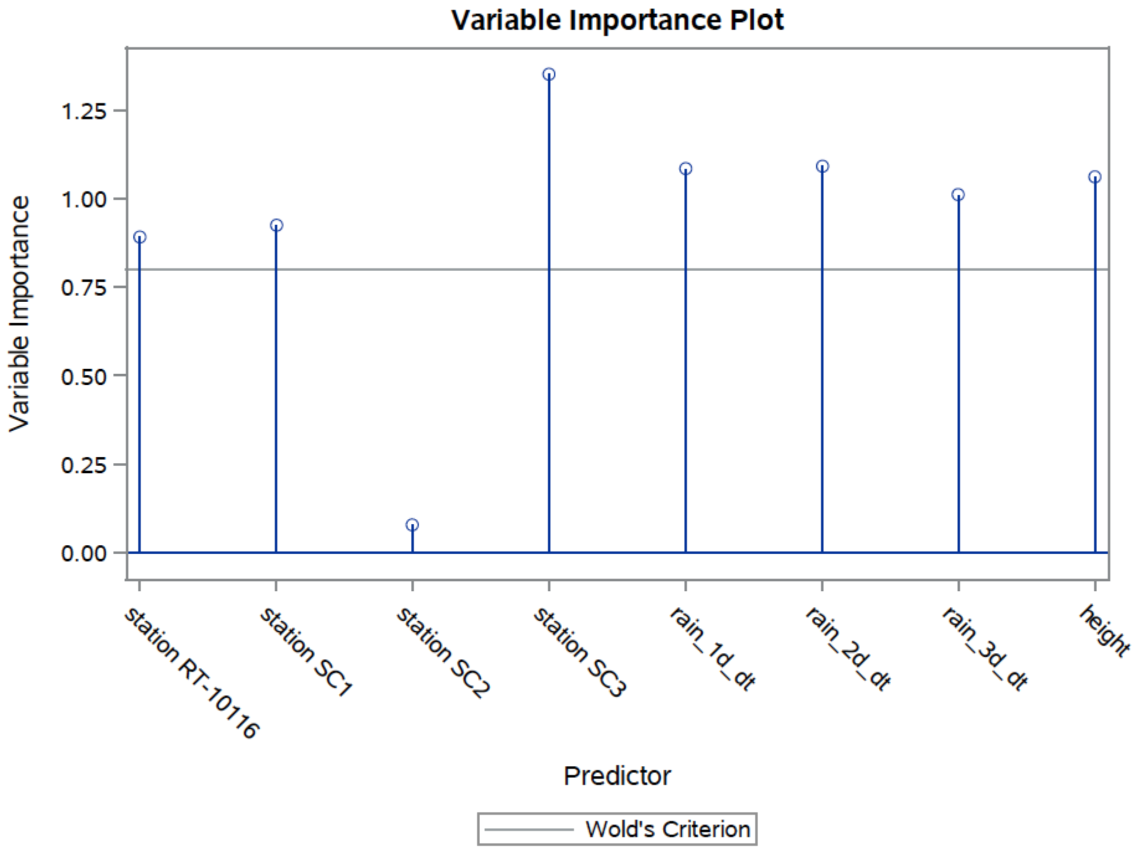


**Figure 3.14:** Variable importance plot based on the Variance Importance for Projection (VIP) statistics of Wold for Model 6, the final model

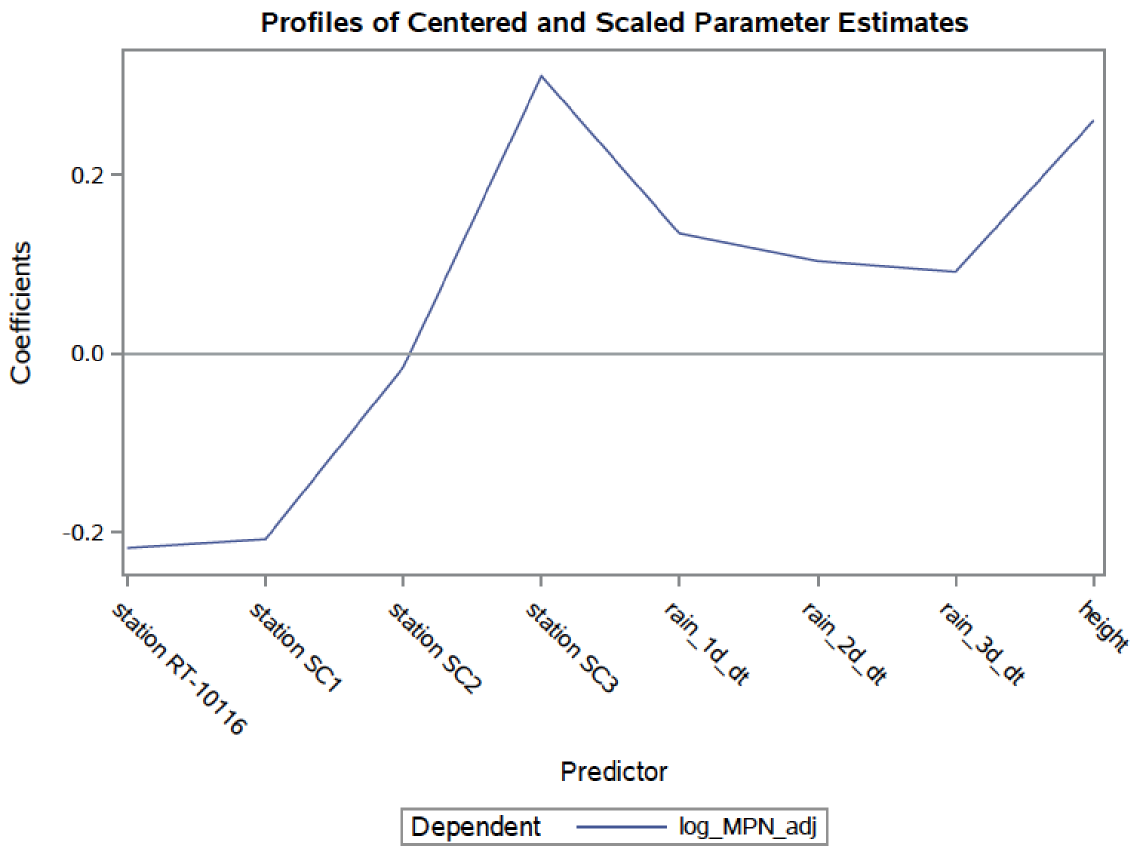


**Figure 3.15:** Regression coefficients profile of parameter estimates

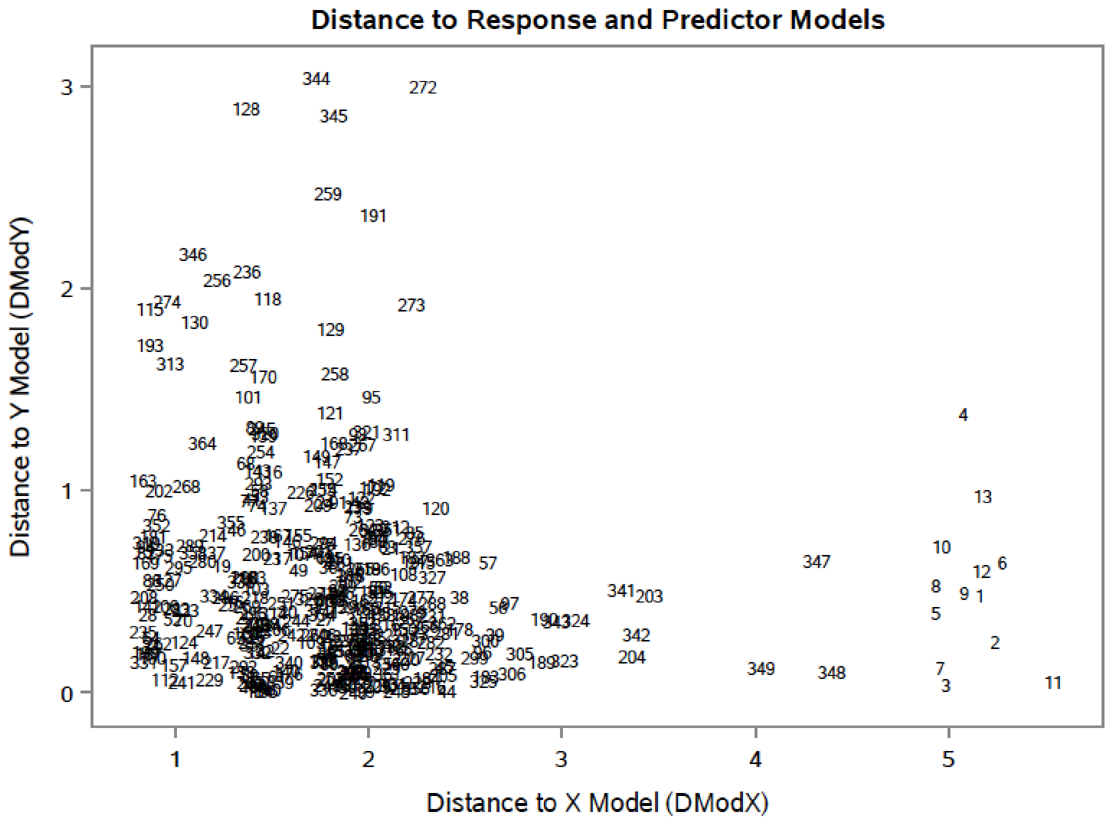


**Figure 3.16:** The “distance to response and predictor models” plot gives the distance from each point to the PLS model with regard to the predictors and responses respectively.

**Table 3.8:** Parameter Estimates

| | **log_MPN_adj** |
|---|---:|
| Intercept | 3.668300 |
| Station RT-10116 | -2.280029 |
| Station SC1 | -0.086197 |
| Station SC2 | -0.064866 |
| Station SC3 | 1.283954 |
| Rain_1d_dt | 0.484188 |
| Rain_2d_dt | 0.271817 |
| Rain_3d_dt | 0.190731 |
| Height | 0.250721 |

**Table 3.9:** Analysis of Variance Table, Testing if the Final Model Explains the Variance of the Response Variables

| Source | DF | Sum of Squares | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| Model | 8 | 629.061249 | 78.632656 | 38.14 | <0.0001 |
| Error | 355 | 731.958159 | 2.061854 | | |
| Corrected Total | 363 | 1361.019409 | | | |

# Chapter 4

## Discussion

As seen in the percent variation accounted for by the partial least squares factors (Table 3.7), 91.69% of the variation in precipitation summed for two days prior to *Enterococcus* sample collection (rain_2d_dt) can be explained by the model. This is the highest percent variation accounted for by the partial least squares factors among all the predictor variables. Because 91.69% is higher than precipitation summed for one day prior to *Enterococcus* sample collection (rain_1d_dt) (76.65%) or precipitation summed for three days prior to *Enterococcus* sample collection (rain_3d_dt) (77.97%), precipitation summed for two days prior to *Enterococcus* sample collection (rain_2d_dt) would be the best precipitation predictor variable to use for future studies looking at influences on *Enterococcus* density levels in the Shem Creek area. Compared to other months, September most frequently exceeded the daily maximum standard for *Enterococcus* density levels in Class SB waters (501MPN/100ml). September, which is also during hurricane season, receives regularly high amounts of precipitation. This explains why both the month of September and precipitation totals were correlated with higher *Enterococcus* density levels.

The correlation loading plot in the final model (Figure 3.12) indicates that station SC3 is highly positively correlated with the transformed *Enterococcus* density levels. In contrast, station SC2 had no correlation, and stations RT-10116 and SC1 are negatively correlated with the transformed *Enterococcus* density levels. The station correlations follow a positive to negative pattern that starts near the headwaters of Shem Creek, where station SC3 is located, and moves to the outflow of the creek, where station SC1 is located. This pattern can be seen by comparing the stations on the correlation loading plot in Figure 3.13 and their locations in Figure 2.2. SC3 is located further inland towards the headwaters of Shem Creek, it is surrounded by extensive marshland, and has far less water volume than the creek has further towards the outflow into the harbor. At the outflow of Shem Creek, there are seawalls on either side of the creek, allowing for restaurants, marinas, and docks to be placed right on the water's edge. The water quality monitoring stations located closest towards the harbor (SC1 and RT-10116, respectively) had a negative correlation with *Enterococcus.* When the tide rises, the water surrounding station SC3 is horizontally distributed, flowing over the extensive marsh area. When the tide falls, the water takes with it the bacteria from the wildlife residing in the marsh, washing it into the creek. In contrast, when the tide rises and falls near the outflow of Shem Creek, the water only changes vertically because of the seawalls preventing horizontal distribution. The creek also has less volume of water further inland, creating higher concentrations of the bacteria than would be seen further down the creek where there is a larger volume of water. The number of times *Enterococcus* density levels exceeded the daily maximum standard for Class SB waters (501MPN/100ml) was higher following days with precipitation less than 0.5 inches compared to days with precipitation greater than 0.5 inches (Figure 4.1). Because the number of exceedances was higher after dry days compared to wet days, this suggests that the water height due to changing tide is a bigger driver for *Enterococcus* density level changes than water height due to changes in precipitation.

Water height was also positively correlated with *Enterococcus* density levels and station SC3. Although the number of septic tanks was not included in the final model, water quality monitoring station SC3, which was highly positively correlated with *Enterococcus,* also had the highest number of septic tanks within a mile radius. The water quality monitoring station SC2, which had no correlation with *Enterococcus* density levels, is located right next to a marina on a bend of the creek and also has a close stormwater discharge outflow. This location acts similarly to a tidal node, where water levels on each side of the point are not the same. The consideration that dumping from the boats in the marina could be keeping the *Enterococcus* density levels stable, regardless of precipitation and water height, was deemed unlikely because of the similar range of bacteria levels found at this station compared with the other stations in the creek.

Stations RT-10116 and SC1, which are located furthest towards the outflow of Shem Creek into the harbor, were negatively correlated with *Enterococcus* density levels. These stations are located where Shem Creek is mixing with the harbor water and diluting the bacteria levels coming from further up in the creek. There is a much higher volume of water here to reduce the bacteria level concentrations. In addition, the seawalls act as a prevention measure for keeping the tidal changes from washing bacteria from the surrounding land area back into the creek. This suggests that building seawalls as a potential mitigation technique for tidal creeks used for recreational purposes that have persistently high *Enterococcus* bacteria levels should be explored further. However, understanding the relationship between impervious surface water runoff and understanding the hydrology of tidal systems would be an essential part of future research exploring seawalls as a mitigation practice for bacterial pollution.

Partial least squares regression is a subset of multiple linear regression and was chosen for this analysis because it is the least restrictive out of the many multivariate methods that can be used for predicting a relationship between predictor and response variables. Unlike more restrictive methods, partial least squares regression extracts factors that are based on a matrix involving both the predictor and response variables (SAS Institute Inc., 2002). Partial least squares regression balances the two purposes of describing the response variation and describing the predictor variation. The advantage of using this method is that each successive factor extracted by the partial least squares regression is an orthogonal factor, meaning it is not correlated with the previous factor (SAS Institute Inc., 2013).

A limitation to this study is that the precipitation data, water height, and specific conductance were not collected in Shem Creek but were collected rather from the downtown Charleston U.S. Customs House. The U.S. Customs House is located across the harbor, approximately 2.25 miles away from Shem Creek (Figure 4.1). Water height at the U.S. Customs House versus at Shem Creek was not expected to change drastically because of the long range of constant tidal fluxes along the coastline. Specific conductance was not used in the final model, but because of the location where it was collected, these values would have been more accurate for the stations closest to the harbor than for SC3, which was further inland. Precipitation values were also collected at the beginning of the study from the Charleston International Airport. The reason these were included was that the data set for the Charleston International Airport was complete with no missing values. Also, this climate station has a full-time employee, making it less likely to have errors in the data. However, because this precipitation data fell out of the model early in the regression analysis and the precipitation data from the U.S. Customs House did not, precipitation in North Charleston is different from rainfall in downtown Charleston. Rainfall was included in the model because it was seen as an important driver for stormwater runoff, influencing bacteria levels in the receiving water body. A rainfall collection gauge located at Sullivan's Island, which would have been closer to Shem Creek, was also considered but was not included in the analysis because of too many missing data points. Because precipitation can vary drastically over spatial areas, incorporating modeling techniques for predicted rainfall based on other climatic factors would be a way for future studies looking at Shem Creek to better represent precipitation values.

According to the South Carolina Department of Natural Resources, Shem Creek is not suitable for aquatic life because of high copper levels and is only partially suitable for recreation because of fecal bacteria levels (SC Department of Natural Resources, 2009). However, locals and tourists use Shem Creek for recreational purposes on a daily basis. Shem Creek is the home to several kayak and paddleboard rental companies that give recreational tours based out of the creek. Because of the concern with high fecal bacteria levels, recreational companies based out of Shem Creek should be aware of the potential risk for gastrointestinal illness or infection especially in immune-compromised clients. According to the results in this study about risk factors for high bacteria levels, illness from exposure to *Enterococcus* in Shem Creek would be more likely to occur after large rain events, at low tide when the water is being pulled from the land, or if exposure to the water occurs further upstream towards the headwaters. Although the public health outcomes are not a causal conclusion explained by the analysis in this study, these results can be used as an informative tool for preventing illness. For example, advertisement for recreational activities in Shem Creek can emphasize use towards the outflow of the creek rather than further inland. There are many residential docs that are located further inland on Shem Creek. Community engaged education about water quality issues in Shem Creek is important for preventing illness for these residents. In addition, kayak and paddleboard rental companies based out of Shem Creek could suggest to clients to paddle towards the harbor rather than towards the headwaters.

Fecal bacterial pollution should be considered when developing new policies impacting zoning laws in the Shem Creek watershed. Zoning categories that incorporate policies limiting the number of buildings per acre could be an effective approach used for targeting the amount of impervious surface in the Shem Creek watershed. However, zoning gives only an estimation of the true amount of impervious surface. Future studies in the Shem Creek watershed could look at depictions of impervious/permeable land area, using methods of digital imaging analysis. In addition, determining the source of bacterial pollution, using microbial source tracking methods, would allow for a better understanding of the complexities associated with *Enterococcus* levels.

In conclusion, zoning and land-use practices between 2010 and 2017 in the Shem Creek watershed have changed by only a small percentage. Change from zoning categories associated with permeable surface to zoning categories associated with impervious surface was calculated to be 3.2% of the total watershed area. This is most likely due to the vast development that was already present in 2010. Multivariate partial least squares regression determined that precipitation and water height were positively correlated with *Enterococcus* bacteria levels in Shem Creek. In addition, water quality monitoring stations located further inland, where the creek was surrounded by extensive marsh, had higher concentrations of *Enterococcus* compared with stations located near the outflow of the creek into the harbor surrounded by seawalls.

Future research looking at the sources for *Enterococcus* in Shem Creek, applying precipitation models for the Shem Creek watershed, and determining if seawalls act as a mitigation technique for tidal creeks with high bacteria levels should be conducted. Implementation of these research findings to landscape planning, land management, and water quality improvement is essential to protecting ecosystem services and the public's health.

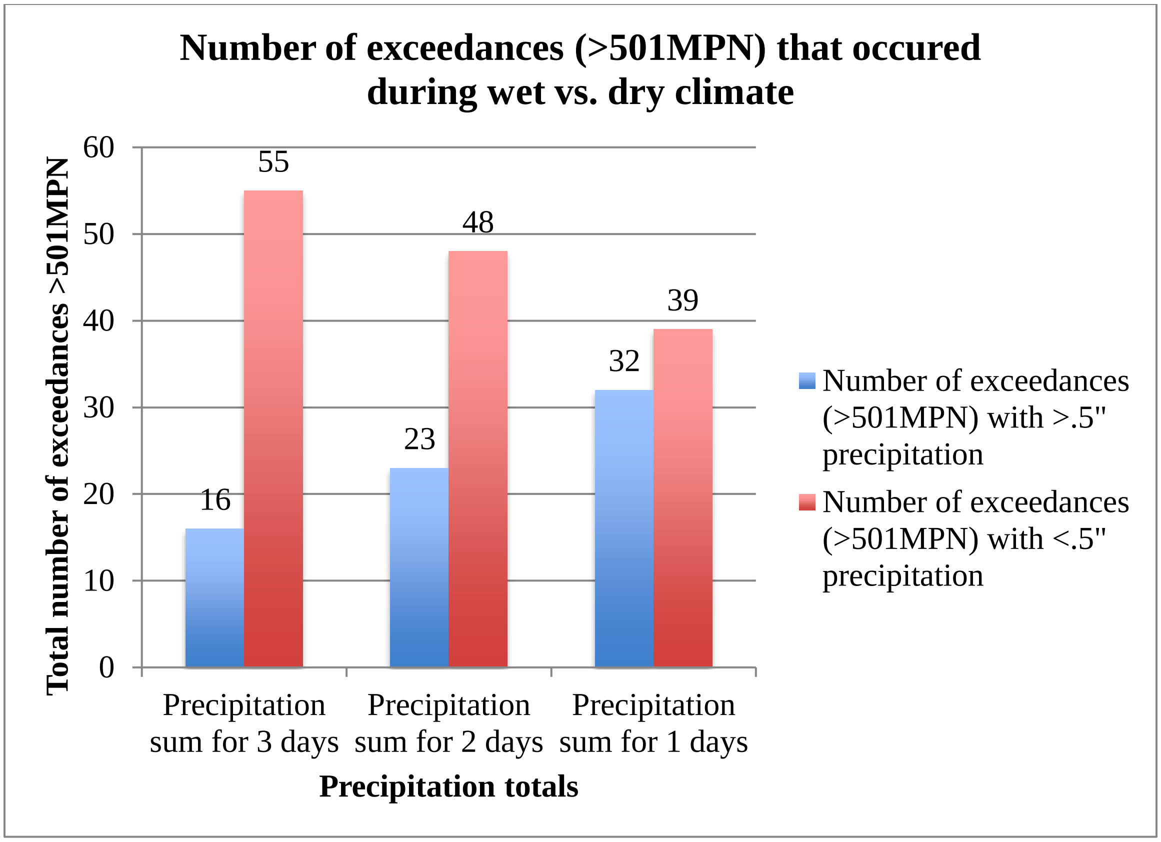


**Figure 4.1:** Number of *Enterococcus* density levels that exceeded the Class SB saltwater recreational limit for a single sample (501MPN/100ml) by wet and dry climate (precipitation <0.5" is considered dry).

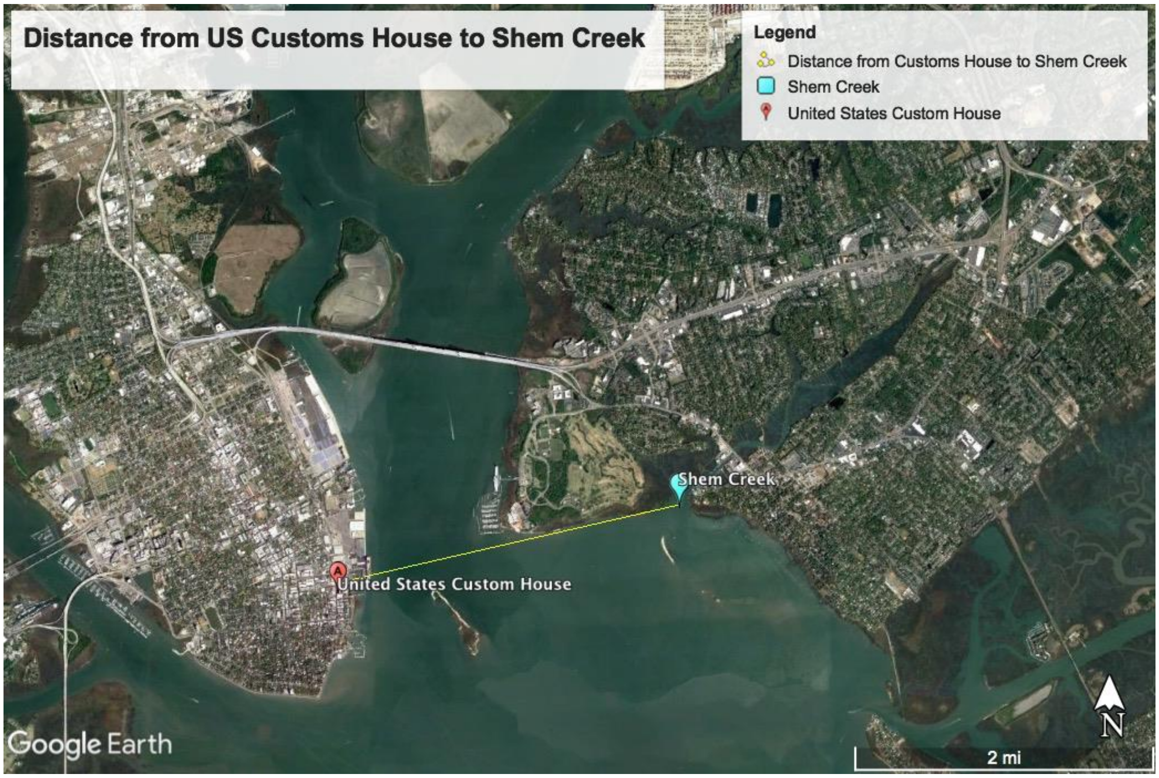


**Figure 4.2:** The distance from US Customs House to Shem Creek

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## Appendix A

## Percent Variation Accounted for by Partial Least Squares Factors for All Models Leading Up to the Final Model Selected and Used for the Final Results

**Table A.1:** Percent Variation Accounted for by the 2 Partial Least Squares Factors for Model 1

| | **Variable** | **Percent Variation Accounted for by the 2 PLS factors** |
|---|---|---|
| Model Effects | Station RT-10116 | 22.9506 |
| | Station SC1 | 19.4476 |
| | Station SC2 | 4.0443 |
| | Station SC3 | 46.1268 |
| | Rain_1d_airport | 29.4620 |
| | rain_2d_airport | 54.3619 |
| | rain_3d_airport | 35.6287 |
| | rain_1d_dt | 30.9234 |
| | rain_2d_dt | 48.3935 |
| | rain_3d_dt | 57.1897 |
| | cond_bottom | 20.0927 |
| | temp | 0.9879 |
| | height | 37.0129 |
| | sep_pt5 | 8.1619 |
| | sep_1 | 50.9534 |
| | **Current** | **14.2970** |
| | **Total** | **31.0492** |
| Dependent Variables | log_MPN_adj | 46.0193 |
| | **Current** | **3.7243** |
| | **Total** | **46.0193** |

**Table A.2:** Percent Variation Accounted for by the 2 Partial Least Squares Factors for Model 2

| | **Variable** | **Percent Variation Accounted for by the 2 PLS factors** |
|---|---|---|
| Model Effects | Station RT-10116 | 24.3254 |
| | Station SC1 | 21.3748 |
| | Station SC2 | 11.8161 |
| | Station SC3 | 51.0769 |
| | rain_1d_dt | 37.3009 |
| | rain_2d_dt | 46.9768 |
| | rain_3d_dt | 40.3355 |
| | cond_bottom | 32.0815 |
| | height | 49.9036 |
| | sep_1 | 54.1360 |
| | **Current** | **12.8982** |
| | **Total** | **36.9328** |
| Dependent Variables | log_MPN_adj | 45.8681 |
| | **Current** | **3.4700** |
| | **Total** | **45.8681** |

**Table A.3:** Percent Variation Accounted for by the 2 Partial Least Squares Factors for Model 3

| | **Variable** | **Percent Variation Accounted for by the 2 PLS factors** |
|---|---|---|
| Model Effects | Station SC1 | 28.6716 |
| | Station SC2 | 11.1737 |
| | Station SC3 | 52.1052 |
| | rain_1d_dt | 35.0868 |
| | rain_2d_dt | 44.0936 |
| | rain_3d_dt | 37.9278 |
| | cond_bottom | 39.6313 |
| | height | 69.4247 |
| | sep_1 | 54.2746 |
| | **Current** | **14.5484** |
| | **Total** | **41.3766** |
| Dependent Variables | log_MPN_adj | 42.2551 |
| | **Current** | **2.7020** |
| | **Total** | **42.2551** |

**Table A.4:** Percent Variation Accounted for by the 2 Partial Least Squares Factors for Model 4

| | Variable | Percent Variation Accounted for by the 2 PLS factors |
|---|---|---|
| Model Effects | Station SC1 | 86.5869 |
| | Station SC3 | 86.5869 |
| | rain_1d_dt | 24.1759 |
| | rain_2d_dt | 23.1569 |
| | rain_3d_dt | 24.7472 |
| | cond_bottom | 44.7062 |
| | height | 67.4352 |
| | sep_1 | 86.5869 |
| | **Current** | **20.7920** |
| | **Total** | **55.4978** |
| Dependent Variables | log_MPN_adj | 45.8772 |
| | **Current** | **3.6376** |
| | **Total** | **45.8772** |

**Table A.5:** Percent Variation Accounted for by the 2 Partial Least Squares Factors for Model 5

| | Variable | Percent Variation Accounted for by the 2 PLS factors |
|---|---|---|
| Model Effects | Station RT-10116 | 16.2292 |
| | Station SC1 | 27.5585 |
| | Station SC2 | 0.2741 |
| | Station SC3 | 54.3680 |
| | rain_1d_dt | 75.2894 |
| | rain_2d_dt | 90.6928 |
| | rain_3d_dt | 81.0070 |
| | cond_bottom | 15.4791 |
| | height | 21.0948 |
| | **Current** | **19.1823** |
| | **Total** | **42.4437** |
| Dependent Variables | log_MPN_adj | 45.7363 |
| | **Current** | **4.8179** |
| | **Total** | **45.7363** |

# Appendix B

## Zoning Classification System

**Table B.1:** Zoning Classification System

| Final Classification | 2017 Zoning Data | 2010 Zoning Data |
|---|---|---|
| Residential | 101 - RESID-SFR, 110 - RESID-MBH, 120 - RESID-TWH, 121 - GROUP-LIV, 130 - RESID-DUP/TRI, 250 - SPCLTY-COMMCONDO, 160 - RESID-CNU, 165 - CONDO COMMON, 167 - CONDO COMMON COMM, 195 - COMM-APP-RES, 200 - SPCLTY-APT, 900 - RES-DEV-ACRS , 910 - COM-DEV-ACRS | APARTMNT-BLD, COMCL/RESIDN, CONDO, COMMON, CONDO-UNIT, DUPLEX, HOTEL-MOTEL, TOWNHOUSE, SMALL-APTS, SNGL-FAM-RES |
| Commercial | 500 - General Commercial, 671 - GOVT-BLDG, 681 – SCHOOLS, 700 - SPCLTY-HTL | COMMERCIAL, OFFICE, RESTAURANT, RETAIL |
| Recreational | 750 - SPCLTY-REC, 140 - MH-PARKS, 711 - MUSEUM-CULT | CULT-ENT-REC |
| Agricultural | 800 – AGRICULTURAL | AGRICULTURAL |
| Vacant | 905 - VAC-RES-LOT, 952 - VAC-COMM-LOT | LAND-ONLY, VACANT-COM, VACANT-RES |
| Undevelopable | 990 - UNDEVELOPABLE | UNDEVELOPABL |
| Other | 210 - SPCLTY-SMA, 220 - SPCLTY-TAMSBERG, 225 - SPCLTY-CNU-TMSBRG, 300 - BUILDNG-ONLY, 460 - AUTO-PARKING, 471 - TELEPH-COMM, 481 - PUBLIC-UTIL, 530 - SPCLTY-RTL, 580 - SPCLTY-RST, 630 - SPCLTY-WHS, 650 - SPCLTY-OFC, 691 – RELIGIOUS, 742 - HOA-PROP | SPCL-PURPOSE |