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Sources and Long-term Trends in Fecal Bacteria Contamination in the Fresh and Marine Waters of the Grand Strand

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Sources and long-term trends in fecal bacteria contamination
in the fresh and marine waters of the Grand Strand

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Submitted in Partial Fulfillment of the
Requirements for the Degree of Master of Science in
Coastal Marine and Wetland Studies in the
Department of Coastal and Marine Systems Science
School of Coastal Environment
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Abstract

Microbial water quality is of significant concern in the two watersheds of the Grand Strand. Polluted runoff, malfunctioning septic tanks, and centralized sanitary sewer failures are common sources of fecal bacteria contamination in coastal areas and pose a threat to human health through recreational use of waterbodies and shellfish consumption. Volunteer water quality monitoring programs are crucial in expanding upon assessments of fecal bacteria contamination by regulatory monitoring. Bacteria monitoring data collected by volunteers and Coastal Carolina University's Environmental Quality Laboratory has been used to identify sites to be investigated by microbial source tracking. Microbial source tracking has been used throughout the Grand Strand to identify nonpoint sources of fecal bacteria pollution. Findings from such studies have been used to develop management plans for reducing fecal pollution in the coastal region.

This thesis focuses on three projects aimed towards improving MST in the waters of the Grand Strand: (1) a cross comparison study between the *Escherichia coli* enumeration methods currently used by local monitoring programs, (2) a microbial source tracking study in Murrells Inlet Estuary to investigate fecal pollution sources at contaminated sites identified by the local volunteer water quality monitoring, and (3) synthesis of reports from local coastal MST studies conducted throughout the Grand Strand over the past two decades. The results from the three research projects presented in this thesis are intended to aid in selection of suitable management approaches and in optimization of future monitoring and microbial source tracking work in the waters of coastal northeastern South Carolina.

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List of Acronyms and Abbreviations

| | |
|---------|---|
| BMP | Best Management Practice |
| CCU | Coastal Carolina University |
| CE | Coliscan [®] Easygel [®] |
| CPE | Coliscan [®] Plus Easygel [®] |
| CFU | Colony Forming Unit |
| C-18 | Colilert-18 [™] |
| EQL | Environmental Quality Laboratory |
| FIB | Fecal Indicator Bacteria |
| MAR | Multiple Antibiotic Resistance |
| MPN | Most Probably Number |
| MST | Microbial Source Tracking |
| NURP | National Urban Runoff Program |
| PF | 3M Petrifilm [™] |
| QMRA | Quantitative Microbial Risk Assessment |
| qPCR | Quantitative Polymerase Chain Reaction |
| RPD | Relative Percent Difference |
| RWQC | Recreational Water Quality Criteria |
| SC DHEC | South Carolina Department of Health and Environmental Control |
| SFH | Shellfish Harvest |
| SOP | Standard Operation Procedure |
| TMDL | Total Maximum Daily Load |

| | |
|--------|---|
| US EPA | United States Environmental Protection Agency |
| WWA | Waccamaw Watershed Academy |
| VWQM | Volunteer Water Quality Monitoring |

Chapter 1

Introduction

1.1. Overview

The coastal communities of the Grand Strand have a significant impact on water quality of the region's waterways. Specifically, growing coastal populations lead to development of natural areas that often degrades microbial water quality of both coastal fresh and marine waters. Impervious surfaces associated with development interfere with the natural processes of filtration and pollution removal that help maintain water quality. Increasing impervious surface coverage increases the rate of stormwater transport of land-based pollution. Growing coastal populations result in an expansion of the sanitary sewer system and may also increase the number of septic systems, some of which may fail or malfunction, leading to microbial pollution. Polluted runoff, malfunctioning septic tanks, and centralized sanitary sewer failures are common sources of fecal bacteria pollution in coastal areas and pose a threat to human health when entering coastal waters.

Risk of gastrointestinal illness is determined by concentrations of fecal indicator bacteria (FIB) in recreational waters and waters used for shellfish harvest. FIBs *Escherichia coli* (*E. coli*), *Enterococcus*, and fecal coliforms are used to identify the

possible presence of fecal pathogens. Humans can become ill by direct contact with contaminated recreational waters or by consumption of shellfish harvested from contaminated waters. Maintaining good microbial water quality can decrease the risk of illness in humans. In the Grand Strand, due to the abundance of coastal waters, contamination by fecal bacteria is a growing concern impacting recreation and shellfish harvesting.

Locally, fecal pollution is a major concern for both recreational and shellfish waters. Several regulatory mechanisms are designed to address protection of waters associated with recreation and consumption of shellfish. The United States Environmental Protection Agency (US EPA) establishes water quality criteria under the authority of the Clean Water Act (CWA) to protect the health of natural waters and the safety and welfare of humans using those waters for drinking water, food resources, and recreation. The Recreational Water Quality Criteria (RWQC) sets standards for fecal bacteria concentrations to minimize the incidence of illness in recreational bathers (US EPA 2012). In turn, South Carolina Department of Health and Environmental Control (SC DHEC) establishes state standards based on the USEPA standards. The RWQC and state water quality standards are used under the Beach Environmental Assessment and Coastal Health (BEACH) Act in monitoring recreational beach water to inform swimming advisories at beaches when water quality standards are contravened. The National Shellfish Sanitation Program (NSSP), managed by the U.S. Food and Drug Administration, determines guidelines for waters where shellfish production occurs. The NSSP was established to set uniform national standards to enable the sale of safe shellfish across state boundaries. Shellfish beds are closed to harvest when standards are exceeded. Section 303(d) of the

CWA requires states to develop a list of water bodies that do not meet water quality standards. Total Maximum Daily Loads (TMDL) must be developed and implemented for the improvement of waters that exceed standards. SC DHEC's 2016 draft of the 303(d) List identifies 54 water bodies in Horry and Georgetown counties that are considered impaired due to FIB concentrations (SC DHEC 2016).

State monitoring of local waterbodies is performed monthly to determine whether water quality standards (WQS) have been contravened. These data are used to develop the 303(d) list. These regulatory measurements are enhanced by the collection of water quality by volunteers on a more frequent basis. Volunteer monitoring programs are crucial in assessing fecal contamination in the Pee Dee Coastal Frontage and Waccamaw River watersheds throughout Horry and Georgetown counties of South Carolina. Bacteria monitoring through the Waccamaw Watershed Academy (WWA) by volunteers and Coastal Carolina University's (CCU's) Environmental Quality Laboratory (EQL) researchers has led to identifying sources and levels of contaminant bacteria in the surf zone of the Grand Strand and the Waccamaw River. Monitoring programs are funded by local municipalities to meet the requirements of the National Pollution Discharge Elimination Program (NPDES) Phase II Stormwater permits issued by SC DHEC. There are currently four volunteer water quality monitoring (VWQM) programs in the two watersheds. These are based in the Waccamaw River, Murrells Inlet, Surfside Beach, and on CCU's campus.

Data collected by VWQM programs can identify sites to be investigated by microbial source tracking (MST). MST uses a variety of methods to determine, and even quantify, sources of microbial pollution, specifically FIB focusing on nonpoint sources of

pollution rather than point sources. While the main concern for pollution was once point sources, improvements in wastewater treatment, industrial operations, and coastal development have caused a shift in pollutant sources. Nonpoint sources, especially runoff, are now the major contributors to pollution in natural waters. Identifying impaired waters is a precursor to developing management plans for reducing fecal pollution.

Three projects were undertaken as part of this thesis research to improve MST in the waters of the Grand Strand. First, a cross comparison study was conducted between the *E. coli* enumeration methods currently used by local monitoring programs (regulatory and volunteer). Accurate enumeration methods are crucial when evaluating microbial water quality to identify contaminated water bodies. If results are inaccurate a site may not be correctly identified as impaired or unimpaired leading to repercussions concerning human health and remediation efforts. Second, a MST study was conducted in Murrells Inlet Estuary to investigate fecal pollution sources at contaminated sites identified by the local VWQM. Murrells Inlet is home to shellfish beds that receive inputs from waters with poor microbial water quality. The detection of the pollution sources could lead to remedial efforts to reduce pollution to safe levels for shellfish harvests. Third, reports from local coastal MST studies conducted over the past two decades were synthesized and a resource webpage was developed with local water resource managers in mind. The population of the Grand Strand is continuing to grow and, as a result of associated development and increased impervious cover, so will the risk of fecal bacteria contamination. By examining results from prior microbial water quality research, a better understanding of the causes of contamination can be obtained and then used to develop suitable management approaches.

The results from the three research projects presented in this thesis are intended to aid in selection of suitable management approaches and in optimization of future monitoring and MST work in the waters of coastal northeastern South Carolina.

1.2. Literature Review

Pollution by fecal bacteria contamination is a major concern in coastal areas like the Grand Strand in northeastern South Carolina where the economy is highly dependent on water-based recreation and tourism. Increased pollution to recreational waters in the southeastern United States has led to increased beach closures and pollution advisories (Mallin 2006). The cause for increased pollution is directly related to increasing coastal populations. More than half of the country's population now lives in coastal counties (Mallin et al. 2001). Land use and land cover have been significantly altered with the growing population and the increase in impervious surfaces; paved roads, parking lots, and buildings have transformed the landscape that was once forests and wetlands (Mallin 2006). This transition has disrupted natural drainage systems and resulted in the fouling of coastal waters (Mallin 2006). Specifically, water quality has been degraded by fecal bacteria contamination.

Studies have linked land use to microbial water quality (DiDonato et al. 2009, Mallin et al. 2001) as well as overall water quality impairments (Mallin et al. 2000). Water quality is inversely related to increased impervious surfaces (Mallin et al. 2000). A significant correlation between watershed populations and fecal coliform and *E. coli* concentrations was identified in estuaries of southeastern North Carolina (Mallin et al. 2000). Previous studies have identified 10% watershed impervious surface coverage as the threshold for potentially impaired waters (Schueler 1994). Mallin et al. (2000) confirmed

the threshold value for estuaries, identifying impaired water quality when greater than 10% of the watershed was impervious cover. Impervious surface coverage alone explained 95% of the variability in average estuarine fecal coliform bacteria concentration (Mallin et al. 2000). Tidal creeks categorized by land use and stream order show a similar relationship between development and water quality (DiDonato et al. 2009). First order creeks show increasing concentrations of FIB with increasing watershed impervious cover (DiDonato et al. 2009). While impervious surfaces are not a direct cause of fecal bacterial pollution, development reduces the natural water purification function of vegetation and soil and contributes to large volumes of untreated water runoff (Mallin et al. 2001). Increased pollution due to runoff from land increasingly covered by impervious surfaces could negatively impact the microbial water quality in coastal northeastern South Carolina.

1.2.1. Federal Regulatory Policy

The historical transition in federal regulatory policy from management of point source pollution to non-point source pollution has increased the need for MST to identify and reduce sources of fecal pollution. When water quality became a major regulatory concern in the U.S. in the 1960's and 1970's, the highest priority pollution sources were typically point source discharges from industry or sewage treatment plants. With improved regulation on point source polluters ushered in by the enactment of the CWA and associated regulations, the remaining major contributors to microbial pollution are now nonpoint sources that can be difficult to identify within a watershed. Regulatory policy at the state and federal level has moved towards addressing the increasing concern of pollution from nonpoint sources through the CWA's NPDES Phase II stormwater program

directed at small municipal stormwater systems (SMS4s) and the Coastal Zone Management Act's Coastal Nonpoint Pollution Control Program.

Federal regulation of natural waters in the United States is mandated by the CWA of 1972. The objectives of the CWA are to restore and maintain the chemical, physical, and biological integrity of the nation's waters by controlling point and nonpoint pollution sources. The CWA requires that NPDES permits be obtained for the discharge of pollutants into surface waters from point sources and nonpoint sources. The original legislation only applied to point sources of pollution but the law was amended by the Water Quality Act of 1987 to include nonpoint sources in response to the results of the National Urban Runoff Program (NURP). Stormwater runoff was identified by NURP as a major contributor to fecal bacterial contamination (US EPA 1983). The study also stated that wetlands provided a promising technique for runoff control (US EPA 1983). NURP demonstrated that development was a contributing factor to fecal bacteria pollution. The inclusion of SMS4s under the NPDES Phase II stormwater program requires municipalities in Horry and Georgetown counties to monitor and manage runoff.

In the coastal zone, additional regulatory policy is in place to protect the unique and complex coastal system. The National Coastal Zone Management Act provides funding for state programs that develop their own Coastal Zone Management Plan (CZMP). In South Carolina, the CZMP is managed by SC DHEC Ocean and Coastal Resource Management (OCRM) which issues permits for uses that have the potential to impact coastal resources. The Coastal Zone Act Reauthorization Amendments of 1990 (CZARA) established the Coastal Nonpoint Pollution Control Program, which is jointly administered by the National Oceanic and Atmospheric Association (NOAA) and US EPA. The program aims to reduce

polluted runoff to coastal waters by requiring coastal states with CZMPs to develop nonpoint pollution control programs. These regulations specifically geared towards protecting the coastal zone are a response to the increasing populations living in the U.S. coastal zone and the recognition that natural resources are being rapidly degraded with increasing populations.

Regulations protecting human health associated with microbial water quality are provided by the NSSP and the US EPA's RWQC. The NSSP is a cooperative program between federal and state governments recognized by the U.S. Food and Drug Administration and the Interstate Shellfish Sanitation Conference (ISSC) (US FDA 2011). The program provides guidelines to promote and improve shellfish sanitation (US FDA 2011). Waters used for shellfish growing and harvest must be monitored for fecal coliform concentrations to ensure the shellfish are safe for human consumption. If the standards set by NSSP and the state regulators are exceeded, shellfish beds can be closed to harvest. SCDHEC monitors a total of 450 sites in 25 shellfish management areas along the South Carolina coast. Six of the management areas are in Horry and Georgetown counties. The NSSP is aimed towards reducing the risk of illness in humans due to poor microbial water quality through shellfish regulation while the RWQC concerns the protection of human health through contact with recreational waters.

The US EPA is tasked by the CWA with developing current RWQC. The first RWQC was published in 1986 and remained the standard until the CWA was amended by the passage of the BEACH Act of 2000 that mandated an update of the RWQC. The US EPA was required to publish new criteria by 2012 and to conduct epidemiological studies in water polluted by urban runoff, determine the applicability of data obtained from coastal

freshwater sites to inland waters, and evaluate new methods including quantitative microbial risk assessment (QMRA) (Fujioka et al. 2015). A panel of scientists tasked with making recommendations for the revised RWQC identified the need to focus on pollution by nonpoint sources as point sources were no longer a major concern but had been the basis for the existing RWQC (Boehm et al. 2009).

The 2012 RWQC did not meet expectations because key recommended studies were not completed, new data to assess risks to bathers exposed to nonpoint sources of FIB were not developed and the criteria did not show marked improvements in strategies for assessing health risks for bathers using all types of recreational waters (Fujioka et al. 2015). Epidemiological studies did not adequately examine sites with nonpoint sources of pollution. Concentrations of nonpoint sources of FIB have not yet been correlated to gastrointestinal illness rates despite being the prominent source of microbial pollution in U.S. waters (Fujioka et al. 2015). A good advisory indicator should be non-pathogenic, rapidly detected, easily enumerated, and have survival characteristics similar to pathogens of concern as well as discriminatory power between hosts (Meays et al. 2004). Scientists have suggested that other organisms, such as *C. perfringens*, be used as indicator organisms (Scott et al. 2002). However, these organisms cannot be used for regulatory purposes without obtaining approval from the US EPA and FIB remain the primary indicator organisms used in MST until further action is taken. Overall, the new RWQC is considered inadequate to meet the needs of current water quality assessments.

1.2.2. South Carolina Regulatory Policy

South Carolina's state water quality standards adhere to the US EPA's RWQC. The state is required to review state water quality standards every three years and the most recent review occurred in 2012 while the new RWQC was still being processed. The water quality standards established by SC DHEC were approved by the US EPA in 2012. While the current state standards may not specifically correspond with the 2012 RWQC, the values established for microbial water quality in recreational waters are still quite similar to current EPA standards (**Table 1-1**). Still, neither set of values have been correlated with nonpoint sources that are the primary contributors in coastal South Carolina and thus the standards may not be entirely accurate for reducing illness rates (Fujioka et al. 2015). The current RWQC is limited in its ability to ensure the safety of recreational water users.

Section 303(d) of the CWA requires states to develop a list of impaired waters based on the results of routine monitoring that is updated every two years. In South Carolina, SCDHEC manages the state 303(d) list of impaired waters. Waters are evaluated on water quality parameters, including FIB parameters to evaluate microbial water quality (SCDHEC 2016). The most recent impaired waters list for South Carolina, which still requires formal approval by the US EPA, identifies 54 water bodies in Horry and Georgetown counties that are considered impaired due to FIB concentrations (SC DHEC 2016). The impaired waters are evaluated based on standards developed in the NSSP and the RWQC (**Table 1-2**). Standards for *E. coli* are used in fresh and marine recreational waters as the primary FIB while standards for *Enterococcus* are used only in marine waters (US EPA 2012). For waters associated with shellfish harvest, fecal coliforms are used (US FDA 2011).

Table 1-1. Water quality criteria for South Carolina waters. US EPA RWQC (US EPA 2012) and South Carolina water quality criteria for recreational waters and shellfish harvesting waters (SC DHEC 2012). RWQC standards are based on an estimated illness rate of 36 per 1,000 primary contact recreators (US EPA 2012). SC DHEC standards are for protection of recreational waters monitored under NPDES permits and shellfish harvesting waters monitored under the NSSP (SC DHEC 2014).

| | US EPA 2012 RWQC (CFU/100mL) | SC DHEC RWQC (MPN/100mL) | SC DHEC Shellfish (MPN/100mL) |
|---------------------------------------|---|---|--|
| Freshwater | | | |
| Monthly average (<i>E. coli</i>) | 126 | 126 | --- |
| Daily Maximum (<i>E. coli</i>) | 410 | 349 | --- |
| Marine & fresh | | | |
| Monthly Average (enterococci) | 35 | 35 | 35 |
| Daily Maximum (enterococci) | 130 | 104 | 104 |
| Tidal saltwater | | | |
| Monthly Average (Fecal coliform) | --- | --- | 14 |
| Daily Maximum (Fecal Coliform) | --- | --- | 43 |

Table 1-2. Number of waterbodies listed as impaired. Impaired waterbodies are by category and FIB based on the draft of SCDHEC 2016 303(d) list of impaired waterbodies. (SCDHEC 2016).

| County | No. of Total Impaired Waterbodies | No. of Impaired Recreational Waterbodies | | | No. of Impaired Shellfish Waterbodies | |
|-------------------|--|---|---------------------|------------|--|------------|
| | | <i>E. coli</i> | <i>Enterococcus</i> | % of Total | Fecal coliform | % of Total |
| Horry | 66 | 8 | 16 | 36% | 14 | 21% |
| Georgetown | 39 | 3 | 2 | 13% | 11 | 28% |

1.2.3. Microbial Source Tracking Methodology

Though the waters identified as impaired by SC DHEC certainly exceed the regulatory criteria, the FIB causing this may not necessarily indicate the presence of pathogens harmful to humans. Another shortcoming of the US EPA's 2012 RWQC is its

failure to provide a sewage specific marker, leading instead to the continued use of traditional FIB (Fujioka et al. 2015). Because traditional FIB are not specific to humans the markers are not clear indicators of human sources. For example, fecal bacteria from other sources, such as pets and wildlife, are often not pathogenic to humans (Roslev & Bukh 2011).

If reported FIB concentrations are high due to sources other than humans, mitigation efforts could be needlessly costly when there is no immediate risk to human health. Also, there are not reliable mitigation techniques to address wildlife sources. To better identify host-animal sources of pollution, MST is used. A variety of MST technologies have been developed to identify potential host-animal source of fecal pollution such as genotypic assays and chemical tracers (Scott et al. 2002).

Traditional FIB are also utilized in MST studies because the RWQC rely on these and hence they provide a linkage to the regulatory realm (USEPA 2012). Although FIB cannot establish that pathogens are present, they continue to be used for MST because they are easier and less costly to detect and enumerate than the actual pathogens (Harwood et al. 2014, Meays et al. 2004). In addition, attempts to detect pathogens that are present in low concentrations may result in false negative measurements, even though the undetected presence still presents a human health risk.

A variety of methods are used in MST including molecular, biochemical, and chemical techniques to track and identify pollution sources. Though many methods have been tested and analyzed, no single particular method stands out as a “gold standard” (Roslev & Bukh 2011). MST techniques have improved over time, but critics insist the field has not reached a point where methods can be discarded or universally recommended

(Stoeckel & Harwood 2007). It has been suggested that a multi-tiered approach to MST utilizing multiple methods and disciplines be used (Roslev & Bukh 2011). MST procedures in the EQL use a weight-of-evidence approach that relies on an index computed from the results of multiple tracers (i.e. FIB, genetic assays, and chemicals) to determine the source of pollution. Using multiple methods provides the validation called for by Stoeckel & Harwood (2007) that is needed to bring MST from a purely research-orientated use to actual applied use. The weight-of-evidence approach has been employed in many MST studies conducted in the waters of the Grand Strand to identify sources of fecal pollution. A particular local application has been in determination of whether fecal bacteria is human-sourced. Human-sourced FIB has been identified in Withers Swash, including high levels associated with sewer-line breaks in the immediate vicinity (Wood et al. 2013). Significant levels of human-sourced fecal bacteria have also been documented in White Point Swash in Briarcliffe Acres (Karkowski et al. 2002). The successful use of MST in these local watersheds is encouraging and indicates it could be useful in other areas along the Grand Strand.

1.2.4. Volunteer Water Quality Monitoring

Water quality monitoring is often the first step in a MST study in order to identify impaired sites that may need further investigation. VWQM has been identified by the US EPA as an acceptable measure for meeting a Minimum Control Measure (MCM) of the NPDES Phase II Stormwater Program (Libes et al. 2012). Under the CWA, municipalities are required to develop and implement stormwater management programs to address MCMs focusing on reducing nonpoint sources of pollution from stormwater runoff. The

six MCMs are: (1) public education and outreach, (2) public participation/involvement, (3) illicit discharge detection and elimination, (4) construction site runoff control, (5) post-construction stormwater management, and (6) pollution prevention/ good housekeeping.

In the Grand Strand, VWQM helps meet some of the requirements of the NPDES Phase II Stormwater Program. VWQM is conducted by citizen scientists throughout the area under one of four programs. The programs are a cost-effective stormwater management strategy providing data over a large spatial and temporal scale while engaging communities in stormwater management (Libes et al. 2012). Technical support for these programs is provided by the Waccamaw Watershed Academy (WWA) which was formed in 2004 to meet local needs for expertise in watershed and wetland science and management. The four programs are in the Waccamaw River, Murrells Inlet, Surfside Beach, and on CCU's campus. Additional information about each of the programs is displayed in **Table 1-3**. The overall goals of the VWQM programs are to: (1) address NPDES Phase II Stormwater Program MCMs for public education and involvement, (2) document long-term water quality trends with a focus on identifying sites with poor water quality, (3) assist with illicit discharge detection, and (4) demonstrate improvements arising from implementation of stormwater best management practices (BMPs) (Libes et al. 2012).

Table 1-3. Descriptions of each of the VWQM programs in the Grand Strand.

| Program | Initiated | Field Leader | Area Covered | Goals |
|----------------|------------------|---|---|--|
| Waccamaw River | 2006 | Waccamaw Riverkeeper | 140 river miles on the Waccamaw River; 12 sites in Horry and Georgetown counties and 6 sites in Brunswick and Columbus counties in NC | Meet TMDL for dissolved oxygen |
| Murrells Inlet | 2008 | Murrells Inlet 2020 | 8 tributaries to the mesotidal estuary in both Horry and Georgetown counties | Support implementation of fecal coliform shellfish TMDL requiring 80% reduction in pathogens |
| Surfside Beach | 2010 | Surfside Beach Stormwater Committee chair | 2 sites in a network of ponds received drainage waters from Horry county eventually discharging into the Atlantic Ocean | Investigate contribution to impaired swashes on 303(d) list for recreational WQS |
| CCU Campus | 2011 | Waccamaw Riverkeeper | 3 sites in a network of stormwater ditches and ponds on CCU campus | Determine water quality contributions from CCU campus to Waccamaw River |

Sampling by the VWQM programs is conducted twice monthly year round. Teams measure dissolved oxygen, temperature, conductivity/salinity/total dissolved solids, pH, turbidity, nitrate, nitrite, ammonia, *E. coli*, and total coliforms (Libes et al. 2012). These data are available to the public through an online database located at: <http://bccmws.coastal.edu/volunteermonitoring/index.html>. Data collected by the VWQM programs has identified sites for investigation by MST to determine sources of pollution in order to reduce fecal bacteria loading.

1.2.5. *E. coli* Enumeration Methodology

Monitoring of FIB is done by both the EQL and VWQM program in Horry and Georgetown counties. The EQL uses IDEXX's Colilert-18™ (C-18), an EPA approved

method to enumerate *E. coli*, while the VWQM program uses Coliscan[®] Plus Easygel[®] (CPE), which is not an EPA approved method but is widely used by volunteer programs in the U.S. At the time volunteer monitoring standard operating procedures (SOPs) for measuring *E. coli* were developed for the WWA's program, Coliscan[®] Easygel[®] (CE) had been validated for volunteer monitoring (O'Brien 2006). These results have since been reconfirmed by Stepenuck et al. (2010). CE was developed for use in natural waters and has a lower detection limit than other low cost commercially available *E. coli* enumeration methods (O'Brien 2006). CPE follows the same procedures and is nearly identical to CE except that it has an additional quantification step using UV light to provide a secondary confirmation of *E. coli* colonies (Micrology Laboratories 2008).

While the C-18 and CE methods have been validated for enumeration of *E. coli*, use of C-18 and CPE at a site monitored concurrently by the EQL and WWA (Myrtle Lake, Surfside Beach) have reported vastly different concentrations for the same site on the same sampling date. The discrepancy in results for Myrtle Lake, which will be discussed in detail in the *E. coli* Enumeration Method Cross Comparison chapter of this thesis, prompted an investigation into the accuracy and comparability of the two methods. The reporting methods for C-18 and CPE are different; C-18 reports in most probable number (MPN) while CPE reports in colony forming unit (CFU). Though the current EPA recreational water quality criteria are presented in CFU's, approved methods for quantification of *E. coli*, such as C-18, and are reported as MPN (SC DHEC 2014, US EPA 2012). This illustrates that the two units are often used interchangeably.

Comparing samples from the same water bodies Cho et al. (2010) found that enumerated *E. coli* reported in a method reading MPN were consistently greater than when

reported using a method reading CFU. Though positively correlated, MPN results were consistently higher than those reported as CFU (Cho et al. 2010). C-18 is reported in MPN while CPE is reported in CFU. From O'Brien (2006), this issue appears to have caused no more than a 6% reduction in *E. coli* in CE as compared to C-18.

The difference in metabolic endpoints has been identified as a possible cause for different results between methods (Gronewold & Wolpert 2008). The issue at Myrtle Lake, however, cannot be attributed to such differences. Unlike comparisons between methods based upon different products of bacterial growth by Noble et al. (2003), CPE and C-18 both rely on the production of the same two enzymes, β -galactosidase and β -glucuronidase, by *E. coli* bacteria. The enzyme reacts with a fluorogenic substrate, MUG (4-methylumbelliferyl- β -D-glucuronide), with the resulting product being visible as bright yellow (C-18) or blue (CPE) fluorescence under long-wave UV light (IDEXX Laboratories 2004, Micrology Laboratories 2008). In CPE, a chromogenic substrate is also used to produce a blue color under visible light (Micrology Laboratories 2008).

C-18 has been documented to produce false positive results. Several bacteria including *Aeromonas* spp., *Pseudomonads*, some *Salmonella* and *Shigella* spp, and *Flavobacterium* spp. are known to cause this phenomenon (Pisciotta et al. 2002). While C-18 is used in freshwater and saltwater for regulatory purposes throughout the U.S., validation of the method was performed primarily in marine waters of California (Pisciotta et al. 2002). An investigation into high *E. coli* counts by C-18 in subtropical marine and estuarine waters revealed a false-positive rate of 27.3% (Pisciotta et al. 2002). A subsequent study of subtropical freshwater samples revealed low false-positives, 7.4%, for C-18 (Chao et al. 2004).

Studies have been performed to compare the performance of C-18 and CE to 3M™ Petrifilm™ (PF), another method used by volunteer monitors in other areas. Several evaluations of the methods have been conducted for use by volunteer monitors. Stepenuck et al. (2010) identified CE and PF as adequate methods for use by volunteer monitors, both exceeding 80% accuracy compared to US EPA approved method, but reported that PF has greater agreement than CE. Vail et al. (2003) also identified PF as a useful method for screening for *E. coli*. However, when compared to Colilert (the predecessor to Colilert-18), PF produced results up to 2 orders of magnitude higher in a study on beach water from Lake Superior and Lake Michigan (Kleinheinz et al. 2012). A 36 month study in streams with variable *E. coli* concentrations over different seasons showed good agreement and low false positive rates for Colilert when compared to the standard membrane filtration method (Method 1603) (Buckalew et al. 2006). Graduate students at Massachusetts Institute of Technology and Auburn University have compared CE and PF to EPA-approved methods and have found the enumeration methods to be similar (Trottier 2010, Yuan 2016). Though the methods seem to have been extensively evaluated, there has not yet been a comparison for validation completed for use by volunteer monitors in natural waters of the southeastern U.S. In addition, CPE has not previously been validated against the other methods. Strains of *E. coli* in local waters may respond differently in these enumeration methods than strains of *E. coli* present in other parts of the U.S. Further investigation is needed to determine which method is best suited to the particular sites monitored in Horry and Georgetown counties by VWQM programs.

1.3. Summary

Microbial water quality in the Grand Strand is a major concern for those who use local waterways for recreation, irrigation, and sources of food and drinking water. Healthy waterways are integral to the natural coastal system and the economic survival of the area that draws 15 million visitors a year. The use of MST to identify the sources of microbial water quality impairments has proven a useful tool in the region and will continue to be important for informing management measures aimed at maintaining good water quality. A thorough understanding of MST methodologies and lessons learned from past local studies both serve as a guide for future water resource management.

This research focuses on fecal bacteria contamination in the Grand Strand. The *E. coli* enumeration cross comparison research helps to identify the method best suited for use by local VWQM programs to identify sites with persistent microbial water quality impairments. The Murrells Inlet estuary MST study demonstrates the use of current methodology used to identify host-animal sources of contamination. The synthesis of past MST studies from the region provides a historical overview of past work in the area and summarizes tools available to local stormwater managers for identifying and remediating water quality impairments.

With increasing coastal development pressure and a major focus on water-based tourism, water quality protection will remain an important topic in the Grand Strand. The goal of this research is to better understand local trends and sources of fecal contamination.

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Chapter 2

***E. coli* Enumeration Methods Cross Comparison**

2.1. Introduction

2.1.1. Overview

A microbial source tracking (MST) study investigating upstream sources to impaired beach sites identified a discrepancy between two different numeration methods. Results generated by the U.S. Environmental Protection Agency (US EPA)-approved method were much higher than those reported by a method used by local volunteer water quality monitoring (VWQM) programs. To determine which method is best suited to the particular sites monitored in Horry and Georgetown counties, a cross comparison of *E. coli* enumeration methods was conducted.

2.1.2. *E. coli* Enumeration Methodology

Water quality monitoring has been a useful tool for identifying water quality trends in the Grand Strand region. Long term monitoring data are being collected by (VWQM) programs in Horry and Georgetown counties and by the Coastal Carolina University (CCU) Environmental Quality Lab (EQL) under the auspice of the Waccamaw Watershed

Academy (WWA). These data have been used to detect illicit discharges and long-term trends and have been used to support MST studies (Anderson & Greoski 2010, Libes et al. 2016, Trapp et al. 2014, Weinreich 2013). VWQM programs under the WWA monitor fecal indicator bacteria (FIB) among other water quality parameters in Murrells Inlet, in Surfside Beach, on the Waccamaw River, and on the CCU campus. Additionally, special projects run by the EQL also collect water quality monitoring data throughout the Grand Strand region.

The EQL and WWA use two different methods for enumerating *E. coli* concentrations. The EQL is certified by SC DHEC to make regulatory-level measurements using an EPA-approved method, IDEXX's Colilert-18™ (C-18). Use of C-18 is generally impractical for volunteer programs that do not often have the resources for using expensive testing methods. The WWA uses Coliscan® Plus Easygel® (CPE) which is not US EPA-approved but is widely used by volunteer programs because of its affordability and ease of use. The method has been validated by O'Brien (2006) and Stepenuck et al. (2010) and is a preferred method for volunteers. This low cost method has a low detection limit and was specifically developed for use in natural waters (Stepenuck et al. 2010). Coliscan® Easygel® (CE), the predecessor to CPE, is used by the Alabama Water Watch volunteer monitoring program whose Quality Assurance Project Plan was approved by the US EPA in Region 4 (Stepenuck et al. 2010). While the method is not approved for other regions, its approval in Region 4 suggests the method is reliable for volunteer monitoring purposes. The method was also included in a recent publication by the Center for Watershed Protection (CWP) as a recommended method for *E. coli* enumeration by volunteer groups (CWP 2016). In the CE method, *E. coli* grown on plated media generate colonies that are blue-colored under

visible light. CPE incorporates a verification step in which the blue colonies are confirmed as *E. coli* by their fluorescence under long wave UV light (Micrology Laboratories 2008).

2.1.3. Method Discrepancy Identified During MST Study

During a MST study conducted in fall 2015 in Surfside Beach's Myrtle Lake, were the VWQM data had documented consistently elevated *E. coli*, CCU's EQL generated results using C-18 that were much higher than those generated from CPE by the volunteers. To verify this, six samples from Myrtle Lake collected from September 2015 to January 2016, were analyzed using both methods, CPE and C-18. Though both methods reported elevated *E. coli* levels above the freshwater recreational water quality criteria, C-18 yielded consistently higher values than CPE. Differences between the two methods varied as much as ten-fold and had relative percent differences (RPD) ranging from 50% to 182% (**Table 2-1**). The EQL has a precision threshold for *E. coli* of $\leq 100\%$ RPD when concentrations are ≥ 150 CFU/mL and $\leq 200\%$ RPD for concentrations < 150 CFU/mL. Of the compared samples, 81% were not within the RPD acceptance threshold established by the EQL.

These results were notable since several published comparative studies have reported *E. coli* concentrations generated by CE were not significantly different from US EPA-approved methods (Colilert or Method 1603) or other commonly used VWQM methods, such as 3M Petrifilm™ (PF) (Stepenuck et al. 2010, Vail et al. 2003, Yuan 2016). In the case of the Waccamaw River, the EQL conducts a monitoring program biweekly that is intentionally offset from the biweekly VMP schedule to provide more temporal coverage except twice per year when monitoring in both programs is conducted one day

apart. This last occurred on November 4 and 5, 2015 when very high *E. coli* levels were detected by both programs immediately following a 4"-rain event. High *E. coli* levels in the Waccamaw River are extremely rare and concentrations are otherwise typically near the detection limit of C-18 and CPE. Amongst the data collected during this unusual event, excellent agreement was observed at 5 sites, with %RPD ranging from 16% to 104% (average = 46%). The EQL's acceptance criteria for lab duplicates is 100% RPD.

Table 2-1. Comparison of results from CPE and C-18 at Myrtle Lake. Samples compared were taken between September 2015 and January 2016. All values are evaluated as being within the acceptance threshold of RPD $\leq 100\%$ as all C-18 values are ≥ 150 CFU/100mL.

| Date of sampling | CPE (CFU/mL) | C-18 (MPN/mL) | RPD | Within EQL Precision Acceptance Threshold |
|------------------|--------------|---------------|------|---|
| 9/08/2015 | 400 | 670 | 50% | Yes |
| 9/22/2015 | 67 | 345 | 135% | No |
| | 200 | 345 | 53% | Yes |
| 11/03/2015 | 1000 | 7556 | 153% | No |
| | 1500 | 7556 | 134% | No |
| 11/17/2015 | 116 | 1496 | 182% | No |
| | 500 | 1496 | 133% | No |
| 12/08/2015 | 482 | 3591 | 153% | No |
| | 1167 | 3591 | 102% | No |
| 01/12/2016 | 367 | 1285 | 111% | No |
| | 533 | 1285 | 83% | Yes |
| | 267 | 1285 | 131% | No |

In Murrells Inlet, MST work conducted by the EQL at VWQM suites with chronic *E. coli* impairments, albeit on different days, has generated results that are broadly similar to those generated by the VWQM program, but are suggestive of somewhat higher C-18 values. In summary, the discrepancies between the C-18 and CPE results were not widespread and appeared to be site specific.

2.1.4. Previous Method Validations

Issues with the US EPA-approved *E. coli* enumeration methods have been reported. For example, Cho et al. (2010) found that *E. coli* concentrations measured by a most probable number (MPN) method were consistently higher than those measured using a method based on counts of colony-forming unit (CFU). This could account for some of the discrepancy in results observed in Surfside at Myrtle Lake as C-18 reports in MPN while CE and CPE report out in CFU. But based on published comparative studies, such as O'Brien (2006), this issue appears to cause no more than a 6% difference in *E. coli* in CE as compared to C-18. Though several studies have determined results reported in MPN and CFU tend to be significantly different, the two are often used interchangeably. Most notably the current US EPA recreational water quality criteria are presented in CFUs, whereas C-18, the most commonly used approved method for quantification of *E. coli*, is reported as MPN (SC DHEC 2014, US EPA 2012).

Another possible cause for the difference between CPE and C-18 results could arise from differences in metabolic endpoints (Gronewold & Wolpert 2008). This does not apply to the use of CPE and C-18 as both rely on the production of the same two enzymes, β -galactosidase and β -glucuronidase, by *E. coli* bacteria. Both methods identify *E. coli* by a chromogenic reaction identifying the production of the enzymes. β -galactosidase enzymes produced by the bacteria's metabolism of the media results in a change in color under visible light and the production of β -glucuronidase results in fluorescence under long-wave UV light (IDEXX Laboratories 2004, Micrology Laboratories 2008).

A possible cause for higher C-18 results could be from false positives. Studies have revealed that C-18 can generate false-positive results for *E. coli* (Chao et al. 2004, Pisciotta

et al. 2002). Several bacteria including *Aeromonas* spp., *Pseudomonads*, some *Salmonella* and *Shigella* spp, and *Flavobacterium* spp. can produce a false-positive result for the method (Pisciotta et al. 2002). While C-18 is used in freshwater and saltwater for regulatory purposes throughout the U.S., validation of the method was performed primarily in marine waters of California (Pisciotta et al. 2002). An investigation into high *E. coli* counts by C-18 in subtropical marine and estuarine waters revealed a false-positive rate of 27.3% was attributed to interference from species of marine bacteria (Pisciotta et al. 2002). However, a subsequent study of subtropical freshwater samples tested with bioMérieux's analytical profile index (API®) revealed fewer false-positives, 7.4%, for C-18 (Chao et al. 2004). These studies indicate that C-18 may have site specific enumeration differences.

Studies have been performed to evaluate the use of C-18, CE, and PF, another method used by volunteer monitors. Several evaluations of the methods have been conducted for use by volunteer monitors. Stepenuck et al. (2010) identified CE and PF as adequate methods for use by volunteer monitors, both exceeding 80% accuracy relative to Method 1603, but reported that PF has greater agreement with EPA approved methods than CE. Vail et al. (2003) also identified PF as a useful method for preliminary detection of *E. coli*. However, when compared to Colilert (the predecessor of C-18 required a longer incubation period), PF reported values up to 2 orders of magnitude higher in a study on beach water from Lake Superior and Lake Michigan (Kleinheinz et al. 2012). A 36 month study in streams with variable *E. coli* concentrations over different seasons evaluated the Colilert method against the confirmed standard membrane filtration method (Method 1603) (Buckalew et al. 2006). The results showed high agreement between test methods across all variables as well as low false-positive rates (Buckalew et al. 2006). Yuan (2016)

evaluated CE and PF against Method 1603 and found no significant difference between methods. While it is encouraging that these studies have no detected significant differences amongst C-18, CE, and PF, none evaluated CPE and none were conducted in coastal plain waters of the southeastern United States.

2.1.5. Expected Outcomes

Given the preliminary observations of discrepancies between C-18 and CPE at some of the long-term VWQM sites, a study was performed to evaluate some potential causes including the possibility that strains of *E. coli* native to the natural waters of northeastern South Carolina generate false positives with C-18 and/or false negatives with CPE. As part of this effort, another detection method used by VWQM programs, PF, was included to determine if it is better suited for local use than CPE. The study design was based on tests of the following null hypotheses and objectives:

- 1. C-18 generates results that are not significantly different from CPE or PF.** *E. coli* were enumerated in samples at five sites characterized by high *E. coli* concentrations using each of these three test methods.
- 2. PF results are not significantly different from CPE.** Results from the two commercially available methods that are widely used by volunteer monitoring programs were tested for significant difference. A significant difference could indicate that the use of CPE would need to be reevaluated, especially if the PF results are better correlated with C-18.

- 3. Agreement between test methods is independent of *E. coli*, turbidity, and conductivity levels.** Agreement between test methods by relative percent difference was correlated with *E. coli*, turbidity and conductivity levels to determine whether a relationship exists.

- 4. CE results are not significantly different from CPE.** All VWQM data collected from January 2015 through June 2016 was evaluated for differences between fluorescing and non-fluorescing colony counts. The influence of incubation time on these results was also evaluated. For CPE, fluorescence of *E. coli* colonies should appear after 12 hours of incubation. It is recommended that plates be read after 18 hours and no more than 20 hours of incubations (Micrology Laboratories 2008). If read after 20 hours, the fluorescence can spread throughout the plate and obscure individual colony fluorescence leading to low results.

Mallin et al. (2000) found significant relationships between enteric bacteria concentrations with salinity and turbidity. Salinity, a measure of total dissolved solids, was inversely related to enteric bacteria concentrations possibly because of shortened survival in saline waters (Mallin et al. 2000). Turbidity was positively correlated with enteric bacteria due to the bacteria's ability to adsorb to particulate matter (Mallin et al. 2000). The behavior and structural characteristics of enteric bacteria allows for it to adsorb to particulate matter that provides shelter and food for the bacteria thus increasing its survival in a turbid environment. Investigating the relationship between agreement and these parameters will allow the VWQM programs to reevaluate the use of CPE.

2.2. Materials and Methods

To obtain the widest diversity of *E. coli* strains, sampling was conducted from May 2016 through September 2016 at five sites regularly monitored by VWQM and the EQL. The five selected sites, identified by local volunteer monitoring and EQL research for exhibiting consistently elevated *E. coli* levels, provided adequate fecal bacteria levels for a comparison of methods. The sites are: (1) a tidal brackish lake (Myrtle Lake in Surfside Beach), (2) two tidal tributary creeks (HS and BHR in Murrells Inlet), and (3) two freshwater tributaries to a blackwater river (Crabtree Canal on the Waccamaw River and Highway 544 West on the CCU campus). The wide variety of sites was used to determine whether the method issues are site specific possibly due to the presence of different strains of bacteria, the influence of particulate transport (turbidity), or saline waters.

During each of eight sampling dates, two grab samples were collected in sterile collection bottles at each site. This collection was performed by the volunteer monitors and EQL staff. Samples were transported on ice to the EQL and stored under refrigeration until analyzed. Hold times from collection until analysis did not exceed 8 hours and were kept as consistent as possible between methods. Companion water samples were collected for laboratory analysis of turbidity and salinity/conductivity.

Samples were analyzed for *E. coli* using three enumeration methods: CPE, PF, and C-18. For each site on each sample day, two of each test was performed including a field duplicate at each site to evaluate the study's hypotheses. Tests were prepared as indicated in the instruction guides and EQL Standard Operation Procedures (SOPs) for each method (3M 2014, EQL 2014, IDEXX Laboratories 2004, Micrology Laboratories 2008, WWA

2016). Analysis of salinity/conductivity and turbidity were conducted according to EQL SOPs (EQL 2016, EQL 2015).

Statistical analyses were performed to test for significant differences between the results from each of the methods. All data were first transformed by taking the natural log in an effort to normalize the data. Both parametric and nonparametric tests were performed. Only the nonparametric results are reported although the parametric test results were similar. Regressions were used to test for relationships between the methods with turbidity and salinity/conductivity.

2.3. Results and Discussion

2.3.1. Field Duplicate Comparison

Field duplicates were collected and analyzed at each sampling site on each sampling date. These field duplicates represent replicates for each enumeration method. Before averaging the two replicates from each test, the replicates were correlated and then analyzed to determine if the results were significantly different. The EQL has established a precision acceptance threshold for *E. coli* enumeration by C-18 using relative percent difference (RPD), which is a standard quality control measure used in water quality testing. Percent RPD was calculated using the equation:

$$\%RPD = \left(\frac{|x_1 - x_2|}{\bar{x}} \right) \times 100\%$$

Where x_1 and x_2 represent the results of the two replicates for an individual test and \bar{x} represents the average of the two results. Acceptable RPD values for the EQL are $\leq 100\%$ RPD when *E. coli* concentrations are ≥ 150 CFU/mL and $\leq 200\%$ RPD for < 150 CFU/mL.

When field duplicates were compared by RPD, most values were within the precision acceptance threshold. Distribution of RPD for each method is displayed in **Fig. 2-1**. All C-18 replicate results were within the acceptance threshold. CPE and PF each had replicate results exceeding the acceptance threshold. CPE had 3 sets of results above the threshold, representing 7.5% of the total samples. PF had one set of results above the threshold, representing 2.5% of the total samples.

Overall, agreement between methods assessed by RPD was good. To further confirm these results, statistical analysis was performed on the replicate results. A nonparametric Spearman's Rho correlation demonstrated that the replicates for each test were strongly positively correlated ($r_s = > 0.900$, $p = 0.000$) in all cases. Additionally, replicates between tests all displayed a strong positive correlation ($r_s = > 0.900$, $p = 0.000$). A Wilcoxon Signed Rank test revealed replicates of each were not significantly different from each other ($p > 0.05$). However, the test revealed that replicates between tests (i.e. C-18 replicate 1 vs. CPE replicate 1) were significantly different for all comparisons ($p < 0.05$).

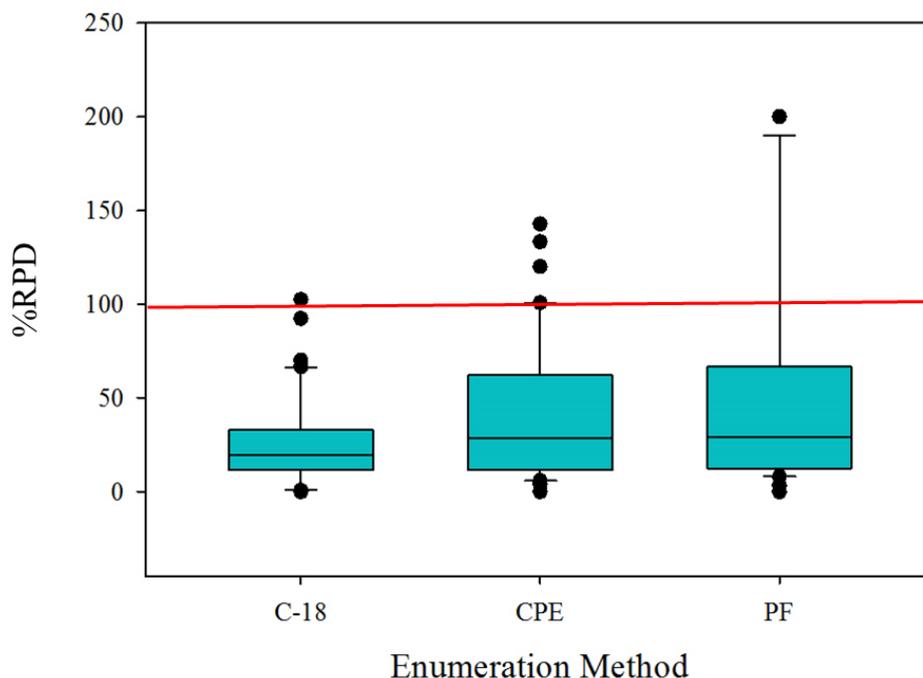


Figure 2-1. Distribution of %RPD between replicates by method. The red line represents the 100% RPD precision acceptance threshold for *E. coli* enumeration in the EQL. Values above this line indicate instances where the threshold was exceeded. However, lower *E. coli* results increase the acceptable threshold to 200%. Some results above 100% RPD do not necessarily exceed the precision acceptance threshold. (n=40)

2.3.2. Method Comparison

E. coli enumeration results from the three test methods were analyzed to determine whether results were significantly different between tests. After averaging the replicates, the three test methods were compared. A nonparametric Spearman's Rho correlation shows all three methods have a strong positive correlation ($r_s = >0.900$, $p < 0.05$). Analysis of the average method results by Wilcoxon Signed Rank test shows the three methods are significantly different. Average C-18 results are significantly greater than average results generated by CPE ($p = 0.000$) and results generated by PF ($p = 0.000$). These results reject the null hypothesis; C-18 generates results that are significantly different from CPE or PF

(Fig. 2-2). Additionally, PF generates results significantly greater than CPE ($p=0.000$), rejecting the second null hypothesis.

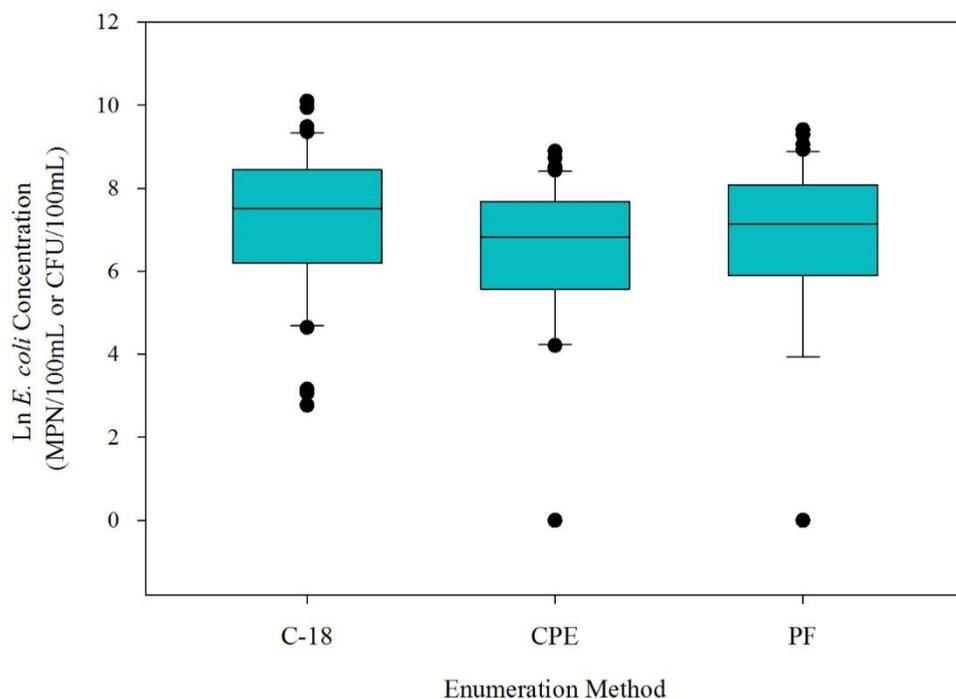


Figure 2-2. Distribution of average *E. coli* concentration by method. Methods are significantly different from one another ($n=40$, $p < 0.05$).

These results indicate the US EPA-approved method, C-18, generates higher average results than either of the two volunteer methods. These results reinforce the previous study by Cho et al. (2010) where MPN and CFU were strongly correlated, but the MPN-based results were consistently higher. Ideally, the methods should not be reporting significantly different enumeration results. The difference between methods indicates C-18 could possibly be reporting false positives, thus overestimating *E. coli* concentrations. Conversely, the volunteer methods may be underestimating the *E. coli* concentrations if false negatives are being reported. If the US EPA-approved method is overestimating *E.*

coli concentrations, waters may be falsely identified as impaired. Costly remediation may not be necessary if *E. coli* concentrations are actually lower than the reported values. While the volunteer methods are not approved at the federal or state level, enumeration data obtained by volunteers is integral to local water resource management. Underestimation by volunteers may inhibit the ability to detect potential water quality problems.

2.3.3. Agreement between Methods by Relative Percent Difference

Agreement between methods was evaluated by RPD, a standard quality control measure used in regulatory water testing. Percent RPD for agreement was calculated using the equation:

$$\%RPD = \left(\frac{|x_1 - x_2|}{\bar{x}} \right) \times 100\%$$

Where x_1 and x_2 represent enumeration method values of two different methods and \bar{x} represents the average of the two values. The EQL precision acceptance threshold for *E. coli* is $\leq 100\%$ RPD when concentrations are ≥ 150 CFU/mL and $\leq 200\%$ RPD for < 150 CFU/mL. These criteria were used to evaluate the results between methods. RPD and agreement are inversely related; high RPD indicates poor agreement between methods. RPD results are displayed in **Table 2-2**. Agreement was greatest between CPE and PF, the two volunteer methods. C-18 had greater agreement with PF than with CPE. These results are similar to those of Stepenuck et al. (2010) when comparing EPA-approved methods to volunteer methods.

Table 2-2. RPD statistics between methods. RPD results of 200% represent the occurrence of one method reporting the absence of *E. coli*.

| | C-18 vs CPE | C-18 vs PF | CPE vs PF |
|-----------------------|-------------|------------|-----------|
| Mean | 81.37 | 57.02 | 43.01 |
| Min | 20.90 | 0.00 | 0.00 |
| Max | 200.00 | 200.00 | 200.00 |
| Percentiles 25 | 53.80 | 22.35 | 16.68 |
| 50 | 73.41 | 51.44 | 36.77 |
| 75 | 97.69 | 75.24 | 59.88 |

Quality control criteria was exceeded for only 8 of the 40 samples (20%). Of those cases, 3 were the results of the volunteer methods detecting an absence of *E. coli* resulting in RPDs of 200%. The other five cases were the result of significantly greater results from C-18 than from CPE. In only one of the cases did RPD between C-18 and PF also exceed 100%. These results reinforce the findings by statistical analyses that C-18 has greater agreement with PF than with CPE. Agreement was greatest between CPE and PF, the two volunteer methods. The distribution of RPDs between methods can be seen in **Fig. 2-3**.

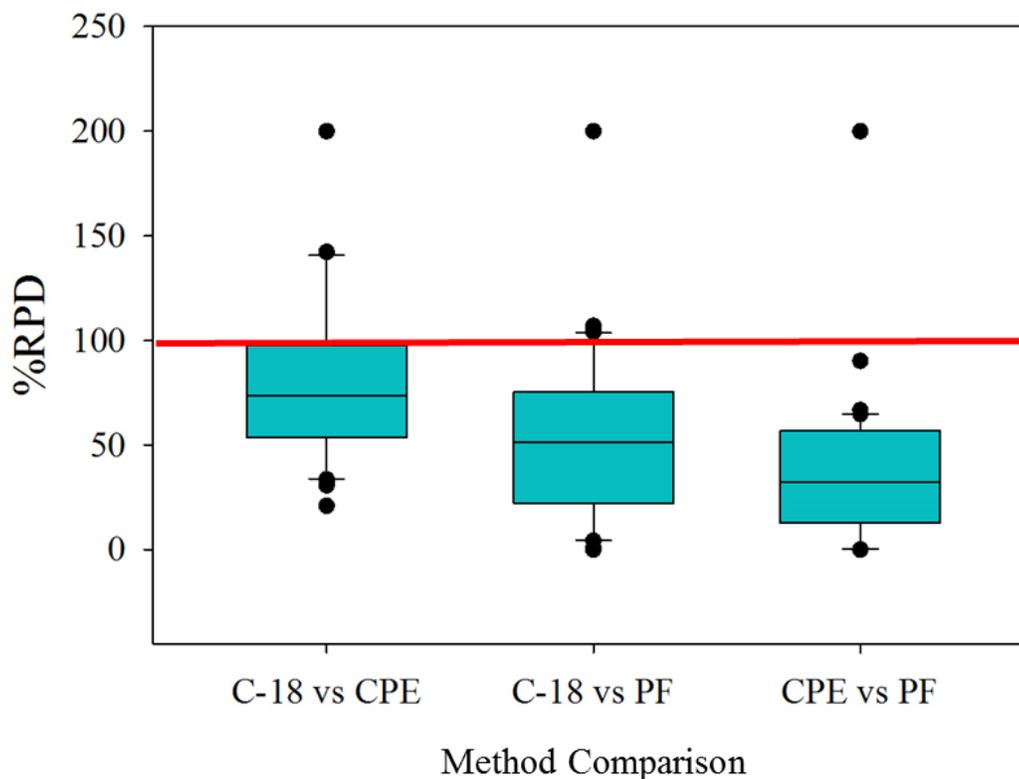


Figure 2-3. Distribution of RPD between methods. High RPD indicates poor agreement between methods compared. . The red line represents the 100% RPD precision acceptance threshold for *E. coli* enumeration in the EQL. (n=40)

2.3.4. Relationships between Method Agreement and Other Potential Controlling Parameters

E. coli concentration, turbidity, and conductivity levels could potentially influence the accuracy of an enumeration method. To test this hypothesis, enumeration results were correlated with potential controlling parameters. Turbidity was not significantly correlated with results of any of the enumeration methods. Average *E. coli* concentrations were positively correlated with enumeration results of all three methods ($r_s = >0.980$, $p = 0.000$). Conductivity was also positively correlated with enumeration results of all three methods

($r_s = < 0.400$, $p = < 0.05$). These results indicate higher conductivity and higher *E. coli* concentrations are correlated with higher reported *E. coli* concentrations whereas *E. coli* concentrations reported by each method are not correlated with turbidity or average *E. coli* concentrations.

The three agreement scores were determined by %RPD then correlated with *E. coli* concentration, turbidity, and conductivity levels to determine if any relationship exists. Nonparametric correlations using Spearman's Rho revealed a significant relationship only with turbidity. Agreement between C-18 and CPE was positively correlated with turbidity ($r_s = 0.354$, $p = 0.025$) as was agreement between C-18 and PF ($r_s = 0.328$, $p = 0.039$) (**Fig. 2-4**). Agreement between CPE and PF was not significantly correlated with any of the water quality parameters. These results partially reject the null hypothesis concerning relationships between method agreement and *E. coli* concentration, turbidity, and conductivity levels. Agreement is independent of *E. coli* concentration and conductivity but is not independent of turbidity for test methods compared to C-18. Agreement between CPE and PF, however, is independent of all parameters. The positive relationship indicates greater turbidity may lead to higher RPDs, meaning agreement between C-18 and the volunteer methods decreases with higher turbidity.

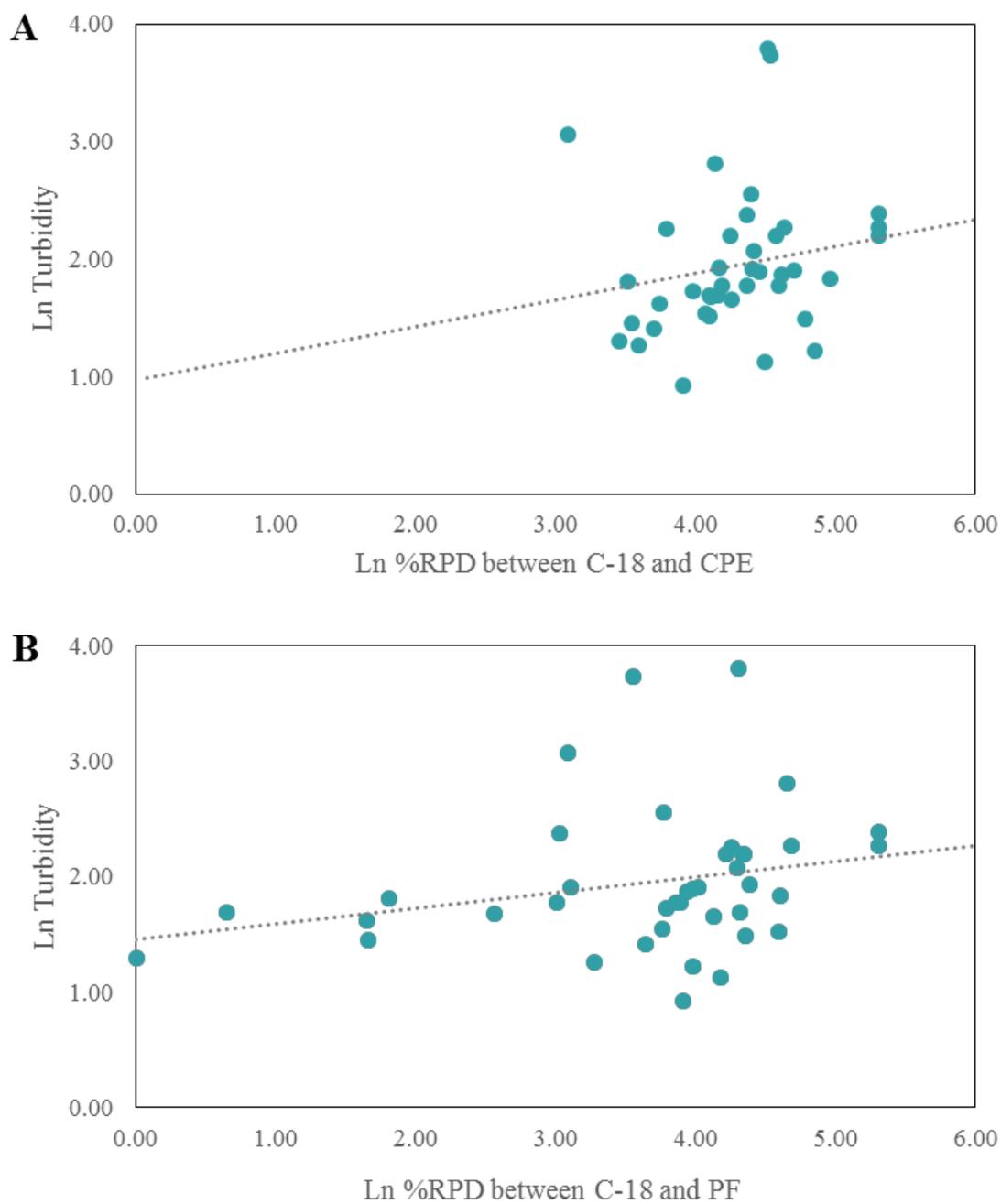


Figure 2-4. Correlation of %RPD and turbidity by sample case ID. Relationship between (A) %RPD of C-18 and CPE with turbidity ($n=40$, $r_s= 0.354$, $p=0.025$) and (B) %RPD of C-18 and PF with turbidity ($n=40$, $r_s= 0.328$, $p=0.039$). Blue trend lines represent the correlation coefficient. Trends throughout the sampling demonstrated a positive correlation between RPD and turbidity.

2.3.5. Relationship between Method Agreement and Site Location

Agreement between methods was also tested against site location to determine whether Myrtle Lake, or any of the other sites, were anomalous. Using a rank-based nonparametric one-way ANOVA, the Kruskal-Wallis test, agreement was found to be independent of site location. These results indicate that agreement between methods does not seem to be site specific. However, when analyzed by site type, there was a significant effect on RPD between C-18 and CPE ($p=0.043$). The data was grouped into three site types: freshwater tributary, tidal tributary, and tidal lake. The tidal lake group, containing only Myrtle Lake, appears to have significantly lower %RPD than the other two groups (**Fig. 2-5**). The results from the tidal lake have better agreement between C-18 and CPE than observed in the other site type groups. Unequal sample sizes between groups does not influence the significant results of the statistical test.

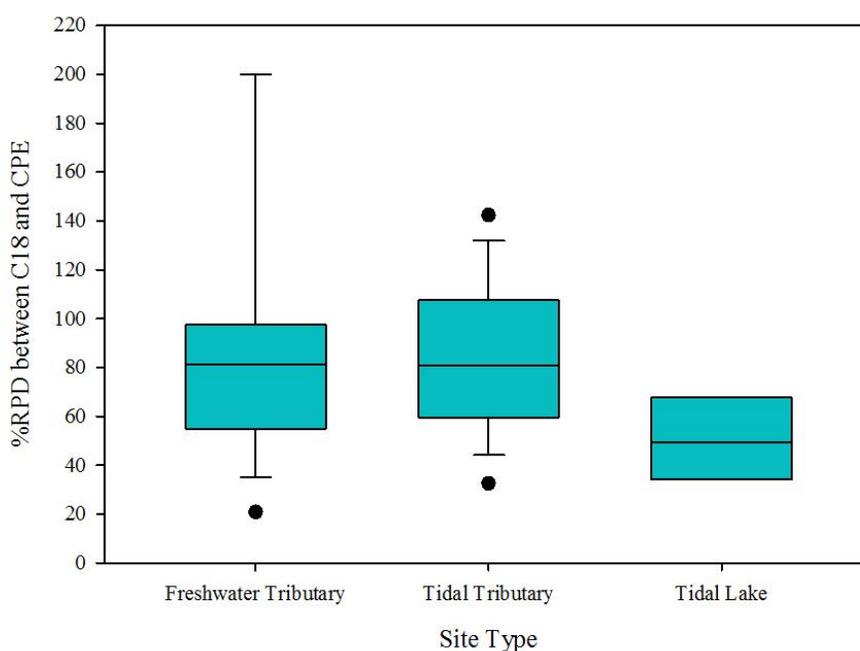
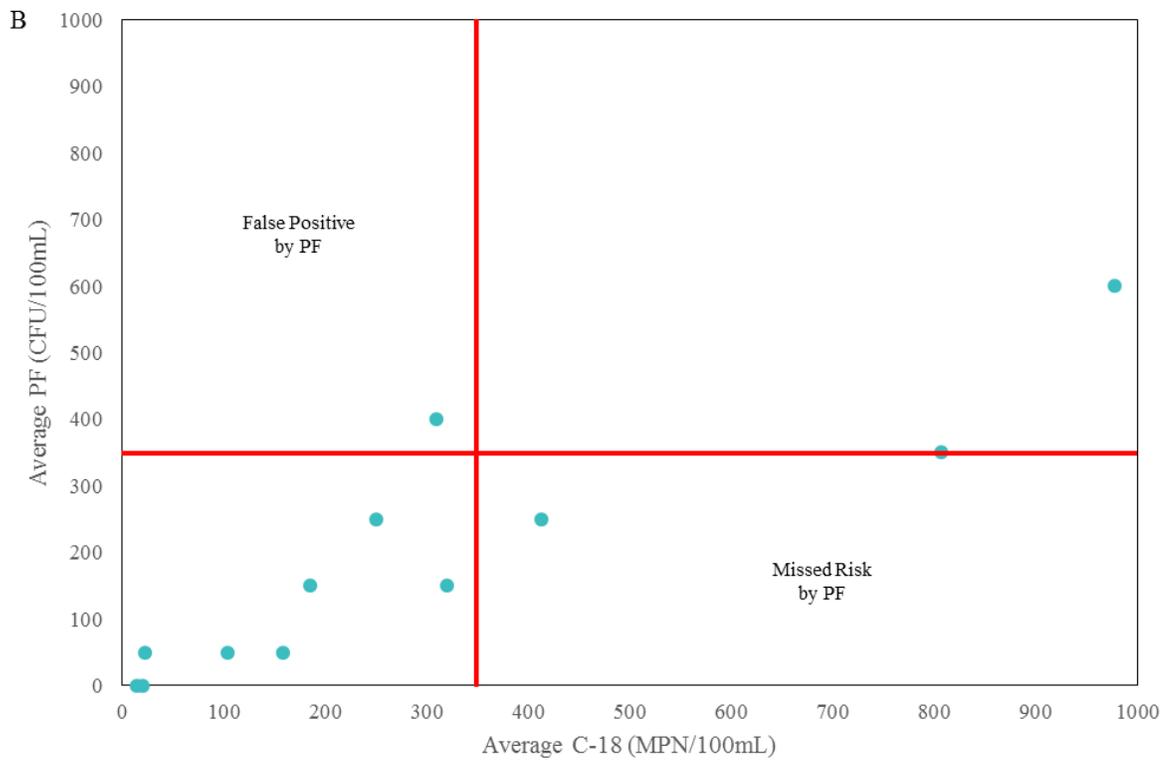
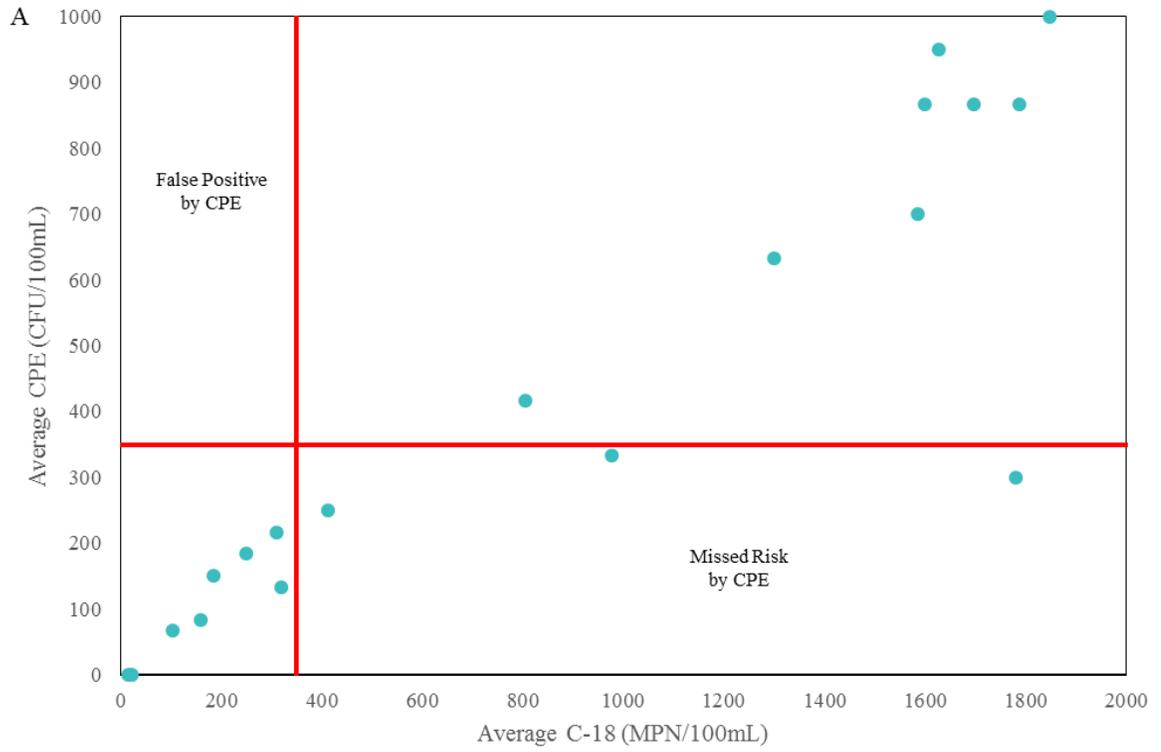


Figure 2-5. Distribution of %RPD between C-18 and CPE by site type. The tidal lake group is significantly different from the other groups ($p=0.043$).

2.3.6. Method Agreement with Water Quality Standard

Assessing agreement between methods should also be examined from a policy standpoint. A primary concern is the possibility of one method artificially identifying an *E. coli* concentration that contravenes the water quality standard (WQS). This study used the SC DHEC single sample maximum for *E. coli* in recreational water monitored under NPDES permits of 349 CFU/100mL (SC DHEC 2014). *E. coli* concentrations above the WQS indicate waters impaired by fecal bacteria.

Of forty cases, four had disagreement between methods regarding contravention of the WQS (10% of samples). Between the three methods there were eight instances of disagreement represented by four specific samples. These disagreements can be classified as either a missed risk or a false positive (**Fig. 2-6**). A missed risk indicates that a method did not identify a concentration above the WQS when it was identified by another method. False positives indicate one result contravened the WQS while another method reported results below the WQS. All three disagreements between C-18 and CPE were missed risk values reported by CPE. The two instances of disagreement between C-18 and PF were one missed risk by PF and one false positive by PF. Between CPE and PF, there were three instances of false positive results by PF.



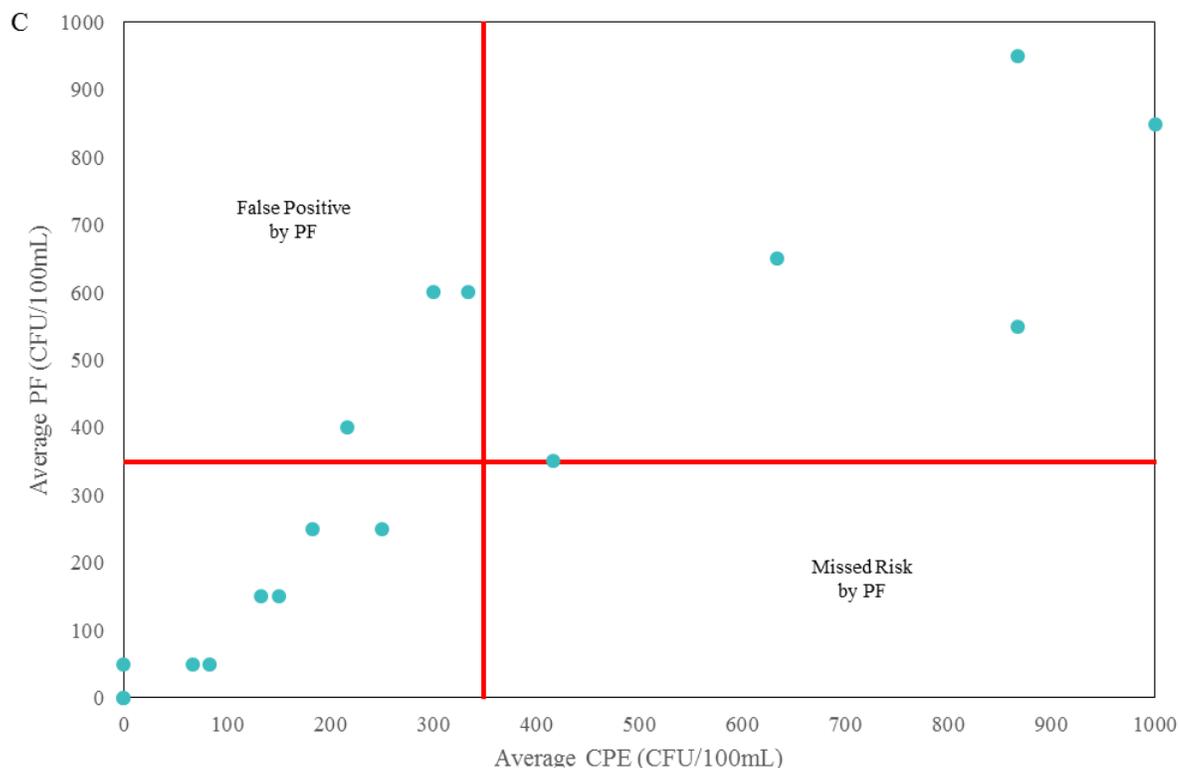


Figure 2-6. Agreement between enumeration methods with WQS standards. The red lines indicate the 349 CFU/100mL WQS. Correlations between C-18 and CPE (A), C-18 and PF (B), and CPE and PF (C) with values identified as a false positive or missed risk. Scale of graphs has been altered to best show missed risks and false positives identified. Graphs do not include all sample data but do include all missed risks and false positives.

Further investigation into these disagreements is provided in **Table 2-3**. While the two replicates for C-18 were always in agreement, replicates for the two volunteer methods disagreed 50% of the time. Specifically, CPE had three instances where individual replicates disagreed. However, it is important to note that all replicates of volunteer methods reported values within 100% RPD of the WQS (0-1047 CFU/100mL). Overall, disagreement between methods was limited to four samples, representing only 10% of the total samples. When approaching agreement from a policy standpoint concerning WQS, the methods appear to have good agreement.

Table 2-3. Results for samples exhibiting disagreement. Enumeration results for individual replicate and averaged *E. coli* concentrations for samples displaying disagreement. Replicates represent filed duplicates. Values in red exceed the WQS.

| Date | Site | C-18 (MPN/100mL) | | | CPE (CFU/100mL) | | | PF (CFU/100mL) | | |
|---------|-----------|---------------------|-------|---------|--------------------|-------|---------|-------------------|-------|---------|
| | | rep 1 | rep 2 | average | rep 1 | rep 2 | average | rep 1 | rep 2 | average |
| 6/1/16 | 544 | 908 | 1046 | 977 | 400 | 267 | 334 | 600 | 600 | 600 |
| 6/14/16 | Myrtle Lk | 327 | 292 | 310 | 200 | 233 | 217 | 800 | 0 | 400 |
| 8/23/16 | HS | 959 | 2603 | 1781 | 100 | 500 | 300 | 400 | 800 | 600 |
| 9/13/16 | HS | 426 | 399 | 413 | 100 | 400 | 250 | 300 | 200 | 250 |

2.3.7. Analysis of Historical Volunteer Data to Evaluate CE vs CPE

Since samples were collected concurrently with the volunteer monitoring program, results could be compared. As shown in **Table 2-4**, significant discrepancies between the volunteer's results and those obtained in the EQL using CPE were observed. However, duplicate results generated in the EQL were well within the precision acceptance threshold for each of the three methods tests, CPE, PF and C-18.

Table 2-4. Comparison by %RPD of results for samples from Myrtle Lake. CPE results compare the volunteer's results to the two replicates obtained in the EQL. EQL results compare between replicates of each method and then between average values of the three methods. Values in red exceed the EQL QC criteria for *E. coli* enumeration. The 200% RPD reported on 6/14 between replications by PF indicates one replicate reported an absence of *E. coli*.

| Date | CPE Results | | | EQL Results with All Enumeration Methods | | | | | |
|------|---------------------------|---------------------------|-----------------------|--|-----------------------|---------------------|----------------------------|---------------------------|--------------------------|
| | volunteer vs. EQL 1 | volunteer vs. EQL 2 | EQL 1 vs. EQL 2 | C-18 1 vs. C-18 2 | CPE 1 vs. CPE 2 | PF 1 vs. PF 2 | C-18 ave vs. CPE ave | C-18 ave vs. PF ave | CPE ave vs. PF ave |
| 6/14 | 40% | 55% | 15% | 11% | 15% | 200% | 35% | 26% | 60% |
| 6/28 | 100% | 133% | 50% | 28% | 50% | 67% | 82% | 72% | 12% |
| 7/12 | 172% | 170% | 8% | 23% | 8% | 91% | 59% | 98% | 45% |
| 7/26 | 143% | 147% | 8% | 25% | 8% | 29% | 64% | 79% | 17% |
| 8/9 | 160% | 164% | 11% | 33% | 11% | 15% | 69% | 67% | 3% |
| 8/23 | 189% | 188% | 6% | 11% | 6% | 14% | 40% | 37% | 2% |
| 9/13 | 143% | 133% | 18% | 18% | 18% | 40% | 31% | 0% | 31% |
| 9/27 | 0% | 0% | 0% | 18% | 0% | 17% | 34% | 4% | 30% |

While good agreement was found between method replicates and between methods in the EQL (last three columns in **Table 2-4**), the volunteer data had poor agreement with the results obtained in the EQL for CPE. On the last sample date (9/27), the volunteer sample was read in the EQL rather than by the volunteer at home which may account for the high agreement. Further investigation led to identifying a problem with the UV light source used for the dual confirmation step in determining *E. coli* concentrations by CPE. The volunteer reported a low percentage of fluorescing colonies of the total blue colonies. **Fig. 2-7** shows the significant difference in fluorescence between the plate read in the EQL and the plate read by the volunteers.

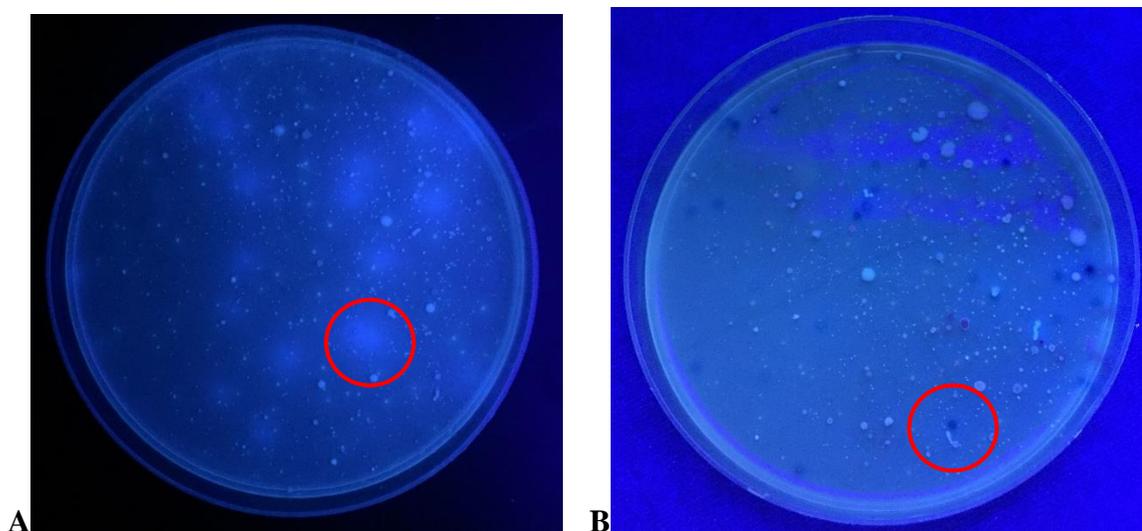


Figure 2-7. Images of plates under long wave UV light. Image A is of a plate read in the EQL where blue fluorescence is evident. The red circle indicates a fluorescing blue colony Image B is of a plate read by the volunteer where blue fluorescence is not evident. The red circle indicates a blue colony which does not display fluorescence. These plates represent sample replicates from Myrtle Lake on August 9, 2016.

The UV-light used by the EQL to produce the bright fluorescence in panel A of **Fig. 2-7** was purchased approximately 10 years ago. Bulbs purchased at a later date from this same manufacturer are generating the poor results shown in panel B. Micrology, Inc.

has acknowledged that finding a suitable UV light is currently very difficult. All of the volunteer monitoring data have been collected using bulbs that generate low intensity fluorescence. Although the bulbs are rated to deliver the required 365 nm wavelength light, they produce a yellow halo around the blue colonies, suggesting they are not generating light of the correct wavelength. The EQL has purchased several other light sources, also specified to generate 365 nm, the best of which are only marginally better than the ones in current use.

According to Micrology, Inc. no less than 85% of the blue colonies should fluoresce under long wave UV light. Variability in this percentage is attributed to the variable presence of strains of *E. coli* that do not fluoresce strongly and due to false positives that are correctly identified via a lack of fluorescence. This led to an investigation of historical data collected by WWA's VWQM Program throughout the Grand Strand. Data collected from January 2015 through June 2016 by volunteers in Murrells Inlet, Surfside Beach, the Waccamaw River, and CCU campus were investigated to quantify the scope and scale of discrepancies between *E. coli* concentrations calculated from the total blue colony counts (CE) and the fluorescing blue colony counts (CPE). %RPD was calculated to describe the agreement between the two *E. coli* concentrations. A low agreement between the two *E. coli* concentrations indicates that a low percentage of blue colonies were fluorescing.

Calculations of *E. coli* concentration of CE and CPE were compared at three different concentration levels for the historical VWQM data: (1) all data, (2) concentrations >0 CFU/100mL, and (3) concentrations >100 CFU/100mL (**Table 2-5**). At all levels the two calculated concentrations had a strong positive correlation ($r_s = > 0.700$, $p=0.000$).

However, CE *E. coli* concentrations were significantly greater than those of CPE ($p = 0.000$) by a Wilcoxon Signed Rank test. The significant difference between *E. coli* concentrations show that not all blue colonies are fluorescing. Average percent fluorescence evaluated for all levels were below the 85% fluorescence threshold established by Micrology, Inc.

Table 2-5. Comparison of calculated *E. coli* concentrations. Concentrations of *E. coli* calculated from total blue colony counts (CE) were significantly greater than those calculated from fluorescing blue colonies (CPE). Average percent fluorescence of CPE for all levels were below the 85% fluorescence threshold established by Micrology, Inc.

| | All Data | Data with <i>E. coli</i> concentrations >0 CFU/100mL | Data with <i>E. coli</i> concentrations >100 CFU/100mL |
|-------------------------------------|----------|--|--|
| Sample size | 936 | 658 | 346 |
| Spearman's Rho Correlation | r_s | 0.863 | 0.790 |
| | p | 0.000 | 0.000 |
| Wilcoxon Signed Rank test | p | 0.000 | 0.000 |
| Average Percent Fluorescence | 77% | 68% | 64% |

Agreement between *E. coli* concentrations (%RPD) was tested for significant correlations with *E. coli* concentration, turbidity, conductivity, and incubation time. RPD was positively correlated with *E. coli* concentration calculated from fluorescing blue colonies ($r_s = 0.123$, $p = 0.000$) as well as with *E. coli* concentrations (as calculated from total blue colonies) ($r_s = 0.516$, $p = 0.000$). This relationship means there is better agreement – or a lower RPD – when *E. coli* concentrations are lower. A possibility here is that increasing numbers of blue colonies lead to overlap in fluorescent halos and hence undercounting. RPD is positively correlated with turbidity ($r_s = 0.093$, $p = 0.004$) and conductivity ($r_s = 0.157$, $p = 0.000$). Agreement is greater when turbidity and conductivity are lower. Incubation time was not significantly correlated with RPD.

The relationship between agreement (%RPD) and location was evaluated by performing an ANOVA. Site location was found to have had a significant effect on natural log transformed %RPD ($F=15.025$, $p=0.000$). A post hoc Tukey B grouped sites by %RPD into six subsets. The sixth subset contains sites with the greatest RPD and includes Myrtle Lake in Surfside (SB 2), the CCU site at Hwy 544 (CCU 3), and Waccamaw River site at Hagley Landing (WR 7). While the Myrtle Lake and Hwy 544 sites often have high *E. coli* levels, Hagley Landing does not. The high %RPD at Hagley Landing arose from samples that had few (1-5) blue colonies of which none fluoresced, resulting in a 200% RPD being reported. The fifth subset contains the other sample site in Surfside Beach, Lake Dogwood (SB 3). Though Lake Dogwood has lower *E. coli* concentrations it also displays poor agreement between the two *E. coli* concentrations.

Both of the Surfside sites have low percentage of fluorescing blue colonies (only two sites in Surfside are monitored). Overall, these two sites have the second and third highest RPD among all VMP sites (**Fig. 2-8**). However, this can partially be attributed to the inclusion of sites with low *E. coli* concentrations which skews the data. When examining sites with *E. coli* concentrations consistently greater than 100 CFU/100mL, Lake Dogwood is not included. The box plot displayed in **Fig. 2-9** shows percent non-fluorescence for sites with consistently elevated *E. coli* concentrations. Similar distribution can be seen at sites where plates are read by the same person (MI 5 and MI 6, CCU 1 and CCU 3). This suggests the discrepancy between the volunteer and EQL results are largely associated with how the readers are interpreting whether fluorescence is present when using UV lights that do not produce strong fluorescence. For example, some readers may assume that most of the blue colonies should fluoresce and hence accept the ambiguous

fluorescence as acceptable, while other readers who do not assume that most of the blue colonies should fluoresce would not confirm that same colony as fluorescing. To resolve this matter, the Surfside volunteers evaluated PF for use at the Myrtle Lake site and the EQL continued purchasing lights in an effort to find ones that generate suitable light.

After testing multiple long-wave UV lights to obtain acceptable fluorescence with the use of CPE, the EQL determined that currently available light sources are not adequate. Upon contacting Micrology Laboratories, the manufacturer of CPE, a new formulation of the media was developed that provides greater fluorescence under UV light. This new formulation performed well with all UV lights previously tested obtaining greater than 85% fluorescence at a variety of sampling sites. The new formulation is currently being used by the VWQM programs. After a year of collecting enumeration data using the new formulation of CPE, the VWQM will reevaluate the percent fluorescence at each monitoring site to determine whether historical data should be revised to reflect accurate colonies counts. Currently, the VWQM programs have a method that appears to be appropriate for enumeration *E. coli* in northeastern South Carolina.

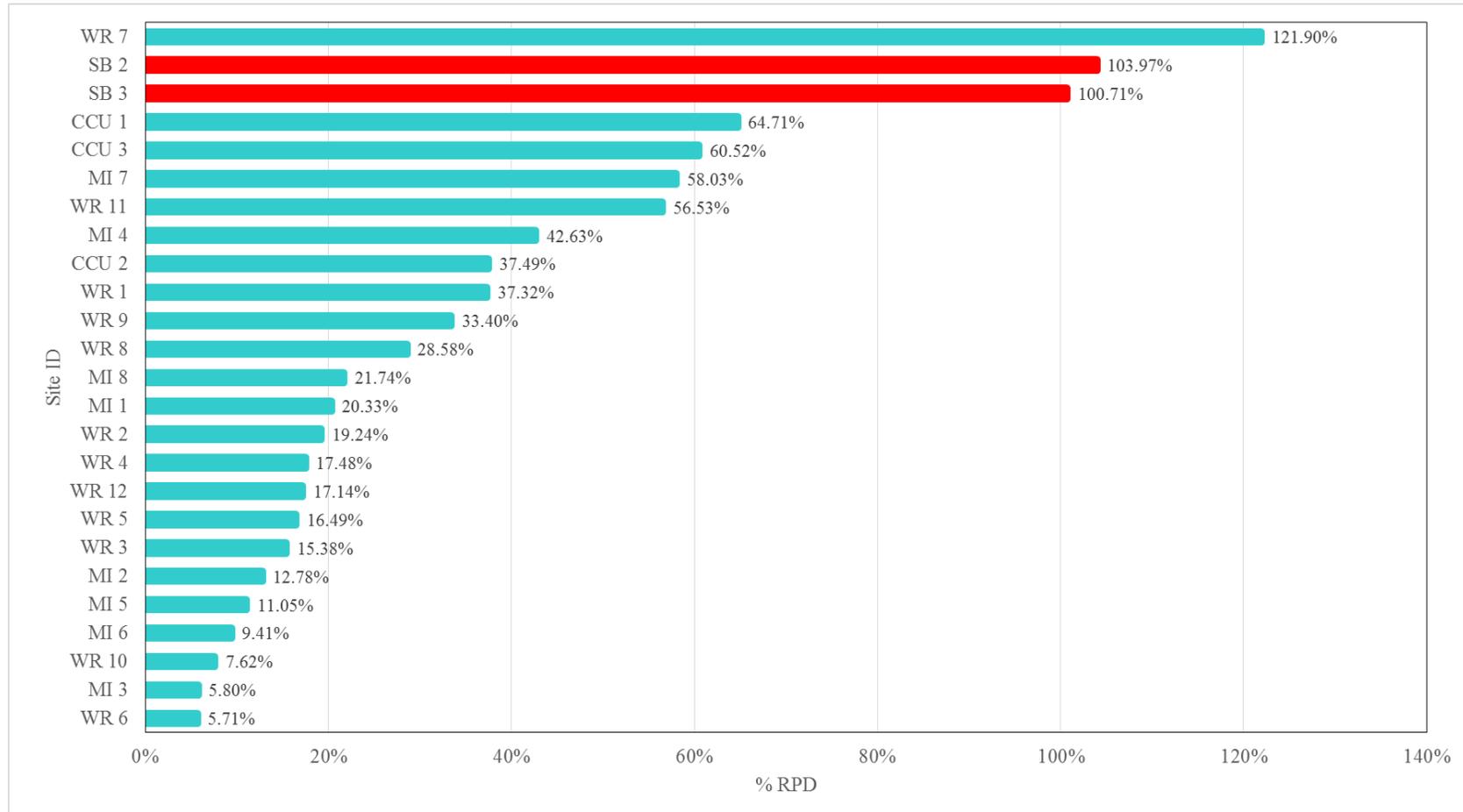


Figure 2-8. Mean RPD between *E. coli* concentrations with CE and CPE. Chart demonstrates the distribution of sites by RPD. The red bars represent the two Surfside Beach sites; SB 2 represents Myrtle Lake and SB 3 represents Lake Dogwood.

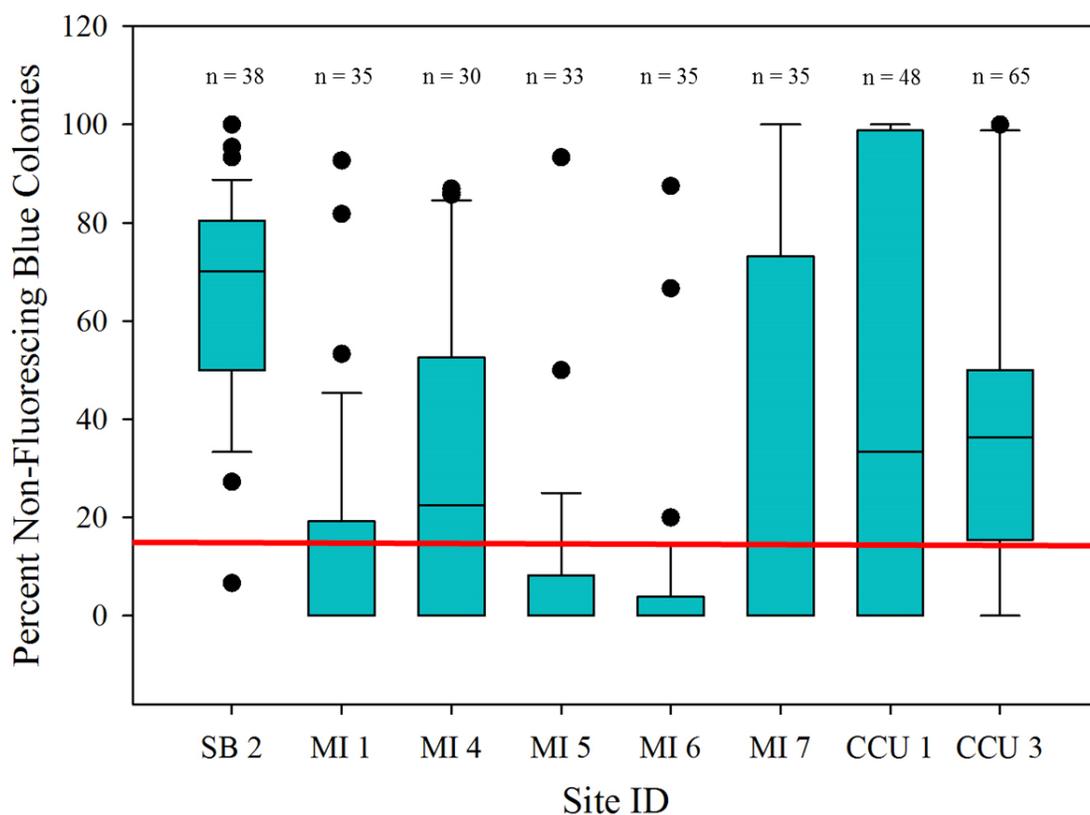


Figure 2-9. Distribution of percent non-fluorescing blue colonies. Sites included have *E. coli* concentrations consistently greater than 100 CFU/100mL. Red line represents 15% non-fluorescing colony threshold established by Micrology Inc. Values above 15% non-fluorescence are considered unusual.

2.4. Conclusions

The goal of this research was to determine whether strains of *E. coli* native to the natural waters of northeastern South Carolina generate false positives with C-18 and/or false negatives with CPE. While this question remains unanswered, it is obvious that C-18 and CPE generate significantly different results. Additionally C-18 generates significantly different results than both volunteer methods (CPE and PF). Whether this issue is specific to northeastern South Carolina requires further research.

The question remains as to whether methods producing results reported in MPN are directly comparable to those reported in CFU. Despite using methods relying on the production of the same enzymes, C-18 and CPE report significantly different results. Enumeration values for C-18 reported in MPN are calculated based on 95% confidence intervals. While this may explain some of the variance between method results, there is still the problem of state and federal agencies using the two terms interchangeably. While C-18 has been extensively validated for use of enumerating *E. coli*, there appear to be inconsistencies with reported values. Though agreement between methods evaluated by both %RPD and the WQS is good, C-18 reports significantly greater values than either of the volunteer methods. If C-18 is in fact overestimating *E. coli* concentrations, there are implications for regulatory actions. *E. coli* concentrations are used to identify impaired water bodies, establish Total Maximum Daily Loads for remediation efforts, and to evaluate the effectiveness of those efforts. An overestimation of *E. coli* concentrations could lead to costly remediation that may not be necessary.

Though the issue of agreement between methods was originally thought to be site specific to Myrtle Lake, this does not appear to be the case. Overall, Myrtle Lake had the best agreement between test methods. The VWQM program data reveals that among the VWQM sites, Myrtle Lake has poor agreement between fluorescing and total blue counts. However, the other Surfside Beach site also has poor agreement. The current assumption is rather than site specific, the issue may be volunteer team specific and related to unsuitable UV light produced by current light sources rather than a unique strain of bacteria.

The results of this study may lead to a reevaluation of CPE as the preferred volunteer method for enumerating *E. coli* for WWA's VWQM program. Low agreement between the current method, CPE, and the EPA-approved method, C-18, indicates CPE may be underestimating *E. coli*. The poor agreement between *E. coli* concentrations calculated from VWQM data also indicates the method may be underestimating *E. coli* due to the dual confirmation step with fluorescence. A possible solution may be to use CE, thus eliminating the problematic dual confirmation step used by CPE which is absent with CE. However, with the development of a new formulation of CPE by Micrology Laboratories, the VWQM program may have found a suitable method for enumeration *E. coli*. This new formulation performs well with the UV lights previously used by the VWQM program and will be evaluated throughout the next year. PF had greater agreement with C-18 and could possibly be a method better suited for volunteer monitoring. The method is low cost, easy to use, and does not require the use of a UV light for confirmation of *E. coli*. However, PF has a higher detection limit and may not be suitable for all sites. The use of PF for volunteers in the Grand Stand needs to be further investigated.

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Chapter 3

Murrells Inlet Estuary Microbial Source Tracking Study

3.1. Introduction

The Murrells Inlet estuary is a moderately tidal, euhaline estuary on the northern coast of South Carolina. Classified as shellfish harvesting (SFH) waters by South Carolina Department of Health and Environmental Control (SC DHEC), the estuary is subject to monitoring under the shellfish monitoring program under SC DHEC and the National Shellfish Sanitation Program (NSSP) (SC DHEC 2005). Impairments cause closures of shellfish beds to harvest. As of 2014, 71% of the total 3,108 available shellfish acres in Murrells Inlet are approved and open for harvest (WRCOG 2014). However, 23.7% of the available shellfish acres are restricted, closed for direct harvest but where shellfish can be harvested and relocated to approved areas, and 5.0% are prohibited and closed to harvest for any purposes related to human consumption (WRCOG 2014). The map of monitoring stations (**Fig. 3-1**) shows prohibited beds which are closures established adjacent to permitted wastewater discharges, marina facilities, or areas containing multiple point sources of pollution (SC DHEC 2016). These prohibited beds are a response to point source pollution rather than nonpoint source pollution from runoff. Under the monitoring program, long-standing impairment for fecal coliform, the fecal indicator bacteria (FIB) used for water quality criteria in SFH waters, has been observed in the estuary and led to eight water

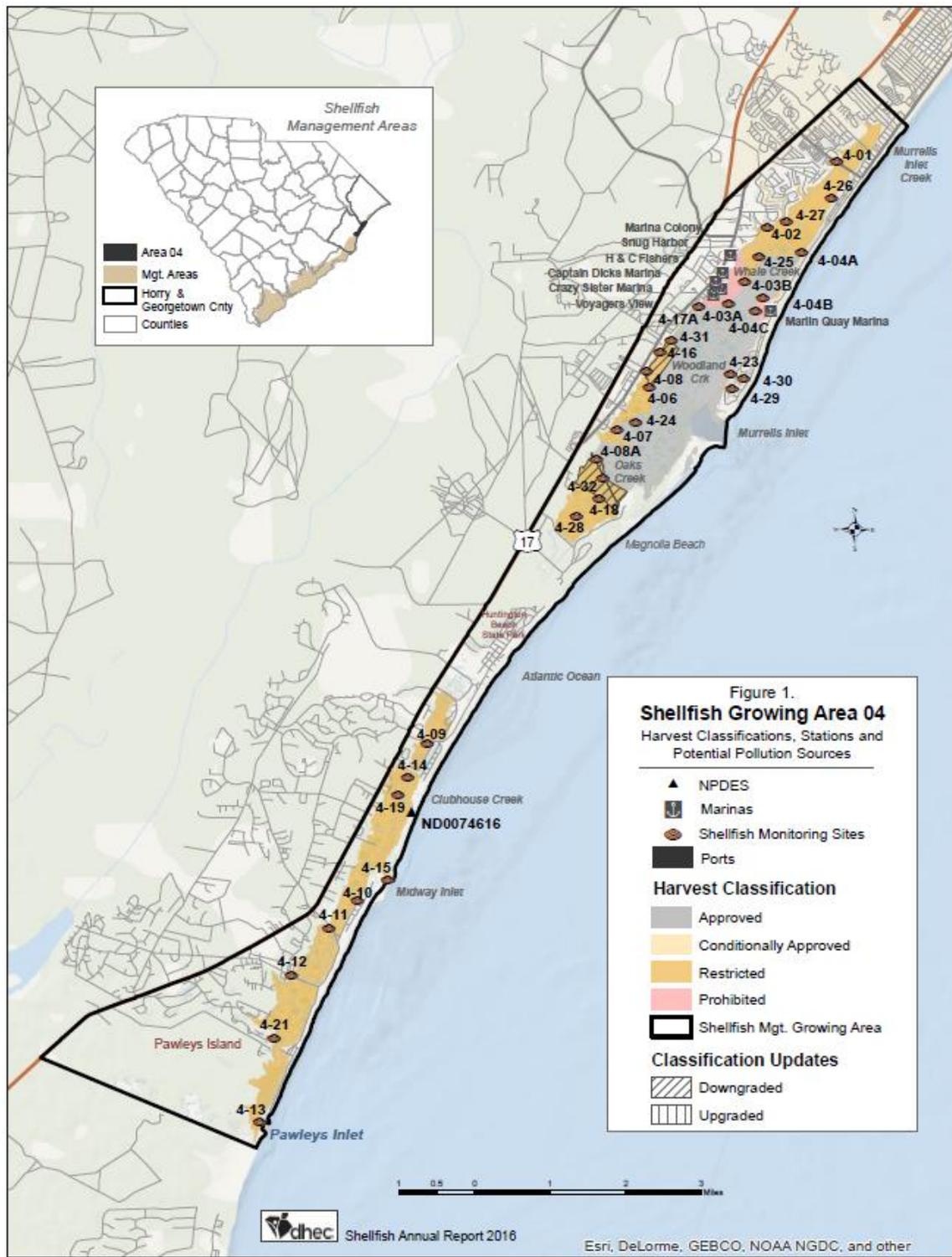


Figure 3-1. Map of Shellfish Growing Area 04 (SC DHEC 2016). The map shows harvest classifications, stations, and potential pollution sources throughout the management area which includes Murrells Inlet.

quality monitoring stations within the Murrells Inlet Estuary being listed as impaired on the states 2004 303(d) list (SC DHEC 2004). While FIB, such as *E. coli* and fecal coliform, are not pathogenic themselves, they are indicators of the presence of feces of many warm-blooded mammals including wildlife, livestock, domesticated animals, and humans (Meays et al. 2004). Shellfish are filter feeders and can become contaminated when poor microbial water quality conditions exist. The consumption of contaminated shellfish can lead to illness in humans and thus SFH waters must be monitored to ensure the safety of shellfish for human consumption (US FDA 2011).

In 2005, SC DHEC approved a Total Maximum Daily Load (TMDL) requiring approximately an 80% reduction in fecal bacteria loading in several areas of the estuary (SC DHEC 2005). The goal of the TMDL is to develop and implement a management plan to reduce fecal coliform bacteria loading so shellfish harvesting beds in the Murrells Inlet estuary system can reopen once water quality standards are met (SC DHEC 2005). The TMDL established that stormwater runoff from nonpoint sources is the primary contributor to fecal coliform contamination in Murrells Inlet (SC DHEC 2005). Wildlife are believed to be a major source based on Multiple Antibiotic Resistance (MAR) analysis results that found little evidence for human-sourced fecal bacteria (Kelsey et al. 2003, Libes et al. 2014). Though fecal bacteria from nonhuman sources are not necessarily pathogenic to humans, the distinction between sources is not made when assessing fecal bacteria concentrations for meeting water quality standards; the evaluation is quantitative not qualitative. Current water quality standards are based primarily on FIB concentrations without considering the potential risk to human health from a specific source.

A volunteer water quality monitoring (VWQM) program was initiated in 2008 to provide additional insight into upstream sources of fecal bacteria to Murrells Inlet. This program is conducted under the aegis of Murrells Inlet 2020, which provides the field leader, and the Waccamaw Watershed Academy (WWA), which serves as technical support. The program is jointly funded by Horry and Georgetown counties. Through the years, volunteers have documented persistently elevated *E. coli* concentrations at three volunteer monitoring sites located in Georgetown County. Monitoring sites HS, BHR, and BB, identified in **Fig. 3-2**, are all located at the termination of tributary streams to Murrells Inlet. These sites and their subwatersheds were selected for investigation by Microbial Source Tracking (MST) to determine the source of fecal contamination.

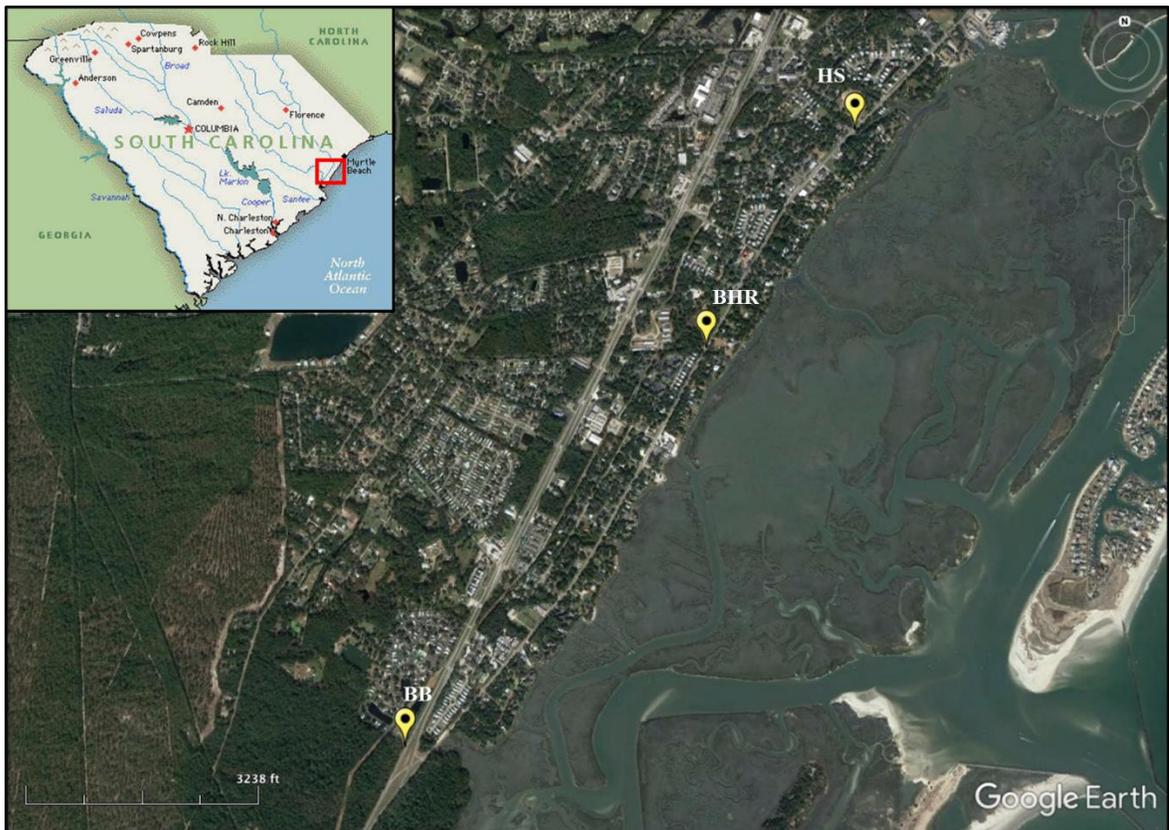


Figure 3-2. Map of three Murrells Inlet VWQM sites. These sites have shown persistently elevated *E. coli* concentrations. Map source: Google Earth.

Determining the source of fecal bacteria is necessary for the cost-effective implementation of management practices to reduce fecal bacteria loading. Yet, specific contributors to non-point sources of pollution, such as stormwater runoff, are often difficult to identify (Meays et al. 2004). MST can help identify specific contributors to nonpoint sources which allows for a targeted management approach to reduce bacteria loading. No strong evidence for human sources was identified in a MST study performed in the Horry County portion of Murrells Inlet (Trapp et al. 2014) or in a study using MAR analysis in 2002 by Kelsey et al. (2003). Strong evidence for human-sourced fecal bacteria in Murrells Inlet has yet to be identified by MST (Libes et al. 2014).

A MST study was conducted in the southern end of Murrells Inlet during the summer and fall of 2015 to identify sources of fecal bacteria loading. The primary goal of the study was to determine whether human-sourced bacteria was a major contributor to fecal bacteria pollution in the three selected subwatersheds. Secondary goals of this research were to determine the roles of stormwater flows and sediments in fecal bacteria loading to the estuary. Five specific null hypotheses were investigated through this study:

1. **Human-sourced bacteria do not comprise a significant component of fecal bacteria present.** To test this hypothesis, genotypic markers of *Bacteroides* (GenBac) and human-sourced *Bacteroides* (BacHum) and caffeine were quantified in water samples.
2. **Weather does not have a significant effect on FIB concentrations.** Dry and wet weather sampling results were compared to evaluate the effect of wet weather on FIB concentrations. Sampling under different weather conditions can help identify

a source. While elevated FIB concentrations during wet weather could indicate nonpoint sources attributed to runoff, higher dry weather FIB concentrations could indicate a point source such as failing septic tanks or a leaking sewer line.

3. **Site location does not have a significant effect on FIB concentrations.** Sampling results from volunteer monitoring sites were compared to upstream sites to determine whether FIB concentrations are significantly different between upstream and downstream sites.
4. **FIB concentration is independent of turbidity and salinity levels.** FIB concentrations were correlated with turbidity and salinity levels to determine whether a relationship exists between the water quality parameters.
5. **FIB concentration in sediments is not significantly affected by weather.** Dry and wet weather sediment results were compared to evaluate whether sediments act as a sink or a source for bacteria. Higher concentrations during dry weather could indicate the sediments act as a source while higher wet weather concentrations could indicate the sediments are a sink.

3.2. Materials and Methods

Three sites located in Georgetown County were identified by the Murrells Inlet VWQM Program as having consistently elevated *E. coli* levels. Each site is at the termination of a tributary stream to the inlet and represents a separate subwatersheds discharging into the estuary. For this phase of the study, the three volunteer monitoring sites as well as two upstream sites were selected for sampling as shown in **Fig. 3-3**. Site

descriptions and coordinates are presented in **Table 3-1**. The upstream sites were selected to eliminate potential sources. A second study is planned to further track suspected sources that could not be eliminated by this first study.

The Mariner/Wesley subwatershed contains the BHR volunteer monitoring site (BHR-VM) as well as an upstream site, BHR-1. The Vaux Hall subwatershed contains the HS volunteer monitoring site (HS-VM) and an upstream site (HS-3). The Bike Bridge subwatershed contains only the volunteer monitoring site (BB-VM). BHR-VM and BB-VM are located near sewage lift stations. Sampling at these two sites in particular is necessary to confirm or deny a potential human source.

Samples were collected during three dry and three wet events during the summer and fall of 2015. Fecal bacteria concentrations tend to be highest in the summer months providing better chances for detection. Dry events are defined as sampling being preceded by a 72-hour dry period according to U.S. Environmental Protection Agency (US EPA) stormwater protocols (Smoley 1993). Wet events are defined as an event of at least 0.1” of rainfall occurring in a four-hour period preceded by 72 hours of dry weather. Rain accumulation data were reported by the weather station at Crazy Sister Marina (<https://www.wunderground.com/personal-weather-station/dashboard?ID=KSCMURRE10#history>).

Table 3-1. Sample site descriptions and locations.

| Site | Description | Latitude | Longitude |
|--------|---------------------------------------|---------------|--------------|
| HS-VM | HS volunteer monitoring site | 33°33'8.23"N | 79°2'24.26"W |
| HS-3 | Upstream site in open ditch | 33°33'3.74"N | 79°2'37.24"W |
| BHR-VM | BHR volunteer monitoring site | 33°32'38.16"N | 79°2'51.63"W |
| BHR-1 | Upstream site on southern tributary | 33°32'37.97"N | 79°3'7.59"W |
| BB-VM | Bike Bridge volunteer monitoring site | 33°31'45.26"N | 79°3'45.05"W |

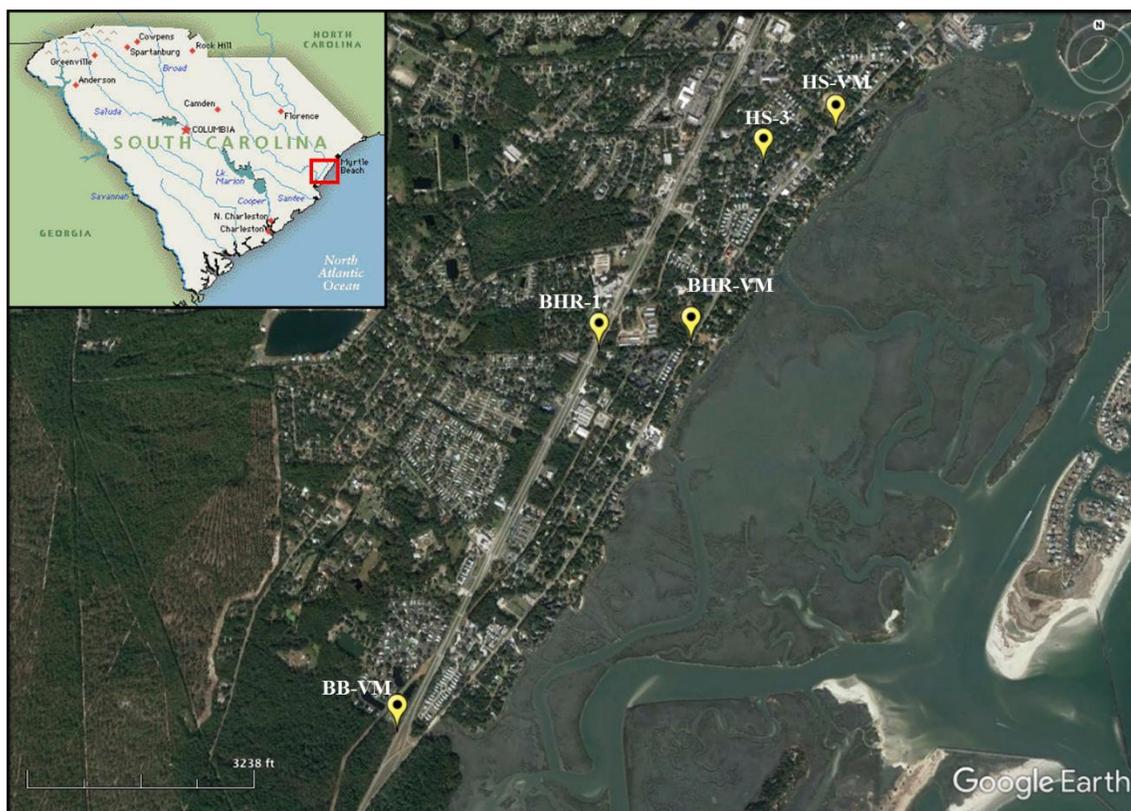


Figure 3-3. Map of Murrells Inlet showing selected sampling sites.

Dry weather samples were collected via grab sample where water was present. Wet weather samples were collected on ebbing tides within 3 hours of the start of rain by grab at downstream sites and by first flush Nalgene stormwater samplers at upstream sites. The downstream sites (BHR-VM, HS-VM, and BB-VM) all displayed tidal influence in preliminary hydrographs collected prior to the study from July to August 2015. The tidal behavior of these sites prohibited the use of first flush Nalgene stormwater samples. Preliminary hydrographs for BHR-1 and HS-3 were used to determine appropriate installation of the samplers to capture first flush samples. Sediment samples were collected by punch coring to 1 cm during both dry and wet weather sampling. Samples were transported on ice to Coastal Carolina University's Environmental Quality Lab (EQL) for

processing and then stored under refrigeration for subsequent analysis. Hold times from collection until analysis did not exceed eight hours.

Multiple tracers were used to provide a weight-of-evidence approach. No single tracer alone can provide evidence of a human source, so a variety of chemical and biological tracers were used in this study. Samples were analyzed for fecal bacteria, genetic source tracers, and chemical tracers. The latter included: salinity, turbidity, and caffeine. Salinity was used as a tracer of water mass, turbidity as a tracer of eroded and resuspended sediment that is a well-documented agent of bacteria transport (Jamieson et al. 2005, Schillinger & Gannon 1985), and caffeine as it is excreted in human urine and can be an indication of human sewage possibly from a leaking sewer line or failing septic systems (Sauvé et al. 2012).

Two separate methods of enumerating FIB concentrations in water samples were used in this study. Both fecal coliform and *E. coli* were enumerated. Fecal coliform was selected because it is the FIB used in monitoring SFH waters. *E. coli* is the FIB used for enumeration of fecal bacteria in recreational freshwater and also the FIB enumerated by the VWQM program that identified elevated bacteria concentrations at the monitoring sites. Enumeration of fecal coliform was performed using A-1 media and multiple-tube fermentation to confirm samples contained a level of FIB consistent with regulatory impairment (SM 9221). IDEXX Colilert-18™ was used to enumerate *E. coli* and total coliform concentrations in water and sediment samples (SM 9223B). For dry weather samples a 1:10 dilution was used for analysis using Colilert-18™. For wet weather samples a 1:100 dilution was used for the analysis. The difference in dilution is based on the expected increase in fecal bacteria concentration during a storm event.

E. coli in sediments was enumerated using IDEXX's Colilert-18™ after being resuspended in sterile buffer water. A sample of 1 gram of sediment was resuspended in 99 mL buffered sterile water using a gentle shaking procedure similar to the method of Craig et al. (2002). Results for sediment samples were normalized to grams by dry sediment and organic contents of the sediment as determined by Loss of Ignition results (EQL 2012a).

Genotypic assays for GenBac and BacHum were performed using Quantitative Real-Time Polymerase Chain Reaction (qPCR) analysis according to EQL standard operating procedures (SOPs) (EQL 2015a, EQL 2015b, EQL 2015c, EQL 2015d).

Chemical tracers of turbidity and salinity were also analyzed using EQL SOPs (EQL 2016, EQL 2013). Caffeine was analyzed using an ELISA test kit from Abraxis (EQL 2012b).

3.3. Results & Discussion

Each site was sampled a total of six times with three dry weather events and three wet weather events from August to October 2015. A summary of sampling dates with antecedent rain conditions is provided in **Table 3-2**.

Table 3-2. Summary of sampling dates with antecedent rain conditions.

| Collection Date | Collection Time (MIL DST) | Rain just prior to sampling (inches) | Date of Antecedent Rain | Antecedent rain (inches) |
|-----------------|---------------------------|--------------------------------------|-------------------------|--------------------------|
| 8/29/2015 | 10:15 to 10:47 | None | 8/26/2015 | 0.61 |
| 8/30/2015 | 14:20 to 14:42 | 0.19 | 8/26/2015 | 0.61 |
| 8/31/2015* | 12:10 | 2.48 | 8/30/2015 | 0.50 |
| 9/17/2015 | 10:43 to 11:14 | None | 9/10/2015 | 0.02 |
| 9/24/2015 | 17:08 to 17:35 | 0.43 | 9/17/2015 | 0.02 |
| 10/1/2015 | 11:05 to 11:40 | None | 9/25/2015 | 0.59 |
| 10/1/2015 | 21:35 to 22:06 | 0.31 | 9/25/2015 | 0.59 |

* BHR-1 was sampled one day after the other sites as rain was insufficient on 8/30/15 to fill the first flush sampler.

3.3.1. Human Sources

Both caffeine and the genetic tracker BacHum were analyzed to determine whether a human-source of fecal bacteria was present in Murrells Inlet. Excreted in human urine, caffeine is used as a tracer for human wastewater. A threshold concentration of >0.4 ng/mL has been proposed by Sauv   et al. (2012) as evidence for the presence of significant human fecal contamination. Only two of the dry weather samples had detectable levels of caffeine while most wet weather samples had caffeine detections (see **Fig. 3-4**). Detection of caffeine levels exceeding 0.4 ng/mL only occurred during wet weather sampling. All wet weather samples from BHR-1 and HS-3 had detectable caffeine. Concentrations at HS-3 during wet weather always exceeded 0.4 ng/mL. Wet weather concentrations of caffeine were higher than those observed at the north end of Murrells Inlet (Trapp et al. 2014). A univariate analysis of variance tested the effects of weather (wet vs. dry) on caffeine concentrations. Results indicate caffeine concentrations were significantly greater during wet weather sampling.

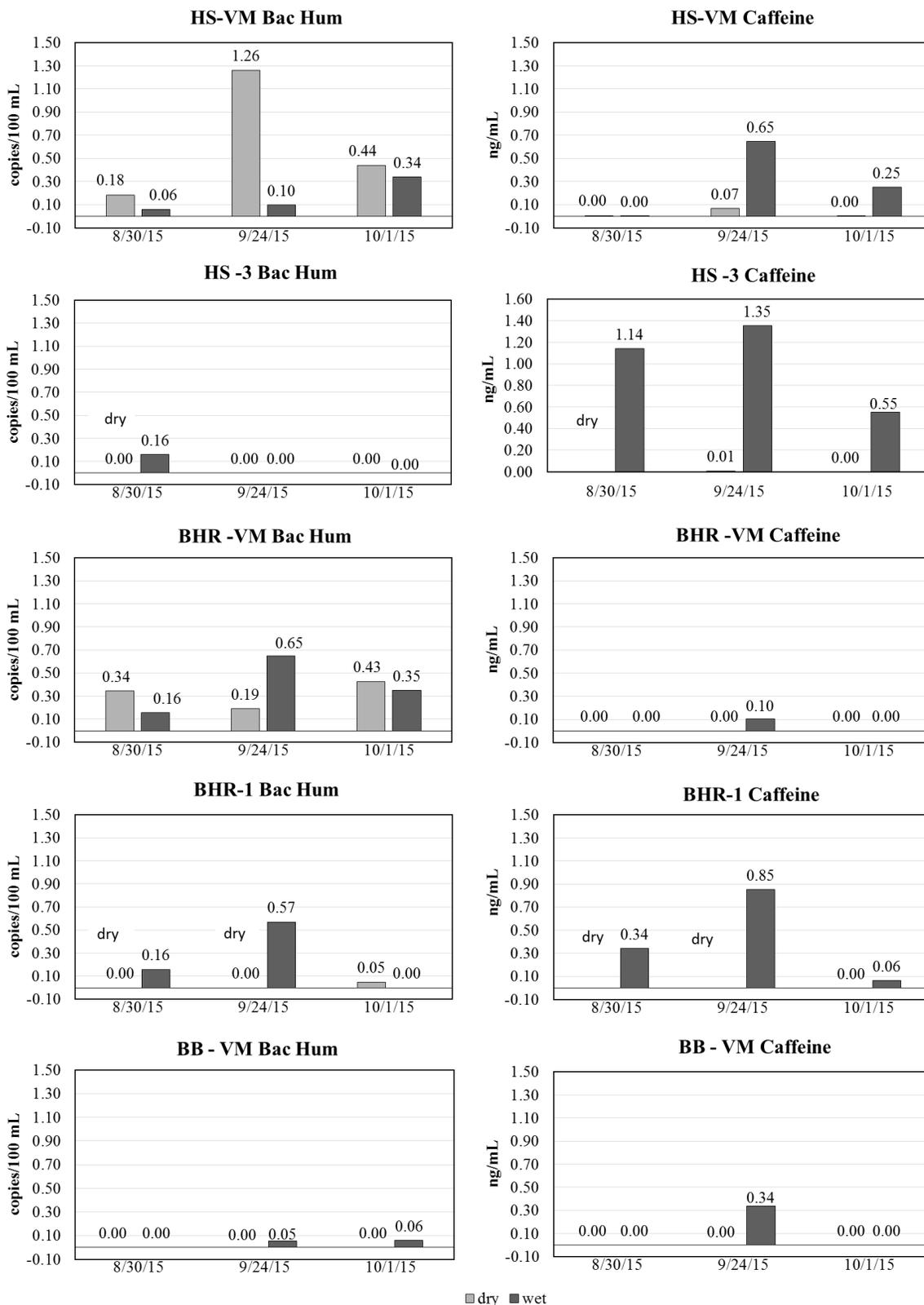


Figure 3-4. Results of caffeine and BacHum analyses by site. Average relative percent difference (%RPD) for replicates performed for the caffeine analysis for this research is 42%.

The genetic tracer BacHum was detected during both wet and dry weather sampling but only at low levels. BacHum was detected in 11 of 15 wet weather samples and 7 of 12 dry weather samples. All samples from BHR-VM and HS-VM had detectable BacHum for both dry and wet weather samples. However, only one sample at HS-VM exceeded 1 copy per 100 mL. Though all the BacHum detections were low, the levels were higher than those observed in north Murrells Inlet (Trapp et al. 2014).

Regulatory water quality standards have not been established for these tracers. A weight of evidence approach was used to determine whether humans were a major contributor to fecal contamination in Murrells Inlet. Using a method developed by Wood et al. (2013), concentrations of BacHum and caffeine were rank ordered and then aggregated as sums and averaged to generate indices. These indices were assigned a qualitative ranking of evidence present for a specific tracer: minor, significant, strong, and very strong. The rankings and results are displayed in **Table 3-3** through **Table 3-7**.

Table 3-3. Quartile rankings for caffeine and BacHum. These rankings were used specifically for this project to create qualitative ranking.

| Parameter | Scores | | |
|-----------|---------|-------|-------|
| | Ranking | Lower | Upper |
| Caffeine | 1 | 0.01 | 0.09 |
| | 2 | 0.10 | 0.49 |
| | 3 | 0.50 | 0.99 |
| | 4 | 1.00 | >1.00 |
| BacHum | 1 | 0.01 | 0.09 |
| | 2 | 0.10 | 0.19 |
| | 3 | 0.20 | 0.49 |
| | 4 | 0.50 | >0.50 |

Table 3-4. Qualitative ranking based on four-point maximum score. Rankings are applied to the overall ratings in **Tables 3-5** through **3-6**. Non-detects for these parameters were assigned a “0” rank.

| Rating | Scores | | |
|-------------|--------|-------|-------|
| | Lower | Upper | Range |
| Minor | 0.0 | 1.0 | 1.0 |
| Significant | 1.0 | 1.9 | 0.9 |
| Strong | 2.0 | 2.9 | 0.9 |
| Very Strong | 3.0 | 4.0 | 1.0 |

Table 3-5. Rankings for caffeine results. See **Table 3-3** for quartile rangers and **Table 3-4** for definitions of overall qualitative ratings.

| Site | Wet | | | Dry | | | Average | | | Overall rating |
|--------|---------|---------|---------|---------|---------|---------|---------|-----|-----------|----------------|
| | 8/30/15 | 9/24/15 | 10/1/15 | 8/29/15 | 9/17/15 | 10/1/15 | Wet | Dry | Wet & Dry | |
| HS-VM | 0 | 3 | 2 | 0 | 1 | 0 | 1.7 | 0.3 | 1.0 | Significant |
| BHR-VM | 0 | 2 | 0 | 0 | 0 | 0 | 0.7 | 0.0 | 0.3 | Minor |
| BB-VM | 0 | 2 | 0 | 0 | ND | 0 | 0.7 | 0.0 | 0.4 | Minor |
| HS-3 | 4 | 4 | 4 | dry | 1 | 0 | 4.0 | 0.5 | 2.6 | Strong |
| BHR-1 | 2 | 3 | 3 | dry | dry | 0 | 2.7 | 0.0 | 2.0 | Strong |

Table 3-6. Rankings for BacHum results. See **Table 3-3** for quartile rangers and **Table 3-4** for definitions of overall qualitative ratings.

| Site | Wet | | | Dry | | | Average | | | Overall rating |
|--------|---------|---------|---------|---------|---------|---------|---------|-----|-----------|----------------|
| | 8/30/15 | 9/24/15 | 10/1/15 | 8/29/15 | 9/17/15 | 10/1/15 | Wet | Dry | Wet & Dry | |
| HS-VM | 1 | 1 | 3 | 2 | 4 | 3 | 1.7 | 3.0 | 2.3 | Strong |
| BHR-VM | 2 | 4 | 3 | 3 | 2 | 3 | 2.8 | 2.7 | 2.8 | Strong |
| BB-VM | 0 | 1 | 1 | 0 | 0 | 0 | 0.7 | 0.0 | 0.3 | Minor |
| HS-3 | 2 | 0 | 0 | dry | 0 | 0 | 0.7 | 0.0 | 0.4 | Minor |
| BHR-1 | 2 | 4 | 0 | dry | dry | 1 | 2.0 | 1.0 | 1.8 | Significant |

Table 3- 7. Qualitative overall ratings for human-source tracers at each site.

| Site | Caffeine | BacHum |
|--------|-------------|-------------|
| HS-VM | Significant | Strong |
| BHR-VM | Minor | Strong |
| BB-VM | Minor | Minor |
| HS-3 | Strong | Minor |
| BHR-1 | Strong | Significant |

Using this approach, neither caffeine nor BacHum displayed very strong evidence for a human-source. To confidently identify a human source of fecal bacteria, these two tracers should corroborate one another (**Table 3-7**). The two parameters were not significantly correlated ($r=0.130$, $p>.05$). Sites with strong evidence of one tracer did not have strong evidence for the other. Caffeine detection could be the result of sources other than human urine. The disposal of unconsumed caffeinated beverages and medications can contribute to concentrations found in surface waters (Edwards et al. 2014). These sources would lead to a false positive detection of human-sourced FIB. These results lead to the acceptance of the null hypothesis that human bacteria do not comprise a significant component of fecal bacteria present in the south end of Murrells Inlet.

3.3.2 Weather Effect on FIB Concentrations

Fecal bacteria concentrations were estimated from measurements of fecal coliform and *E. coli*. The results are presented in **Fig. 3-5**. Results of nonparametric correlations indicated the two FIB concentrations were correlated throughout the sampling ($r_s = 0.784$, $p = 0.000$) and are presented in **Fig. 3-6**. All samples had detectable levels of both FIB and most were high-level detections. All but one of the fecal coliform concentrations contravened the former SC DHEC recreational water quality criteria of 400 MPN/100mL and all but three *E. coli* measurements contravened the US EPA (2012) recreational freshwater quality criteria of 235 MPN/100mL.

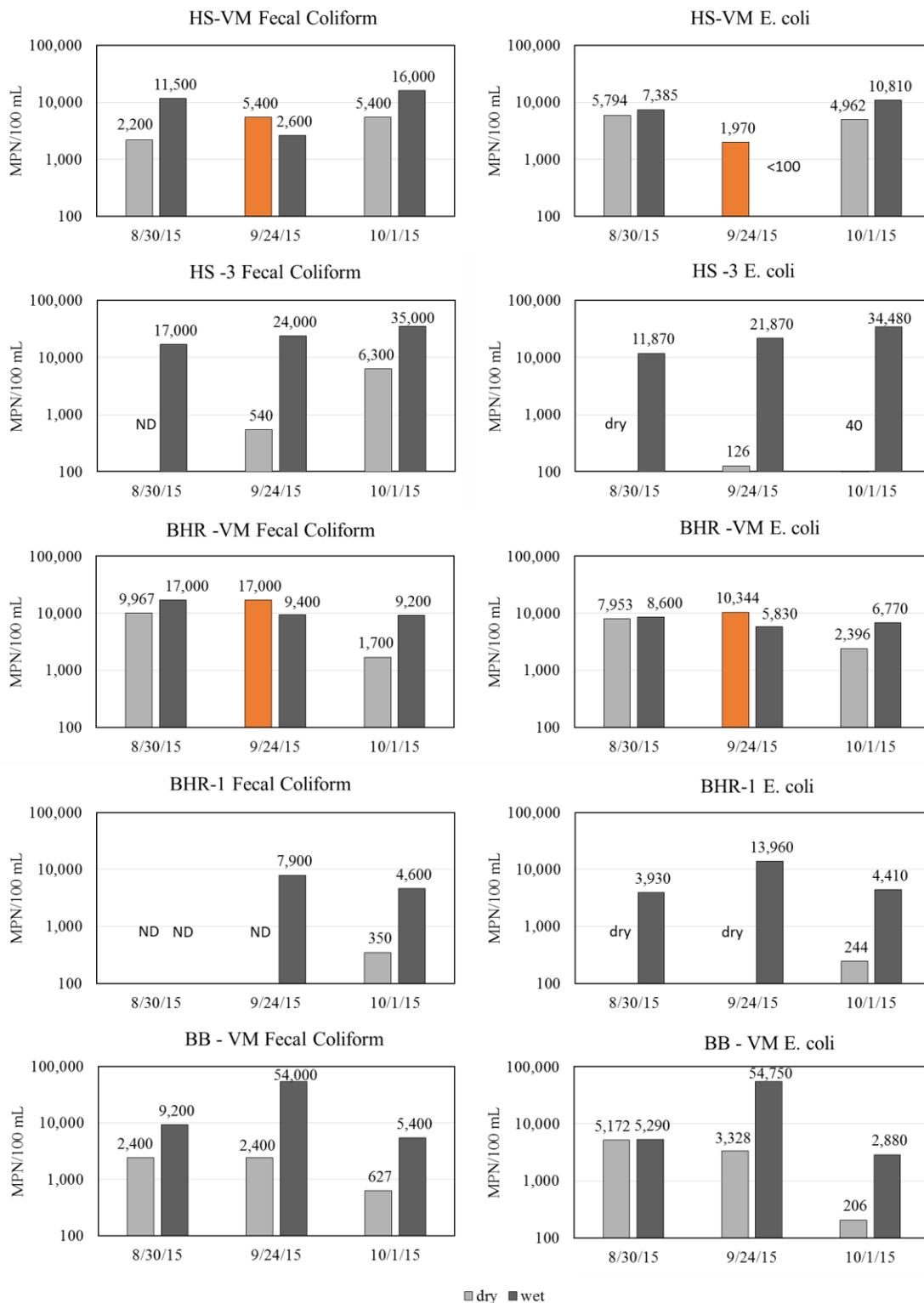


Figure 3-5. Results of fecal coliform and *E. coli* enumeration for all samples. Bars with values indicated as dry represent sampling when no water was present. Bars with values indicated as ND represent a result of no detection. Bars in orange represent dry weather values that were greater than the companion wet weather values. Average %RPD is calculated from samples with field duplicates. Average %RPD is 81% for fecal coliform enumeration and 62% for *E. coli* enumeration in this study.

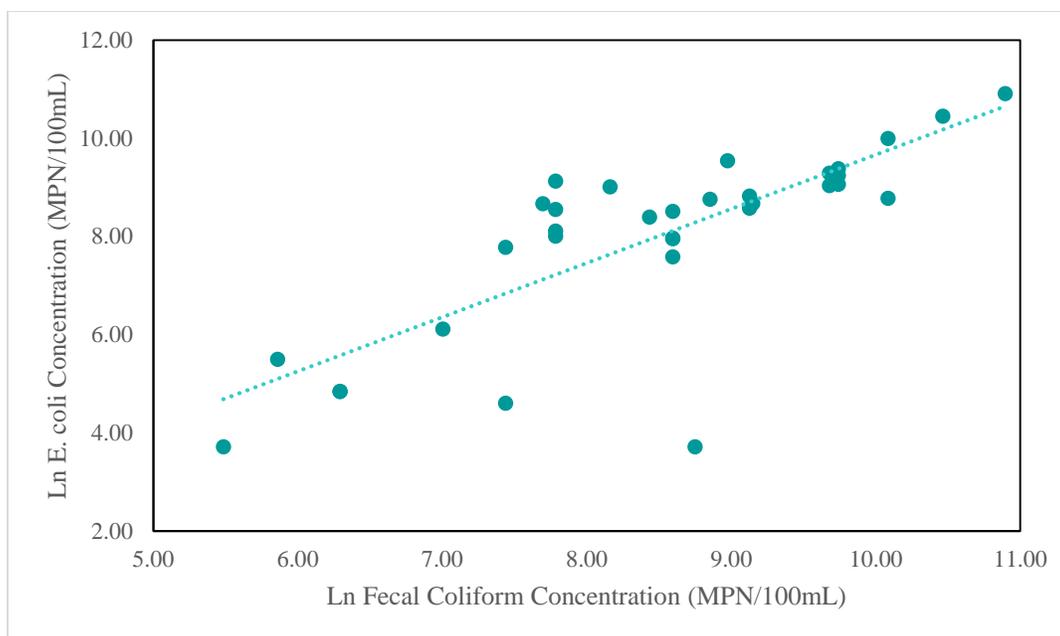


Figure 3-6. Correlation of fecal coliform and *E. coli*. Fecal coliform and *E. coli* were significantly correlated throughout the sampling ($r_s = 0.784$, $p=0.000$). The blue dotted line represents the correlation coefficient.

Overall, wet weather samples had significantly higher FIB concentrations than dry weather samples. In only a few individual cases were dry weather concentrations greater than those observed in wet weather. Univariate analysis of variance tested the effects of weather on the two FIB used in the study. Results showed higher concentrations during wet weather for fecal coliforms ($p= 0.000$) and *E. coli* ($p= 0.008$). Box plots in **Fig. 3-7** show the difference between FIB concentration distribution for wet and dry weather conditions. The overall difference in wet vs. dry weather FIB concentrations suggests that stormwater is a major contributor to FIB contamination in Murrells Inlet, confirming the supposition made by SC DHEC when developing the Murrells Inlet TMDLs previously (SC DHEC 2005). Additionally, these results reject the null hypothesis that weather does not have a significant effect on FIB concentration.

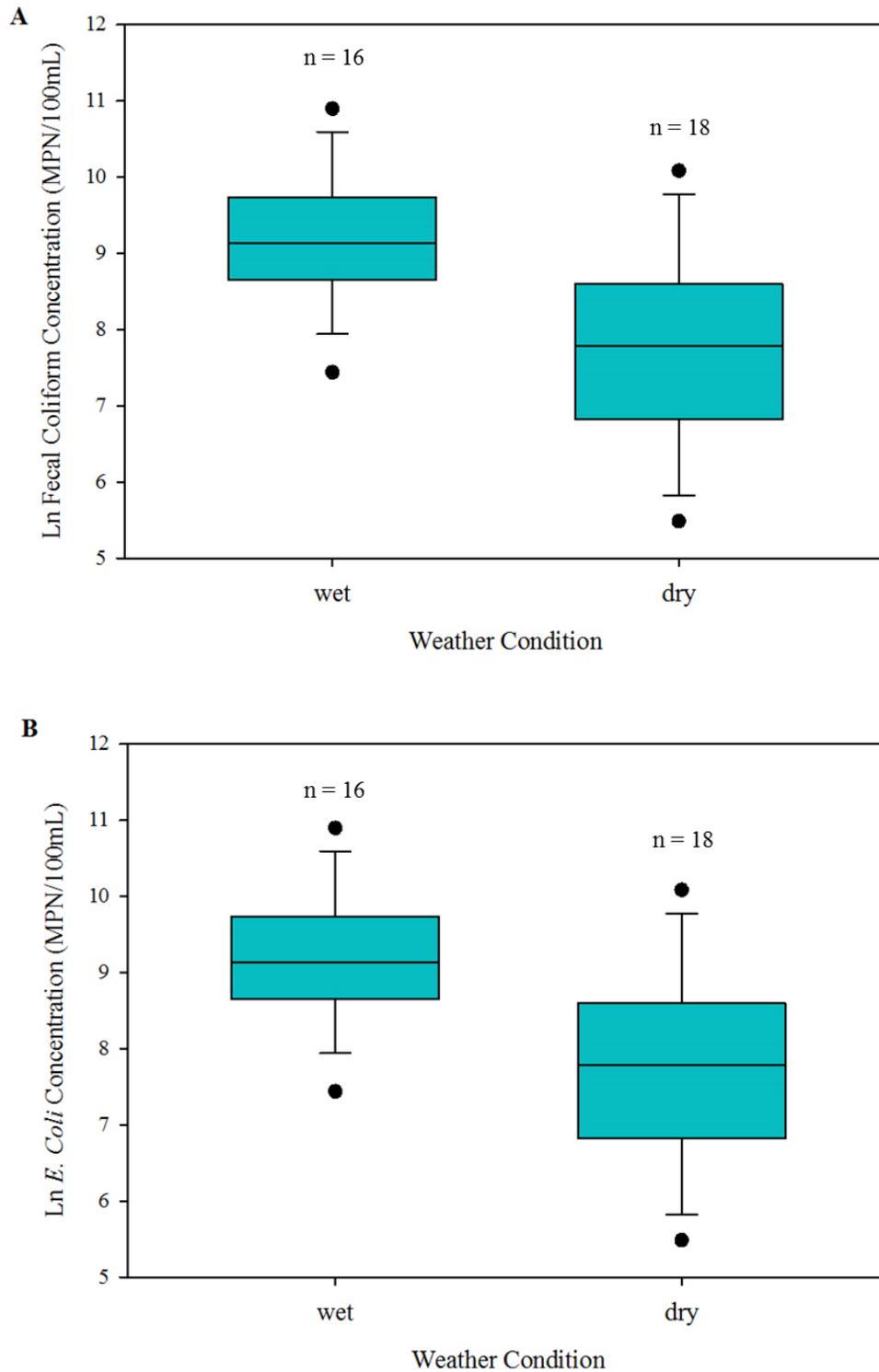


Figure 3-7. Distribution of FIB with respect to weather condition. Significantly higher concentrations of (A) fecal coliforms ($p = 0.000$) and (B) *E. coli* ($p = 0.008$) were detected during wet weather than during dry weather.

The significant effect of weather conditions on FIB concentration indicate the influence of stormwater runoff on microbial water quality. The Center for Watershed Protection (1999) has determined concentrations for typical sources of bacteria (**Table 3-8**). Concentrations detected in water samples for fecal coliform (**Fig. 3-5**) were typical for urban stormwater runoff. Only three wet weather samples (two from HS-3 and one from BB-VM) exceeded typical urban stormwater concentrations. These samples were similar to concentrations typical of a failed septic system. Concentrations detected in samples during this research did not approach concentrations typically consider indicative of a sewer line break. The highest recorded value of fecal coliform measured was a wet weather sample at BB-VM on 9/24/15 of 5.5×10^4 MPN/100mL. While within the range of concentrations related to septic system failure, this measurement is still two orders of magnitude lower than levels indicating a sewer line break.

Table 3-8. Comparison of Bacterial Densities in Different Waste Streams (MPN/100mL). (Center for Watershed Protection 1999).

| Waste stream | Total Coliform | Fecal Coliform | Fecal Streptococci |
|-------------------------|-------------------|-------------------|--------------------|
| Raw sewage | 2.3×10^7 | 6.4×10^6 | 1.2×10^6 |
| Combined sewer overflow | $10^4 - 10^7$ | $10^4 - 10^6$ | 10^5 |
| Failed septic systems | $10^4 - 10^7$ | $10^4 - 10^6$ | 10^5 |
| Urban stormwater runoff | $10^4 - 10^5$ | 2×10^4 | $10^4 - 10^5$ |
| Forest runoff | $10^2 - 10^3$ | $10^1 - 10^2$ | $10^2 - 10^3$ |

3.3.3. Site Effect on FIB Concentration

Differences in FIB concentration between sites can indicate a possible geographic source of FIB. Higher concentrations of FIB at an upstream site could indicate it is acting as a source as FIB will become diluted and subjected to die-off as it flows downstream. Overall, site did not have a significant effect on FIB concentrations for either fecal

coliforms or *E. coli*. Though some sites appear to have higher FIB concentrations, results of univariate analysis of variance for both FIB by site indicate these differences were not statistically different.

Average FIB concentrations for both wet and dry weather sampling by site are shown in **Fig. 3-8**. Some sites, such as BB-VM, had high variability during wet weather sampling. Overall, concentrations were uniformly high during wet weather. The higher values during wet weather indicate sites influenced by runoff. At sites HS-3 and BHR-1 an increase between dry and wet weather FIB concentrations shows the major impact of runoff at these two sites. This may be attributed to few dry weather water samples at those sites.

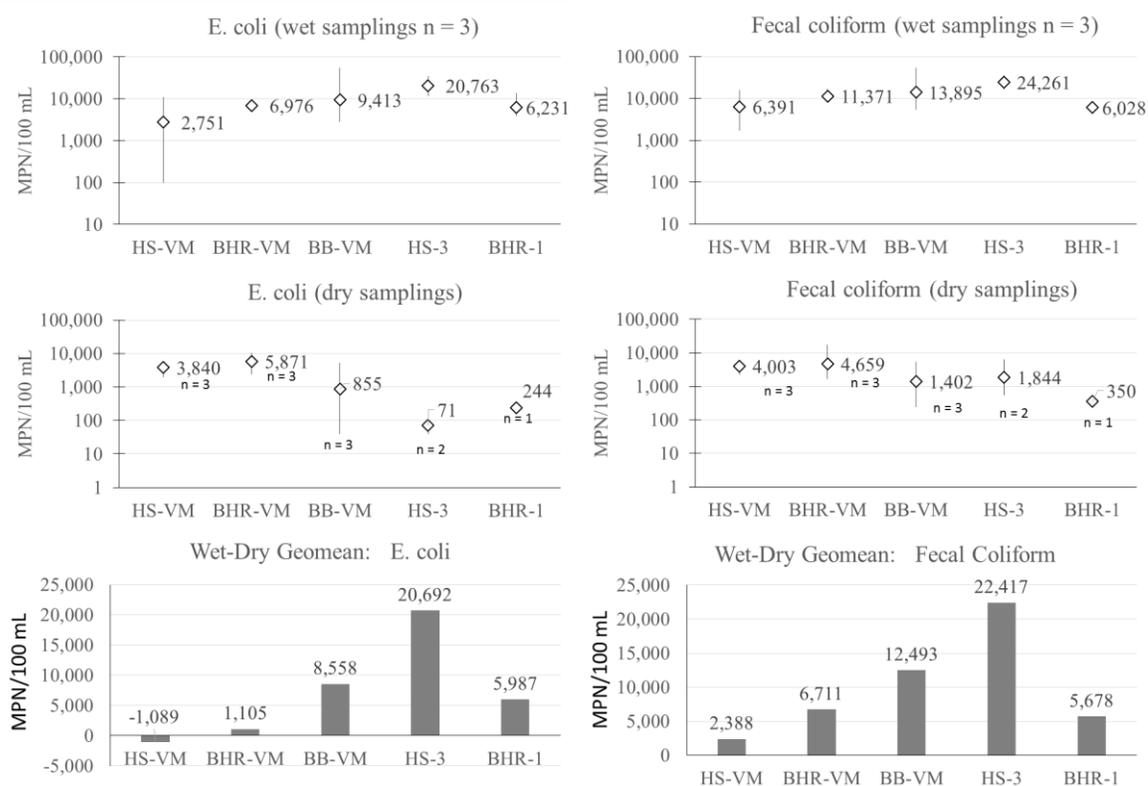


Figure 3-8. Averaged FIB concentrations and wet-dry weather geomeans by site. Vertical lines indicate the range of the values.

Preliminary conclusions suggested that HS-3 may be acting as a source in the Vaux Hall subwatershed during wet weather as FIB concentrations at HS-3 appeared to be greater than those at HS-VM (Libes et al. 2016). Pairwise comparisons of HS-3 and HS-VM show a significant difference during wet weather events ($p = 0.047$) but not during dry weather, indicating HS-3 may be a source of FIB to HS-VM during wet weather.

A site specific source was not identified in the Mariner/Wesley subwatershed. Pairwise comparisons for FIB concentrations at BHR-1 and BHR-VM were only significantly different during dry events for both fecal coliform ($p = 0.040$) and *E. coli* ($p = 0.046$). BHR-VM had higher concentrations than BHR-1 for both during dry weather. This is most likely due to site characteristics of BHR-1 and water not being present for two of three dry sampling dates. In general, the null hypothesis should be accepted: site location does not have a significant effect on FIB concentrations. However, when subwatersheds are examined individually, HS-3 appears to be a possible source during wet weather. No source was identified in the Mariner/Wesley subwatershed and no upstream site in the Bike Bridge subwatershed was sampled for comparison.

3.3.4. Relationship between FIB and Salinity and Turbidity

Relationships between FIB concentration and turbidity and salinity have been documented (Mallin et al. 2000). A negative correlation between salinity and FIB is often evident. The relationship can be explained by two separate effects. First, stormwater tends to flush the system with freshwater as well as FIB from onland sources (Weinreich 2013). Increased freshwater will reduce the salinity of the water. Second, survival of FIB,

especially *E. coli*, is reduced in waters with high salinity (Mallin et al. 2000). Salinity can also be used to identify ambient water sources (e.g. rainwater, groundwater, or saltwater). A positive correlation between turbidity and FIB often exists as increased turbidity is often associated with wet weather events. Not only do FIB adsorb to particulate matter in the water column, but FIB is also present in sediments resuspended by scouring during storms with increased flow. These relationships make turbidity and salinity appropriate low cost tracers for use in MST. Results of turbidity and salinity values are displayed in **Fig. 3-9**.

When evaluating fecal coliform and turbidity, a significant positive correlation is identified ($r_s = .505$, $n = 34$, $p = 0.002$). The same relationship is found between *E. coli* and turbidity ($r_s = .606$, $n = 33$, $p = 0.000$). Correlations are shown in **Fig. 3-10**. Additionally, turbidity was found to be significantly higher during wet weather than during dry weather overall by using a univariate analysis of variance ($p = 0.000$) (see **Fig. 3-11**). The positive correlation of FIB concentrations and turbidity in conjunction with significantly higher turbidity during wet weather indicate a stormwater influence on FIB concentrations.

Correlations of salinity with both fecal coliform and *E. coli* concentrations were not significant. However, weather did have a significant effect on salinity based on results of a univariate analysis of variance ($p = 0.017$). Dry weather salinity measurements were greater than wet weather measurements indicating a system flushing by freshwater during storm events as shown in **Fig. 3-12**.

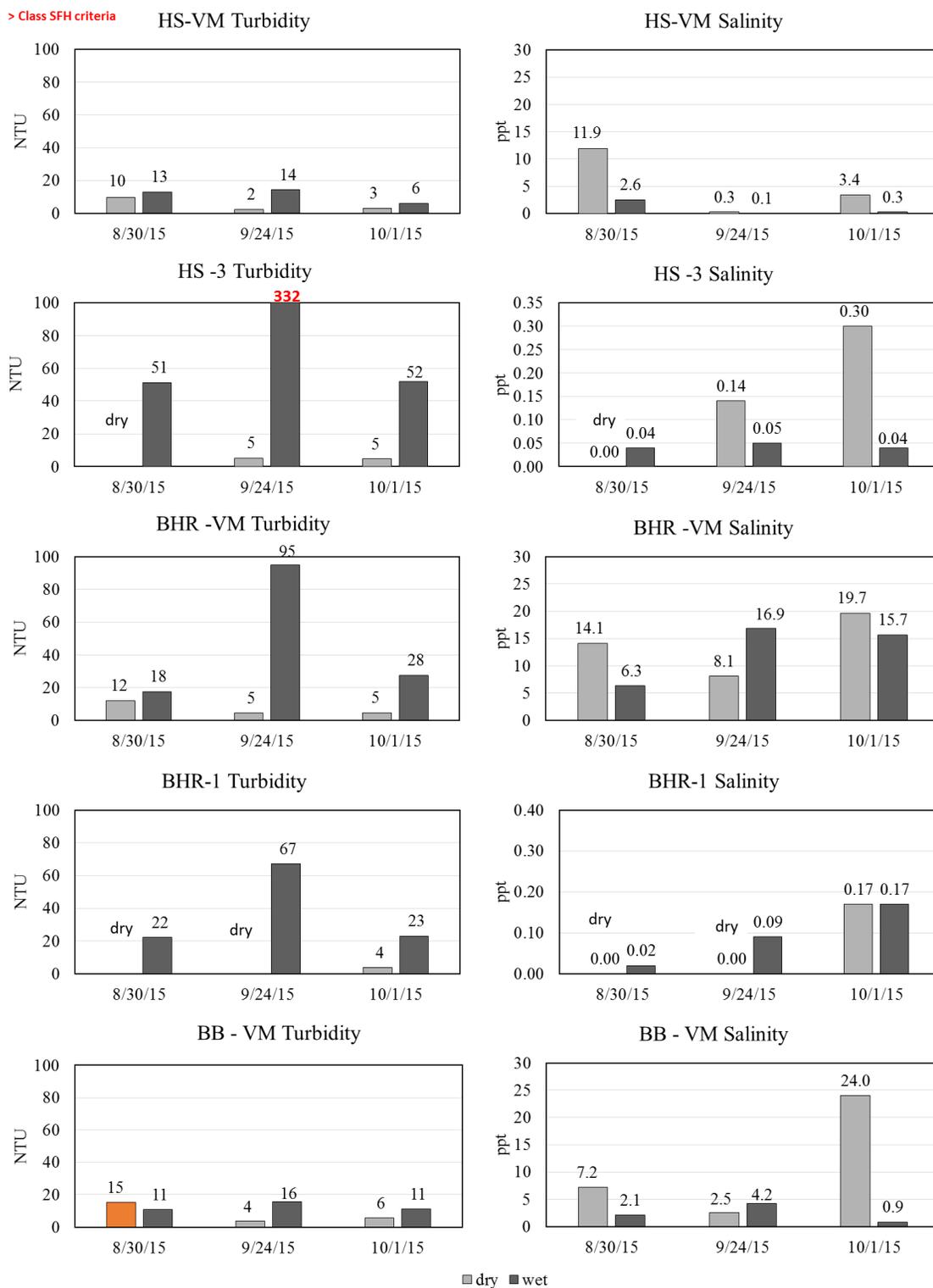


Figure 3-9. Results of salinity and turbidity showing paired wet and dry results. Turbidity results in red exceed the Class SFH water quality criteria of 25 NTU. The orange bar represents a dry weather turbidity measurement that was greater than the corresponding wet weather measurement. Average % RPD calculated from samples with field duplicates is 29% for salinity measurements and 5% for turbidity measurements.

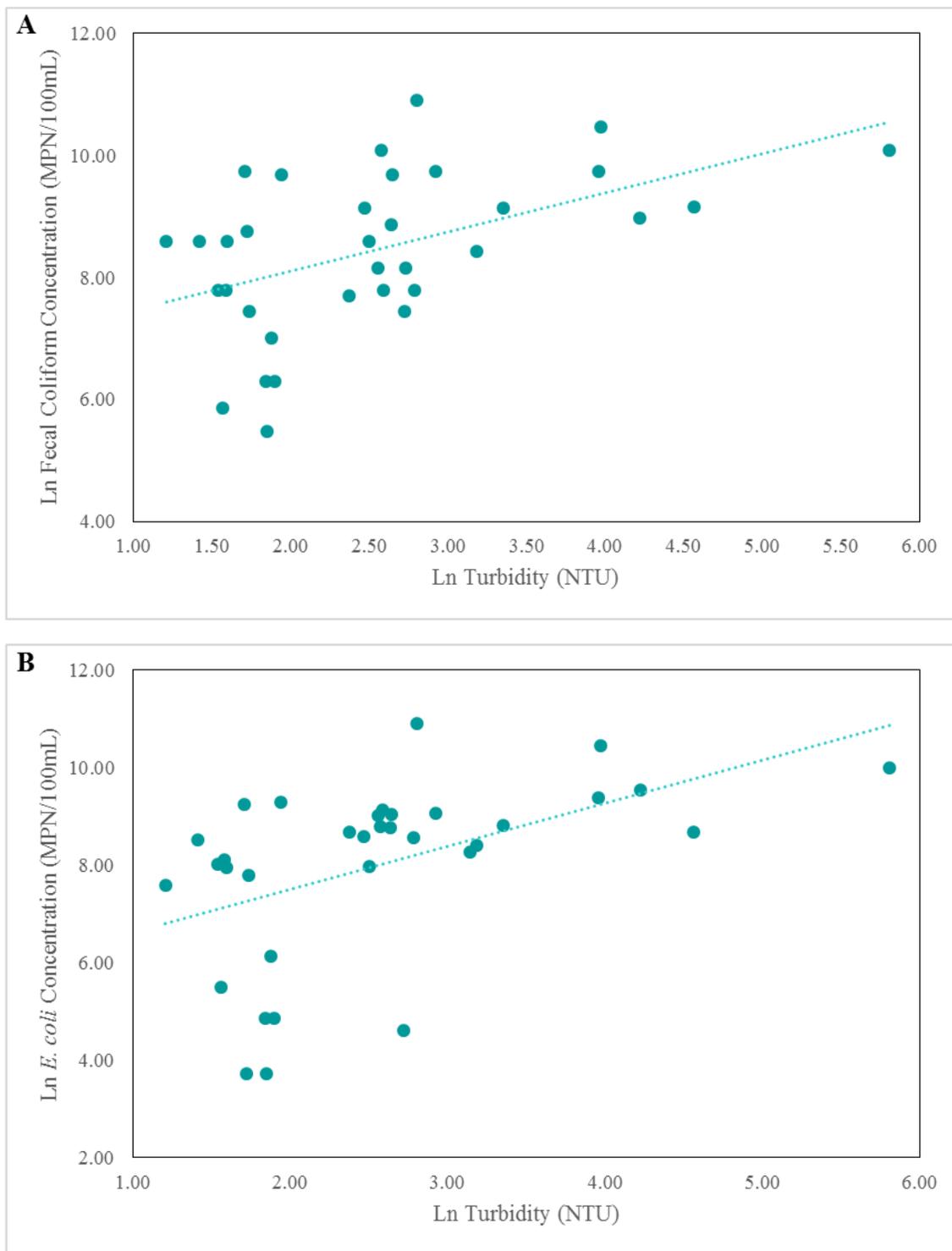


Figure 3-10. Correlation of turbidity with FIB concentrations. (A) Fecal coliform and turbidity are positively correlated ($r_s = .505$, $p = 0.002$). (B) *E. coli* and turbidity are positively correlated ($r_s = .606$, $p = 0.000$). Dotted blue lines represent the correlation coefficient.

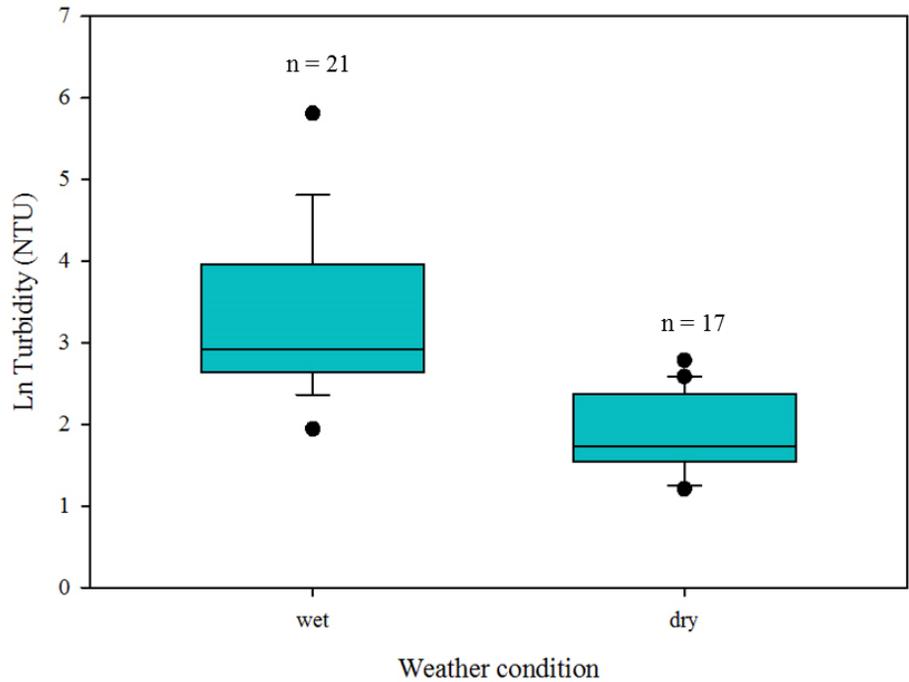


Figure 3-11. Distribution of turbidity by weather condition. Turbidity was significantly greater during wet weather ($p = 0.000$).

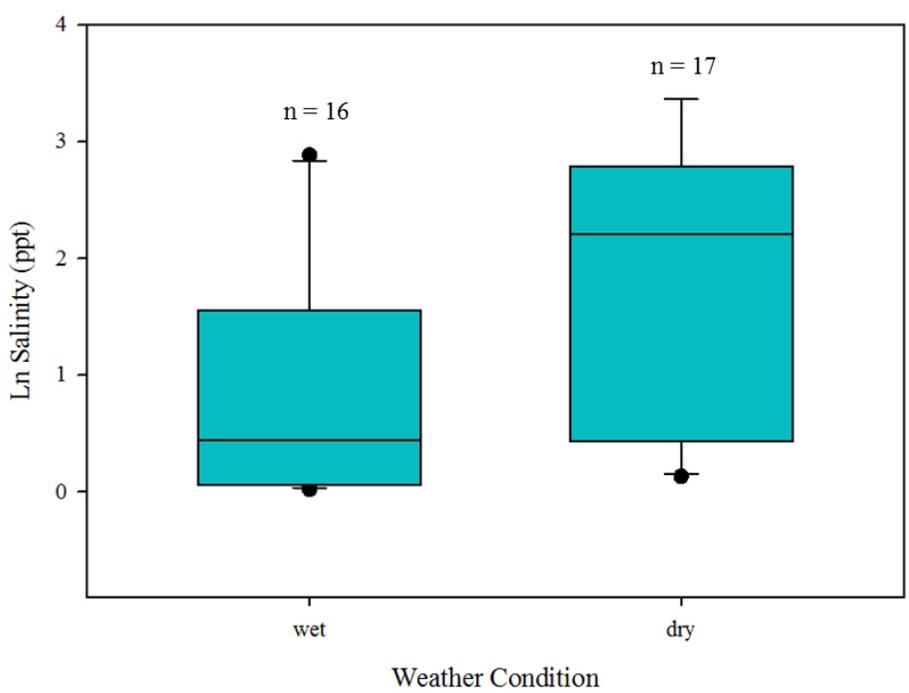


Figure 3-12. Distribution of salinity by weather condition. Salinity was significantly greater during dry weather ($p = 0.017$).

Individual sites had correlations between FIB concentrations and turbidity and salinity not reflected in the overall correlations. For fecal coliform, only salinity at BB-VM was correlated ($r = -0.682$, $n = 9$, $p = 0.021$). For *E. coli*, the correlations vary from site to site (**Table 3-9**). In each case of significant correlation the effect of stormwater runoff can be seen by the negative correlation with salinity and positive correlation with turbidity. While overall the null hypothesis would be accepted, individual cases suggest FIB concentration is not independent of salinity and turbidity.

Table 3-9. Correlation results of *E. coli* concentrations with water quality parameters. Correlation of *E. coli* concentrations with turbidity and salinity were performed by site.

| Site | Correlation of Turbidity with <i>E. coli</i> | Correlation of Salinity with <i>E. coli</i> |
|--------|--|---|
| BB-VM | 0.584* | -0.722* |
| BHR-1 | 0.991** | -0.478 |
| BHR-VM | 0.103 | -0.664* |
| HS-3 | 0.891* | -0.923* |
| HS-VM | -0.910 | 0.490 |

* $p < .05$

** $p < .01$

3.3.5. Role of Sediments

The role of sediments in FIB contamination was also investigated in this study. Sediments can act as a source or a sink for bacteria. Sediments act as a source when stored FIB is released back into the water column. This can occur through resuspension of sediments or by FIB moving independently (Curtis & Trapp 2016). A sink is formed as particulates settle out of the water column, adsorbed fecal bacteria can become buried and will often survive in the sediments. These sediments can then become resuspended by scouring. The mechanism for resuspension of sediments and bacteria with storm flows can be seen in **Fig. 3-13**. Anderson & Greoski (2010) concluded that the role of sediments in

Murrells Inlet in transporting FIB downstream was highly variable and recommended further research.

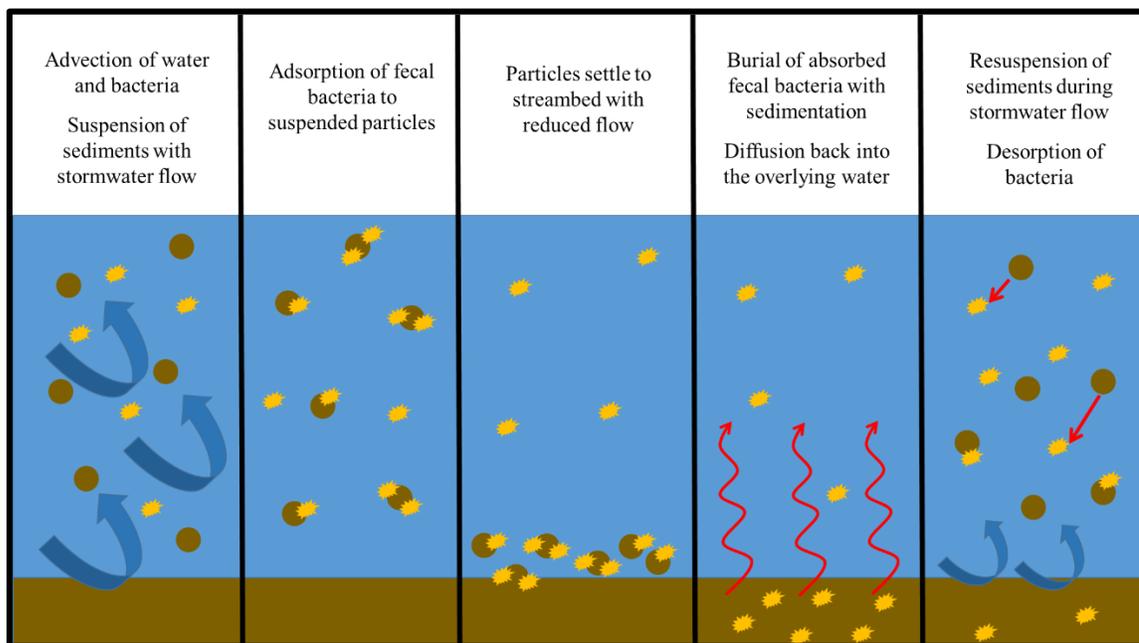


Figure 3-13. Diagram of resuspension during stormwater flows. Sediments and FIB experience resuspension during stormwater flows and can impact concentrations of FIB in the water column.

Unlike the trend in FIB concentrations in water samples showing higher concentrations in wet weather sampling overall, the trend in sediment samples is less consistent (see **Fig. 3-14**). Weather did not have a significant effect on *E. coli* concentrations in sediment samples. Of the 30 sediment samples only eight had elevated *E. coli* (>10,000 MPN/100g). The 30 samples comprise 15 wet-dry paired samples of which 6 had dry>wet results and 9 had wet>dry results. The high variability of *E. coli* concentrations over space and time suggests the roles of sediments transporting FIB downstream is also highly variable.

To further investigate the role of sediments in the transport of *E. coli*, a ranking of *E. coli* concentrations as well as the absolute change in *E. coli* concentrations between dry

and wet weather conditions was completed. The rankings and conclusions drawn from these rankings can be seen in **Table 3-10** and **Table 3-11**. It was assumed that a decrease in *E. coli* concentration indicated a source while an increase indicated a sink. Results examining upstream vs. downstream sites can be seen in **Table 3-12**. Upstream sites are highly variable but downstream sites tend to act as sinks more than sources for *E. coli*.

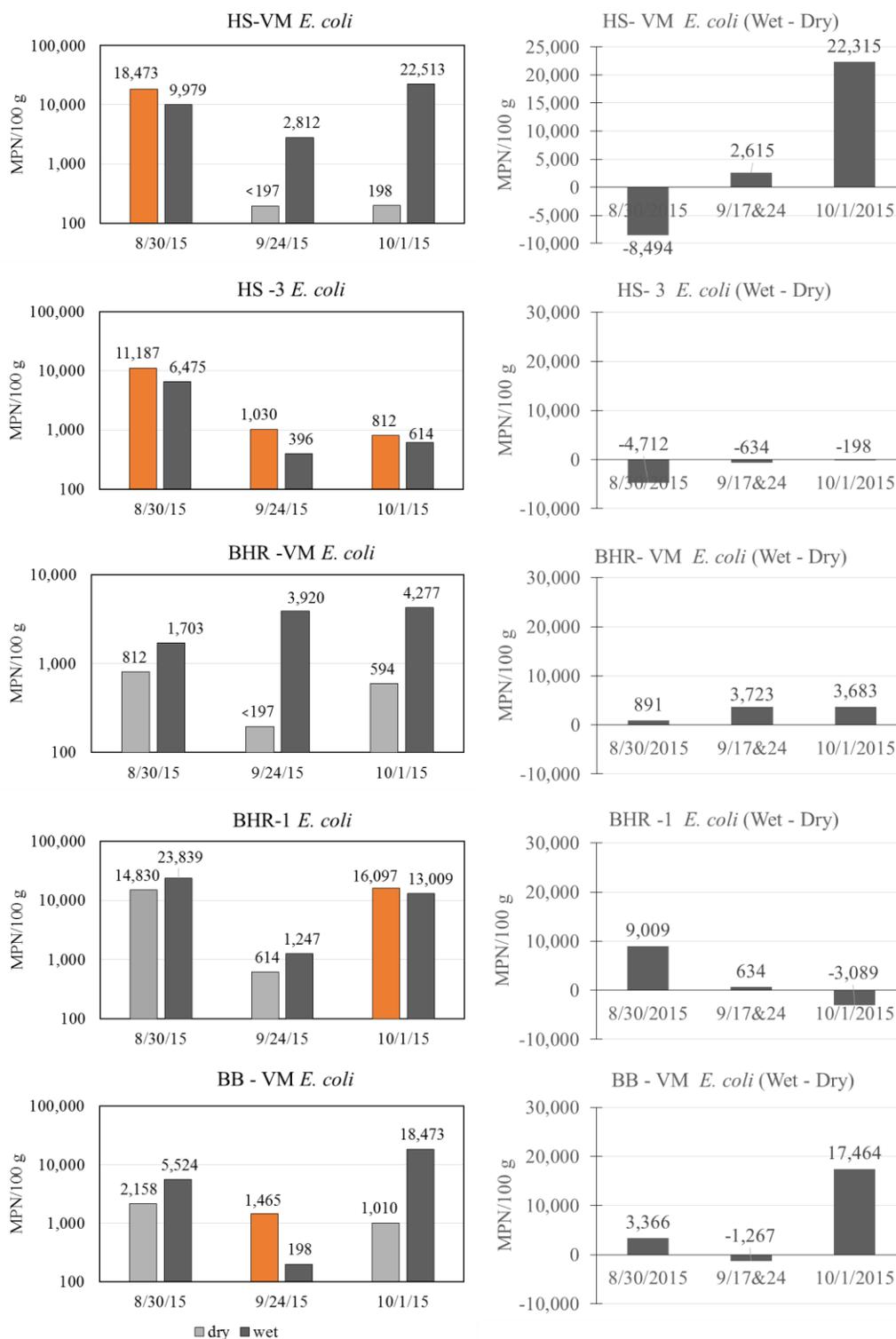


Figure 3-14. Sediment results showing all data for each site for *E. coli*. Bars in orange are dry weather values that were higher than wet weather values. Second column of graphs shows wet-dry means for *E. coli* at each site. No field duplicates for sediment samples were collected so no specific average %RPD exists for sediments. However, the average %RPD for the *E. coli* enumeration method used with water samples is 62% for this study.

Table 3-10. Ranking of *E. coli* in sediments. Rankings are for both *E. coli* concentrations and absolute change in *E. coli* concentrations in sediments. Rankings are used for evaluation in **Table 3-11**.

| Parameter | Scores | | |
|--|---------|--------|---------|
| | Ranking | Lower | Upper |
| Sediment <i>E. coli</i> Concentration | Low | 0 | <1,000 |
| | Medium | 1,000 | <10,000 |
| | High | 10,000 | >10,000 |
| Absolute Change in Sediment <i>E. coli</i> Concentration | Low | 0 | <1,500 |
| | Medium | 1,500 | <4,000 |
| | High | 4,000 | >4,000 |

Table 3-11. Rating of *E. coli* concentrations in sediments by storm event. *E. coli* concentrations for dry and wet samplings for each event were ranked as shown in **Table 3-10**. The change between dry and wet events was also ranked to determine whether the site possibly serves as a sink or source for *E. coli*. Conclusions were drawn at the subwatershed level considering differences between upstream and downstream sites.

| | | HS subwatershed | | BHR subwatershed | | BB subwatershed |
|-------------------------------|--|--|--------|------------------------------------|--------|------------------------------|
| | | HS-3 | HS-VM | BHR-1 | BHR-VM | BB-VM |
| Event 1 8/30/15 0.19 in | Dry <i>E. coli</i> concentration | 11,187 | 18,473 | 14,830 | 812 | 2,158 |
| | Wet <i>E. coli</i> concentration | 6,475 | 9,979 | 22,839 | 1,703 | 5,524 |
| | Change in <i>E. coli</i> concentration | -4,712 | -8,494 | 9,009 | 891 | 3,366 |
| | Possible Source or Sink | Source | Source | Sink | Sink | Sink |
| | Conclusions | Complete scouring throughout tributary | | Transport from unidentified source | | Upstream transport occurring |
| Event 2 9/24/15 0.43 in | Dry <i>E. coli</i> concentration | 1,030 | <197 | 614 | <197 | 1,465 |
| | Wet <i>E. coli</i> concentration | 396 | 2,812 | 1,247 | 3,920 | 198 |
| | Change in <i>E. coli</i> concentration | -634 | 2,615 | 634 | 3,723 | -1,267 |
| | Possible Source or Sink | Source | Sink | Sink | Sink | Source |
| | Conclusions | Upstream transport from HS-3 to HS-VM | | Transport from unidentified source | | Complete scouring |
| Event 3 10/1/15 0.31 in | Dry <i>E. coli</i> concentration | 812 | 198 | 16,097 | 594 | 1,010 |
| | Wet <i>E. coli</i> concentration | 614 | 22,513 | 13,009 | 4,277 | 18,473 |
| | Change in <i>E. coli</i> concentration | -198 | 22,315 | -3,089 | 3,683 | 17,464 |
| | Possible Source or Sink | Source | Sink | Source | Sink | Sink |
| | Conclusions | Upstream transport from HS-3 to HS-VM | | Upstream transport occurring | | Upstream transport occurring |

Table 3-12. Distribution of sink vs. source in upstream and downstream sites. Upstream sites appear to be highly variable while the majority of downstream sites tend to act as a sink for *E. coli* bacteria.

| | | Weak | Medium | Strong | Total |
|-------------------|---------------|-------------|---------------|---------------|--------------|
| Upstream | Source | 2 | 1 | 1 | 4 |
| | Sink | 1 | 0 | 1 | 2 |
| Downstream | Source | 1 | 0 | 1 | 2 |
| | Sink | 1 | 4 | 2 | 7 |

Evaluations were made on the subwatershed level to better understand the dynamic between upstream and downstream sites. In the HS subwatershed, HS-3 appeared to be an upstream source to HS-VM. Concentrations at HS-3 tended to decrease with stormwater flow as concentrations at HS-VM increased, seeming to indicate sediments at HS-3 could be a contributing source of bacteria to HS-VM during stormwater events. In the BHR subwatershed results indicate that BHR-VM is most likely a sink for *E. coli*. However, BHR-1 does not appear to be a source of *E. coli* for the downstream site and may also serve as a sink. This reinforces the results drawn from FIB concentrations in water samples: BHR-1 does not appear to be a source to BHR-VM for FIB. In the BB subwatershed, only one site was sampled so a comparison between upstream and downstream is not possible. Results were highly variable at this site with the site appearing to act as both a sink and a source.

From these results, the null hypothesis is accepted; FIB concentration in sediments is not significantly affected by weather. However, results do help to reinforce previous identifications of HS-3 as a possible source to HS-VM and the elimination of BHR-1 as a source to BHR-VM. The variability of FIB in sediments can be rationalized by the episodic scouring and downstream redeposition of sediments. During resuspension and

redeposition, bacteria are subject to die-off due to predation and exposure to sunlight which may influence the variability displayed in these results.

3.4. Conclusions

The primary goal of this study was to eliminate human contributions as a significant source of FIB contamination in the Bike Bridge, Mariner/Wesley, and Vaux Hall subwatersheds. Additionally, upstream tracing to identify the location of possible sources and determining the role of sediments in fecal bacteria loading in the southern end of Murrells Inlet was incorporated into this study.

This study did not find strong evidence for human-sourced bacteria. Weak detection of BacHum and caffeine despite elevated FIB levels indicate that humans are not a major contributor to fecal bacteria contamination. Based on these results, sewer line breaks and leaking sewer lifting stations can be eliminated as possible sources. It is important to note that some evidence of human-sourced fecal bacteria was identified. However, the detection of two human-source tracers did not occur at the same sites; strong detections of one tracer did not correlate with strong detections for the other. If a human source were present, the two tracers would most likely be detected in the same sample. While these weak detections could possibly be the result of leaking septic tanks upstream from the sample site, detection of caffeine could be attributed to sources not associated with human fecal bacteria such as disposal of unconsumed beverages and medications. Overall, a human source of FIB is unlikely in the south end of Murrells Inlet.

Urban stormwater runoff appears to be the major contributing source in the three subwatersheds evaluated in southern Murrells Inlet. Elevated FIB concentrations during wet weather sampling indicate the effect of stormwater runoff on microbial water quality. Most values for fecal coliform and *E. coli* were typical concentrations of urban stormwater runoff concentrations. Lower *E. coli* concentrations indicate that urban stormwater runoff is a much more likely source of FIB than a possible sewer break. None of the samples approached concentrations typical for a sewer line breaks but were similar to those typical of failed septic systems.

Significant correlations of *E. coli* with salinity and turbidity at individual sites provides further support for stormwater runoff as a major source. *E. coli* concentrations increased with lower salinity and higher turbidity, both of which are often associated with increased stormwater runoff. The overall correlation of turbidity with both FIB concentrations indicate sediment transport may play an important role in Murrells Inlet's fecal bacteria loading.

Of the three subwatersheds sampled, an upstream source site was only identified in the Vaux Hall subwatershed. HS-3 was identified as a possible upstream source to HS-VM in the Vaux Hall subwatershed. However, it is evident that a persistent contamination problem exists in all three subwatersheds as both dry and wet weather samples exhibited elevated FIB concentrations throughout the study. Further investigation during a second phase of the study could reveal other upstream sources.

The role of sediments in fecal bacteria loading is still not clearly explained by the data in this study. High variability over space and time makes identification of sediments as a source or sink difficult. Further study is necessary to make that determination.

Preliminary results drawn from ranking *E. coli* concentrations in sediments and the change in *E. coli* between dry and wet weather events reinforce the high variability among sites. The rankings also identify HS-3 as a possible source of FIB to HS-VM. It is possible that sediments are acting as both a source and a sink. Sediments can act as a source during dry and wet weather conditions (Curtis & Trapp 2016), and as a sink where FIB accumulates and persists (Curtis & Trapp 2014). Sediment sampling should be conducted both longitudinally along the flow path as well as on perpendicular cross-sections to gain a better understanding of spatial variability of sediment bacteria concentration along the stream path. Additional warm weather sampling of paired wet and dry events would help explain the temporal variability.

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Chapter 4

Microbial Source Tracking in the Grand Strand, SC

4.1. Introduction

The purpose of this chapter is to examine the history of microbial source tracking (MST) in the Grand Strand by synthesizing reports of studies performed since urban runoff became a major concern for stormwater managers. MST studies have been performed in the area dating back to the 1970's when the U.S. Environmental Protection Agency (US EPA) was conducting the National Urban Runoff Program (NURP) (US EPA 1983a). Since then, MST has become a popular method for identifying sources of fecal bacteria pollution in the coastal region of Horry and Georgetown counties. Since NURP, fourteen additional MST studies have been performed in the Grand Strand by Coastal Carolina University's (CCU's) Environmental Quality Lab (EQL) and other local researchers.

The MST studies discussed in this chapter address fecal pollution in northeastern coastal South Carolina. MST has been used to reduce fecal bacteria contamination by identifying contaminated sites, investigating and identifying sources of contamination, and evaluating data to develop management strategies. Criteria for clean water standards for both recreation use and shellfish harvest have been established for fecal bacteria

concentrations at the state and federal levels (SC DHEC 2014, US EPA 2012, US FDA 2011). Water bodies consistently exceeding the established water quality criteria are deemed unsafe for recreation or shellfish harvest and are placed on the 303(d) list. MST is used to identify sources of fecal bacteria contamination to remediate water deemed impaired. Understanding the source of fecal pollution is integral to assessing human health risks (Scott et al. 2002).

Most fecal indicator bacteria (FIB) used to assess microbial water quality are present in the feces of warm-blooded animals, though it has been assumed typically only those from human sources pose a significant threat to human health (Scott et al. 2002). Soller et al. (2010) found that while gastrointestinal illness associated with exposure to recreational water contaminated with cattle feces may not be substantially different from waters contaminated with human feces, illness associated with contamination by gull, chicken, or pig feces is substantially lower. These results indicate that identifying a specific source of FIB contamination is integral to reducing human risk. The US EPA recognizes that understanding the predominant source of fecal contamination could help characterize the human health risk associated with recreational water exposure (US EPA 2012). Quantitative Microbial Risk Assessment (QMRA) is a recommended methodology to develop alternative criteria where contamination sources are not predominantly human (US EPA 2012). QMRA examines the risk posed to human health from microbial water quality rather than relying on specific standard criteria for FIB concentrations. Distinguishing between sources has become increasingly important in coastal areas where land use change has increased runoff (Mallin et al. 2001). In the Grand Strand many MST studies are specifically designed to determine if humans are a major contributor to fecal pollution.

Over the years, methodologies have shifted from using single identifying tracers to a comprehensive approach using weight-of-evidence methods and targeted watershed approaches. Methods used for identifying human sources are constantly being improved. During the NURP studies, the fecal coliform/fecal streptococci ratio method was used (US EPA 1983a) While both bacteria are present in the feces of all warm-blooded animals, fecal coliform is present in greater numbers in human feces while fecal streptococci is more numerous in animal feces (Geldreich & Kenner 1969). Geldreich and Kenner (1969) found that a high ratio (>4.0) would indicate a human source while a lower ratio (≤ 0.7) would indicate a non-human source. By the early 2000's, multiple antibiotic resistance (MAR) analysis was being utilized. Possible sources of FIB are determined by the resistance of isolated FIB to antibiotics. It is assumed that human fecal bacteria will have greater resistance to human specific antibiotics and wildlife fecal bacteria will have less resistance (Meays et al. 2004). Results are then compared using cluster analysis to determine sources (Kelsey et al. 2003). Caffeine is a common tracer utilized today. Caffeine is present in beverages and pharmaceutical products consumed by humans and is then excreted in urine (Scott et al. 2002). Presence of caffeine is considered an indicator of human sewage (Scott et al. 2002). Optical brighteners are also used to identify human pollution. Found in laundry detergent, optical brighteners can indicate the presence of human sewage (Meays et al. 2004). Recent developments in genetic tracers have allowed more specific analysis of sources. Real-time polymerase chain reaction (qPCR) analysis can be used to test for species-specific target sequences for *Bacteroidales* associated with all warm-blooded animals, humans, canines, or birds (Roslev & Bukh 2011). CCU's EQL has developed several species-specific assays for qPCR including: warm-blooded animal-sourced

Bacteriodales (GenBac), human-sourced *Bacteriodales* (BacHum), canine-sourced *Bacteriodales* (BacCan), and avian-sourced *Bacteriodales* (GFC Bird). The analysis targets specific sequences to determine the host species.

While individual methods each have advantages and disadvantages, no method has been proposed as the standard for differentiating between sources (Harwood et al. 2014). A combination of methods can be used to best identify a source. Using multiple tracers along with the standard FIB and chemical tracers can then be used in a weight-of-evidence approach (Wood et al. 2013). A targeted watershed, or subwatershed, approach allows higher resolution identification of sources in a specific watershed. This approach divides larger basins into more manageable sections which make pinpointing pollution sources more effective (Wood et al. 2013). Smaller sections can be linked to land use categories that are also useful in determining possible sources.

This chapter focuses on MST studies in six different areas of the Grand Strand: Myrtle Beach, Briarcliffe Acres, Murrells Inlet, Waccamaw River, Surfside Beach, and North Myrtle Beach (see **Fig. 4-1**). Goals and findings of each of the studies are reviewed briefly in **Table 4-1**. This chapter will review each study focusing on the goal of the investigation, MST techniques used, and major findings or recommendations.

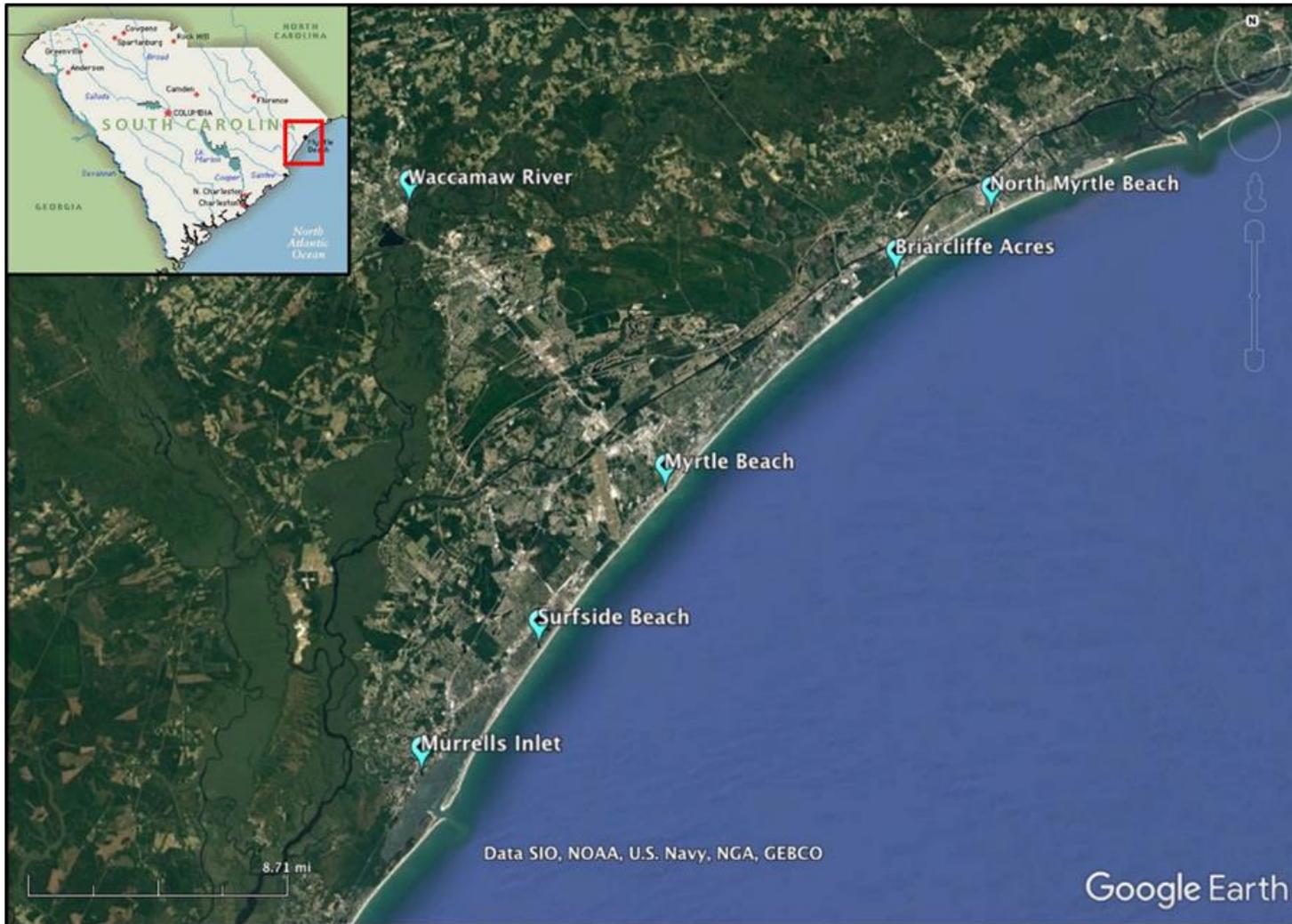


Figure 4-1. Map of study areas throughout the Grand Strand, SC. Map source: Google Earth

Table 4-1. Descriptions of MST studies conducted throughout the Grand Strand.

| Study Area | Study Report | Goal of Study | Study Date | Stormwater Sampling | Tracers Used | Source Identified | Important Findings |
|--|--|--|-------------------|---------------------------------|--|--|--|
| Myrtle Beach | Results of the Nationwide Urban Runoff Program | Determine whether urban runoff is impacting national water quality | 1975-1978 | Stormwater influence identified | FC/FS ratio | Human source | Identified need for policy focus shift from industrial wastewater to urban stormwater runoff |
| Withers Basin, Myrtle Beach | Water Quality in the Withers Swash Basin, with Emphasis on Enteric Bacteria | Assess water quality of streams before and after storm runoff | 1991-1993 | Stormwater influence identified | FC/FS ratio | Multiple sources likely; no single source detected | Enteric bacteria increased with stormwater flow. Resuspension of sediments identified as a possible source during stormwater flow. |
| Withers Basin, Myrtle Beach | Watershed Assessment Plan | Identify sources of FIB contamination to develop cost-effective and successful TMDLs | 2011-2012 | Stormwater influence identified | FIB Optical brighteners Caffeine qPCR | Human and domesticated animal sources | Identified sediments as a possible source for further investigation. Human source identified as homeless activity. |
| Briarcliffe Acres | Briarcliffe Acres Water Quality Study | Determine whether a link between FIB contamination and septic tank systems exists locally | 2009-2010 | Stormwater influence identified | FIB Optical brighteners qPCR | Human source | Wet weather human source indicates leaking septic systems leading to recommendation to switch to sewer system |
| White Point and Briarcliffe Swashes, Briarcliffe Acres | Final Report: Microbial Source Tracking: White Point and Briarcliffe Acres Swashes | Identify source of pollution to 303(d) listed monitoring site at confluence of two swashes | 2015 | Stormwater influence identified | FIB salinity | N/A | Briarcliffe swash appears to be a greater contributor than White Point Swash to beach sampling site WAC-009A |
| White Point Swash Outfall, Briarcliffe Acres | Storm Water Outfall Study: Horry County Beaches | Identify sources of contamination and recommend options for improvements to water quality | 2000 | Stormwater influence identified | FIB | Human source | Briarcliffe Acres site had a higher percentage of human-sourced fecal bacteria than other sites in Horry County. |

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|---|---|--|-------------|----------------------------------|---|---------------------------------|--|
| Murrells Inlet | Using Multiple Antibiotic Resistance and Land Use Characteristics to Determine Source of Fecal Coliform Bacterial Pollution | Examine effect of land use on fecal coliform densities. Differentiate between human and nonhuman sources. | 2003 | Stormwater influence identified | FIB | Nonhuman source | Regression with land use identified proximity to urban areas and rainfall as predictors for fecal bacteria pollution. |
| BHR and HS tributaries, Murrells Inlet | Microbial Source Tracking of E. coli and Fecal Coliforms in Murrells Inlet, South Carolina | Identify sources of FIB contamination in two tributaries monitored by volunteers with consistently elevated levels of FIB. Determine role of sediments in FIB contamination. | 2010 | No stormwater sampling conducted | FIB Optical brighteners Conductivity Turbidity | Possibly leaking septic systems | Possible upstream sources were identified in both tributaries. Presence of optical brighteners indicates leaking septic systems may be a source. High variability in sediment analyses indicates sediments are not a long term legacy source of FIB. |
| BMP demonstration sites in Murrells Inlet | Effectiveness of Stormwater BMPs in the Receiving Waters of Murrells Inlet | Determine effectiveness of demonstration BMPs and estimate impact on water quality of Murrells Inlet estuary | 2005-2006 | Stormwater influence identified | Fecal coliforms Turbidity Conductivity | N/A | Fecal coliforms increase with stormwater flows but are reduced in BMP stormwater ponds within days after rain. Vegetated wetland ponds improve water quality. |
| Subwatersheds HS, BHR, BB, and HBSP, Murrells Inlet | Murrells Inlet Volunteer Monitoring Program: Upstream Sampling Program Final Report | Understand sources of bacteria present, understand reasons for wide variability, and identify measures to reduce FIB | 2013 | Stormwater influence identified | FIB | Wildlife source | No obvious sources of leaking septic systems identified. Demonstrated effective use of stormwater ponds for removing fecal bacteria pollution. |
| Northern end of Murrells Inlet, Horry County | Murrells Inlet – Microbial Source Tacking Study Report | Determine whether FIB is human-sourced | 2012 – 2013 | Stormwater influence identified | FIB Optical brighteners Caffeine Salinity Turbidity qPCR | Canine and bird sources | Little to no evidence of a human source. While a stormwater runoff influence was identified, some sites display persistent contamination. |

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|--|--|--|-------------|---|--|-------------------------------------|---|
| Subwatersheds HS, BHR, and BB, Murrells Inlet, Georgetown County | Murrells Inlet – Phase I Microbial Source Tracking Study Report | Determine whether FIB is human-sourced. Investigate role of sediments in FIB contamination. | 2015 | Stormwater influence identified | FIB Caffeine Salinity Turbidity qPCR | Nonhuman source | Little to no evidence for human source. High variability in sediment analyses indicates sediments are not a long term source of FIB. |
| Kingston Lake, Crabtree Canal, and Waccamaw River | Identification and Mitigation of non-Point Sources of Fecal Coliform Bacteria and Low Dissolved Oxygen in Kingston Lake and Crabtree Canal | Determine whether stormwater runoff is a major source of pathogenic bacteria. Examine the effectiveness of stormwater ponds. | 1999 – 2001 | Stormwater influence identified | FIB | Human and domestic wildlife sources | Confirmed the presence of chronic pollution problems in addition to a stormwater influence. Demonstrated effective use of stormwater ponds for removing fecal bacteria pollution. |
| Myrtle Lake, Surfside Beach | No official report | Evaluate upstream waters as possible sources to 303(d) listed beach monitoring sites | 2016 – 2017 | No stormwater sampling conducted | FIB Conductivity Turbidity qPCR | No significant human source | Despite high levels of E. coli bacteria detected, a human source was not detected by qPCR. |
| 16 th and 17 th Avenue S, North Myrtle Beach | 16 th and 17 th Avenue S Microbial Source Tracking | Determine significant sources of FIB to storm catch basins with consistently elevated FIB levels | 2016 -2017 | Stormwater sampling conducted but not yet completed | FIB Conductivity Turbidity qPCR | Not yet completed | Not yet completed |
| Cherry Grove Marsh system, North Myrtle Beach | Hog Inlet – Microbial Source Tracking | Identify geographic and host animal source of FIB contamination within Cherry Grove Marsh system | 2016 - 2017 | Stormwater sampling conducted but not yet completed | FIB Caffeine Salinity Turbidity qPCR | Not yet completed | Not yet completed |

4.2. Myrtle Beach Studies

4.2.1. US EPA National Urban Runoff Program Study

The US EPA implemented NURP in the 1970's to use combined water quality studies throughout the country to develop comprehensive knowledge of pollution issues associated with stormwater (US EPA 1983a). The goal of NURP was to determine whether urban runoff was contributing to water quality problems in order to inform decision makers at various government levels on best management practices (BMP) to reduce pollution. Whereas water quality studies had previously focused on wastewater, NURP was primarily focused on urban runoff. As part of NURP, the Waccamaw Regional Planning and Development Council (WRPDC) drafted the 208 Areawide Water Quality Management Plan. During 1976, WRPDC performed a water quality study along the coast from the northern city limit of North Myrtle Beach to the southern city limit of Myrtle Beach giving special attention to Withers Basin in Myrtle Beach (US EPA 1983b). Previous studies had indicated direct stormwater discharges to the ocean may be responsible for not only poor water quality but also beach erosion and unsightly beach appearance (US EPA 1983a). 120 discharge sites in Myrtle Beach were selected for extensive bacteria sampling performed during wet and dry periods (US EPA 1983b). The study relied on early methods of comparing fecal coliform and fecal streptococci concentrations to differentiate between human and animal sources. Findings from NURP indicated fecal coliforms, representing human sources, were of primary concern in urban runoff (US EPA 1983a). The Myrtle Beach component of the study revealed similar results with high bacteria levels reported after storms (US EPA 1983a). The nationwide results of NURP led to the development of

stormwater management and solidified a policy shift from industrial wastewater to stormwater discharge as a primary source of concern for water quality.

4.2.2. USGS Water Quality Study in Withers Swash Basin

With pervious surface cover reduced due to increased development, the City of Myrtle Beach became concerned with the effect of stormwater runoff on water quality (Guimaraes 1995). As a result, the U.S. Geological Survey (USGS) conducted a MST study in the Withers Swash basin during the summer from 1991 through 1993. A primary concern was pollution by enteric bacteria during the summer due to the large seasonal population (Guimaraes 1995). Sampling was conducted at 46 sites within the basin and 5 sites on the beach and in the Atlantic Ocean. Sampling was performed during dry and wet weather to assess water quality before and after storm runoff (Guimaraes 1995). Enteric bacteria (fecal coliform and fecal streptococcus) were analyzed as part of the study which analyzed over 200 physical, chemical, and biological constituents. Enteric bacteria concentrations were found to increase with increased storm flow due to storm runoff. The increased bacteria was partially attributed to resuspension of sediments storing bacteria during increased flow (Guimaraes 1995). Through high concentrations of enteric bacteria were detected, the sporadic contamination made determining a specific source difficult. There were assumed to be multiple sources including septic tanks, garbage containers, waterfowl feces, and domestic animal feces. The study confirmed that development in Myrtle Beach could influence fecal contamination as a result of stormwater runoff.

4.2.3. Watershed Assessment Report for Withers Basin

Withers Basin has continued to be an area of concern for stormwater managers in Horry County. The Beach Environmental Assessment and Coastal Health (BEACH) Act amended the Clean Water Act to provide funding for beach monitoring and assessment programs. Regulatory level monitoring of coastal recreational waters for enterococcus identified numerous sites along the northeastern coastline as impaired (Wood et al. 2013). Sites classified as impaired on South Carolina's 303(d) List require the development of total maximum daily load (TMDL) to reduce contamination. In 2012, a MST study was performed in Withers Basin in preparation for a TMDL to be developed in 2018. MST can be a useful tool to develop cost effective TMDLs and ensure the successful implementation of TMDLs (Wood et al. 2013). The study, funded by the US Army Corps of Engineers and performed by CCU's EQL in collaboration with City of Myrtle Beach stormwater staff, aimed to identify possible sources of FIB in Withers Basin (Wood et al. 2013). Suspected sources included pet waste, waterfowl waste, homeless activity, and leaks from the sanitary sewer system (Wood et al. 2013). To identify the source, a multi-tracer, targeted sub-watershed investigation using a weight-of-evidence approach was implemented (Wood et al. 2013). Samples were taken during three wet and two dry events and analyzed for an array of water quality parameters as well as chemical and genotypic tracers. Analyses for optical brighteners, caffeine, and qPCR assays for GenBac, BacHum, and BacCan were completed to identify possible sources. The results of the study narrowed the suspected source list down to pet waste and homeless activity, though pet waste appeared to be a more significant source (Wood et al. 2013). Another significant source identified was a sewer line break that was subsequently repaired, thus demonstrating a real-time use of

MST. Additionally, higher bacteria concentrations during wet weather than during dry weather indicated inputs from both overland runoff and resuspension of sediments harboring fecal bacteria (Wood et al. 2013). The project partners acknowledged that due to the limited scale of the effort, more sampling is needed in order to confirm correlations between land use and water quality results to better inform management interventions (Wood et al. 2013).

4.3. Town of Briarcliffe Acres Studies

4.3.1. Stormwater Outfalls Study for Horry County Beaches

The Town of Briarcliffe Acres has been the site of several MST studies. These studies have been conducted since 2000 to identify the source of pollution well documented to be occurring at a nearby regulatory beach monitoring site (WAC-009A). Horry County contracted Davis & Floyd, Inc. to perform a MST study to identify sources of contamination from stormwater outfalls in 2000. By sampling throughout Horry County to provide reference data, the study aimed to identify sources and recommend options for improvement of water quality based on those findings (Davis & Floyd 2002). Of particular interest is the basin draining Briarcliffe Acres sampled at the southern end of White Point Swash. Water and sediment samples were collected during dry and wet weather and analyzed for *Enterococcus* and fecal streptococci. Results indicated a more likely human source at Briarcliffe Acres in comparison to other sites throughout the Grand Strand (Davis & Floyd 2002). Despite its proximity to the ocean, most of the 200 homes in Briarcliffe

Acres utilize septic systems. Davis & Floyd Inc. (2002) identified the septic systems as a potential source of fecal bacteria in White Point Swash, and subsequently the coastal ocean. The researchers suggested Briarcliffe Acres eliminate the septic systems and connect directly to a municipal sewer system (Davis & Floyd 2002).

4.3.2. Briarcliffe Acres Water Quality Study

The connection between poor microbial water quality and the Briarcliffe Acres septic system was further investigated in 2009-2010. Horry County sponsored a water quality study to assess a possible change from septic to sewer. The study was a collaborative effort by Thomas & Hutton Engineering Co., CCU's EQL, and Virginia Polytechnic Institute. Sampling was performed at six sites including 3 discharge sites and 3 possible contributing sites during four dry and three wet weather events. Analyses included standard water quality parameters (conductivity, temperature, dissolved oxygen, pH, turbidity, biological oxygen demand, total suspended solids, and ammonia), enteric bacteria (fecal coliform bacteria and enterococcus), qPCR (general *Bacteriodes* and human *Bacteriodes*), and optical brighteners. Researchers found high correlation between FIB and human tracers of optical brighteners and human genes, especially during wet weather samples (Thomas & Hutton 2011). Researchers recommended a shift from septic to sewer in Briarcliffe Acres. Because the switch would be costly, additional recommendations were made in order to reduce microbial pollution to White Point Swash. The report advised proper maintenance of the septic system, such as regular pumping, and installation of water-saving devices, as well as homeowner education (Thomas & Hutton 2011). This

study served to reinforce the issue of septic systems contributing to poor water quality in Briarcliffe Acres and to coastal water bodies.

4.3.3. White Point and Briarcliffe Acres Swashes MST

Continuing poor water quality at beach monitoring site WAC-009A led to the site being placed on the federal 303(d) list of impaired water bodies (Libes 2016a). The site is at the confluence of White Point Swash and Briarcliffe Acres Swash. A MST study was performed to determine which swash was the primary contributing source of FIB (Libes 2016a). CCU's EQL was contracted by Horry County to perform the study. During the summer and fall of 2015, sampling was conducted during five dry and five wet weather events at the WAC-009A site, as well as two upstream sites in each swash. Samples were analyzed for enterococcus and salinity. Overall, wet weather samples had greater concentrations of FIB than dry weather samples (Libes 2016a). Researchers concluded that the Briarcliffe Acres Swash was a more important source of FIB than the White Point Swash by comparing concentrations between the swashes and WAC-009A (Libes 2016a). Additionally, high bacteria results after a King Tide indicated that FIB stored in sediments may have been resuspended and were a contributing source for that particular sampling date (Libes 2016a). While this study did not attempt to identify a specific source in regards to human vs. animal it did identify both drainage basins as contributing FIB sources to the beach monitoring site with the Briarcliffe Acres Swash being an important source of concern.

4.4. Murrells Inlet Studies

Since 2003, there have been five MST studies performed in Murrells Inlet estuary. The estuary system extends for 5.5 miles along the South Carolina coast with the northern part in Horry County and the southern part in Georgetown County. The waters of Murrells Inlet are classified by SC DHEC as suitable for shellfish harvesting (SFH) (SC DHEC 2014). Contaminated shellfish consumption is a pathway of concern, therefore water quality criteria must be met in these waters to keep shellfish beds open to harvest. The area around Murrells Inlet estuary is becoming increasingly more developed which can contribute to poor microbial water quality (Mallin et al. 2001). As such, MST has become an important tool for developing and meeting TMDLs in the Murrells Inlet watershed. A TMDL was developed in 2005 (SC DHEC 2005).

4.4.1. Multiple Antibiotic Resistance and Land Use/Land Cover

In 2003, a MST study was performed by a group of researchers from University of South Carolina who partnered with South Carolina Department of Health and Environmental Control (SC DHEC), South Carolina Department of Natural Resources (SCDNR), Georgetown County Water and Sewer District, and Grand Strand Water and Sewer Authority (Kelsey et al. 2003). Funded in part by a National Oceanic and Atmospheric Administration grant, the study aimed to examine the effect of land use on fecal coliform densities. The researchers used MAR analyses to determine whether fecal bacteria contamination originated from human or non-human sources. Using land use/ land cover data for the surrounding watershed and fecal bacteria analyses results of samples

collected throughout Murrells Inlet, researchers performed a regression to determine predictors of fecal bacteria contamination (Kelsey et al. 2003). MAR analyses were then used to infer host sources of FIB. Land-use variables retained in the regression model indicated that proximity to urbanized land use, septic systems, and sewage system lift stations could be predictors of fecal pollution (Kelsey et al. 2003). MAR analyses revealed that the majority of fecal pollution is non-human. Despite septic tanks being an apparent predictor for FIB contamination, MAR did not reveal the FIB to be human-sourced near areas with a high density of septic tanks (Kelsey et al. 2003). Detection of human-sourced fecal pollution was localized to a single site and possibly the result of a malfunctioning sewage collection system lift station (Kelsey et al. 2003). The researchers identified urban stormwater runoff to be the major source of fecal pollution based on the regression model predictors of rainfall and proximity to urban areas (Kelsey et al. 2003). The study reinforces the concept that increasing development leads to increasing fecal pollution in the coastal area.

4.4.2. Effectiveness of Stormwater BMPs

MST can also be used to determine the effectiveness of stormwater BMPs in improving water quality. CCU's EQL monitored water quality from 2005-2006 at two BMP demonstration sites in Murrells Inlet to estimate the impact of BMPs (Bennet 2007). The cumulative effects of multiple BMPs were evaluated at each of the demonstration sites. At the DNR Boat Ramp parking lot demonstration site a perforated pipe, pervious pavers, and created wetlands were evaluated. At the Morse Park Landing demonstration site being perforated pipes, a created wetlands, grasses swales, an infiltration trench, and pervious

pavers were installed and evaluated. Measurements of water quality parameters (fecal coliform, turbidity, dissolved oxygen, chlorophyll a, pH, and nutrients) were averaged to compare before and after construction of BMPs. Sampling was performed after construction during wet and dry weather events corresponding to six storm events at the outlet flows of the BMPs and flows into the inlet. Results demonstrated that fecal coliform concentrations increased with stormwater flows but concentrations in BMP ponds were significantly reduced with time after rain. BMPs appeared to improve water quality but additional monitoring was required to ensure their effectiveness (Bennet 2007). Additionally, two of the control sites in this study which were previously assumed to be relatively unimpaired were found to contravene SC DHEC standards for fecal coliform and dissolved oxygen (Bennet 2007). This study's recommendation for continued monitoring of both the demonstration sites and the control sites led to the establishment of the Murrells Inlet Volunteer Water Quality Monitoring (VWQM) Program in 2008.

4.4.3. MST of *E. coli* and Total Coliforms in Water and Sediments

Volunteer monitoring in Murrells Inlet has provided a wealth of water quality data. When volunteers identify poor microbial water quality, further investigation can be conducted to identify existing sources. After volunteers reported high FIB concentrations at two tributaries (BHR and HS) a MST study was performed by two CCU students with help from Georgetown County Stormwater (Anderson & Greoski 2010). The goal of the study was to identify sources of pollution in the two tributaries. Specifically, the students wanted to determine if resuspension of fecal bacteria from sediments on the bottom of the tributaries were acting as a source of FIB to the overlying waters. Samples of sediment and

overlying surface water were collected between April and November 2010 from the two volunteer monitoring sites as well as from upstream sites and one control site. A weight-of-evidence approach was used to determine possible sources of contamination utilizing *E. coli*, total coliform, conductivity, turbidity, and optical brighteners (Anderson & Greoski 2010). Upstream sites were identified in both tributaries as possible geographic sources of FIB. This study did not focus primarily on identifying a host-animal source but rather an upstream source. However, presence of optical brighteners indicated leaking septic tanks may also be a source of FIB (Anderson & Greoski 2010). The variability of FIB in sediments throughout the study demonstrates sediments are not a legacy source but could be a reservoir on short timescales (Anderson & Greoski 2010). The students suggested additional sampling to better understand the role of sediments in microbial water quality.

4.4.4. Upstream Sampling Program

An Upstream Sampling Program was conducted in 2013 by the Murrells Inlet VWQM Program. Funded by the Georgetown County Stormwater Department, the program aimed to gain a better understanding of bacteria sources present and reasons for wide spatial and temporal variability observed in the VWQM Program's FIB data (Weinreich 2013). The study also examined the effectiveness of corrective measures, such as stormwater ponds, used to reduce bacteria concentrations. From April to October 2013 the volunteer monitors collected samples at monitoring sites and at upstream sites in four subwatersheds (HS, BHR, BB, and HBSP). Samples were collected twice a month during regular sampling and after major rain events and analyzed for *E. coli* and total coliform using the VWQM Program's standard operation procedure that uses Microology's

Coliscan[®] Plus Easygel[®]. The study identified stormwater runoff transporting wildlife waste as the most significant source to fecal pollution (Weinreich 2013). The results indicated that rainfall events typically increase bacteria concentration while reducing salinity by dilution allowing for bacteria to persist in the estuary (Weinreich 2013). Additionally, testing performed above and below stormwater retention ponds revealed vegetated ponds with longer retention times proved to be more effective in removing pollutants, thus confirming results observed at BMP demonstration sites in Murrells Inlet (Bennet 2007). The study suggests these measures could be helpful in reducing fecal pollution to the Murrells Inlet estuary.

4.4.5. Horry County MST in Murrells Inlet

A MST study in the northern end of Murrells Inlet was conducted by CCU's EQL to determine whether humans were a major contributor to fecal pollution. In order to establish corrective measures to reduce FIB concentrations, Horry County Stormwater commissioned the study to determine the source of fecal bacteria (Trapp et al. 2014). Other potential sources included birds, dogs, and urbanized wildlife. Sampling was conducted in October 2012 and July 2013, providing two dry weather samples and three wet weather samples. Nine sample sites downstream of potential source regions were selected to provide data on the contributions of the major drainage pathways into the estuary. To determine the likely sources of pollution, a weight-of-evidence approach was used relying on genetic tracers (qPCR assays for GenBac, BacHum, BacCan, and GFC-Bird), culture-based enumeration of FIB (*Enterococcus*, *E. coli*, total coliform, and fecal coliform), quantification of chemical tracers (caffeine and optical brighteners), salinity, and turbidity.

A human source seemed unlikely as results for the chemical tracers and detection of BacHum were low and only detected during wet weather (Trapp et al. 2014). Evidence of bacteria from dogs (canines) was more prevalent and higher during wet weather suggesting an upland source transported by stormwater runoff (Trapp et al. 2014). Bird-sourced fecal contamination was widely distributed throughout the samples and seems to be the result of wading birds defecating directly into the waterbodies (Trapp et al. 2014). Overall, wet weather samples had greater concentrations of FIB than dry weather samples indicating stormwater runoff as a source (Trapp et al. 2014). However, at some sites a local dry weather source may be present as concentrations were consistently high despite weather conditions. The researchers suggested additional sampling was needed to confirm fecal pollution sources in the northern end of Murrells Inlet.

4.4.6. Georgetown County MST in Murrells Inlet

A similar MST study was conducted in the southern end of Murrells Inlet for Georgetown County Stormwater. CCU's EQL was tasked with determining whether human-sourced FIB was a significant source to three subwatersheds (HS, BHR, and BB) identified by the Murrells Inlet VWQM Program as having consistently elevated fecal bacteria concentrations (Libes et al. 2016). As Phase I of a two part study, sampling was conducted from August to October 2015 at the three volunteer monitoring sites and two upstream sites during two dry weather and three wet weather events. A weight-of-evidence approach was used with analyses performed for genetic tracers (GenBac and BacHum), FIB (fecal coliform, *E. coli*, and total coliform), caffeine, salinity, and turbidity. Both water and sediment samples were collected in order to determine the role of sediments as a

possible source. Genetic and chemical tracer results suggest there is no significant human source present (Libes et al. 2016). Concentrations of FIB were typically greater during wet weather indicating stormwater runoff as a likely source of pollution (Libes et al. 2016). Variability in sediment results over space and time demonstrate that sediments do not serve as a long term source, but may play some role on a shorter timescale through resuspension by scouring (Libes et al. 2016). This confirmed the results from Anderson & Greoski (2010). A visual investigation upstream of site HS should be conducted as it appears to be a significant source. Unlike the HS site, the upstream site sampled in the BHR subwatershed did not appear to be a significant source of FIB contamination. Sampling of other potential source sites in the subwatershed is recommended to identify a source. Results of Phase I show little evidence for a human source but support a significant influence of stormwater runoff on microbial water quality.

4.5. Waccamaw River Study

4.5.1. Identification and Mitigation of Non-point Sources of Fecal Bacteria

Elevated concentrations of FIB have also been identified in the Waccamaw River. Horry County and the City of Conway partnered with CCU's EQL to perform a Section 319 Program Project to investigate upstream sources of fecal bacteria and low oxygen at two 303(d) listed sites (Kingston Lake and Crabtree Canal) that are tributaries to the Waccamaw River (Libes 2003). Section 319 Programs are funded by the US EPA to help states identify and remediate non-point source pollution. CCU's EQL conducted a MST

study to determine whether stormwater runoff was a major source of pathogenic bacteria. Samples were collected in the tributaries and on the river to evaluate flows to the river. Sampling was conducted on alternating weeks and during storms from 1999-2001. To determine FIB concentrations, analyses of *Enterococcus* and fecal coliform were conducted. MAR analysis of *E. coli* was also performed to differentiate between potential sources. A major finding was a consistently large increase in FIB following storm events indicating stormwater runoff is a significant contributing source (Libes 2003). However, FIB concentrations were consistently elevated with respect to water quality criteria during dry and wet weather, confirming a chronic pollution problem as reflected by SC DHEC's 303(d) listing of both sites. An inventory approach to estimating production rates of potential fecal sources based on local animal populations and septic tanks identified native waterfowl as a significant contributor, whereas MAR analysis indicated that humans and domesticated animal fecal bacteria increased with rainfall, which could indicate leaking septic tanks as a source (Libes 2003). Subsequent to this research, a stormwater retention pond tied to Crabtree Canal was converted to a constructed wetland design. Sampling above and below the wetland was conducted from May to August 2002 after the retrofit was completed. FIB concentrations were analyzed and demonstrated that the wetland reduced contaminant bacteria levels to below state and federal water quality limits within a few days following rain events (Libes 2003). The effectiveness of the wetland in reducing contaminant bacteria could prove useful in improving the water quality of the Waccamaw River.

4.6. Surfside Beach Study

4.6.1. Investigation of Upstream Sources

In 2008, regulatory beach monitoring was used to identify 5 sites in Surfside as Waters of Concern. These sites were added to the 303(d) list of impaired waters in 2012. A VWQM Program was initiated in 2010 to evaluate two upstream waters, Myrtle Lake and Lake Dogwood, as potential sources of downstream impairments. VWQM identified Myrtle Lake, a tidal lake with a large year-round goose population, as a site with consistently elevated bacteria (*E. coli*) concentrations. In order to determine whether the bacteria is human or non-human sourced, a small MST study was conducted in the summer of 2015 with dry weather sampling coinciding with SC DHEC beach sampling. CCU's EQL collected samples for analysis for *E. coli*, *Enterococcus*, turbidity, and conductivity during wet and dry weather in the surf zone at the beach monitoring site (*Enterococcus*) and upstream at Myrtle Lake (*E. coli*). Analysis of caffeine and qPCR was to be performed on samples exceeding the water quality standard for *Enterococcus* of 104 MPN/100mL. These analyses and ongoing monitoring were to provide a weight-of-evidence approach for identifying the contributing source of fecal pollution at Myrtle Lake. Although funding was not available to complete this work qPCR assays for human-sourced bacteria were completed and did not detect significant levels despite high levels of *E. coli* being detected in all samples. In comparison, *Enterococcus* detection displayed a trend with weather: high levels were detected during all wet weather sampling and only once during dry weather. The *E. coli* results, measured by IDEXX's Colilert-18™, during dry weather, were substantially higher than those reported by volunteers using Micrology's Coliscan® Plus

Easygel[®] method. This led to an investigation as to the cause of this difference, which was reported in this thesis.

4.7. North Myrtle Beach Studies

4.7.1. 16th & 17th Avenue South MST

In addition to the study conducted in White Point Swash, two additional MST studies have been conducted in North Myrtle Beach. These were performed in response to occasional elevations of *Enterococcus* at WAC-007 during beach monitoring by SC DHEC and CCU's EQL (Libes 2016b). To assess sources, the City of North Myrtle Beach requested that CCU's EQL conduct a MST study. Preliminary sampling was conducted during the summer of 2015 in the catch basins at two locations along Ocean Boulevard near 16th and 17th Avenues South that are upstream of WAC-007A. Samples were collected four times during dry weather and twice following rain events to verify these sites were sufficiently contaminated to justify collection of samples for qPCR analysis. Both sites provided evidence of significant contamination during dry and wet weather. Sampling was re-initiated in the summer of 2016 with samples collected during severe wet weather events using Nalgene first flush samplers. *Enterococcus* concentrations were again elevated at both sites and qPCR analyses were performed for GenBac and BacHum on three samples from each site that had the highest levels of fecal bacteria contamination. The results are pending.

4.7.2. Hog Inlet MST

In August 2016, a MST study commenced in the Cherry Grove Marsh system. Horry County and the City of North Myrtle Beach requested a study be completed in conjunction with a watershed planning project being conducted as part of a US EPA 319 program project. Both Cherry Grove Marsh and the adjacent Hog Inlet are on the SC DHEC 303(d) list for shellfish impairments due to fecal bacteria contamination and TMDLs are to be completed sometime after 2022 (Burge & Libes 2016). The study's sampling locations were chosen to help identify sources associated with specific host animals and land uses. Specifically, the study aims to characterize stormwater runoff effects on water quality in Cherry Grove Marsh and determine whether the FIB is human-sourced in order to inform remediation efforts (Burge & Libes 2016). CCU's EQL has begun sampling at eight sites around the periphery of Cherry Grove Marsh including a reference site at nearby Dunn Sound adjacent to the undeveloped Waites Island. The study required three wet weather and three dry weather samples be collected via grab sample prior to the start of a channel dredging project. Analysis results will be evaluated by a weight-of-evidence approach including FIB (Enterococcus and fecal coliform), chemical tracers (caffeine, turbidity, and salinity), and genetic tracers (GenBac, BacHum, BacCan, and GFC Bird). Sampling has been completed, but analysis results are still pending.

4.8. Conclusions

The use of MST has a long history in the Grand Strand. Whether being used to differentiate between sources or to identify the impact of stormwater runoff, MST has

proven a useful tool in coastal northeastern South Carolina. Detection of human-sourced bacteria can indicate a possible health risk to humans. Significant detections of human-sourced fecal bacteria are limited to Briarcliffe Acres Swash and to a small portion of Withers Swash. These are areas of concern for local water resource managers. Stormwater runoff has been identified as a major contributor to fecal pollution throughout the Grand Strand since at least the 1970's and continues to be an issue with growing coastal populations. The role of sediments in microbial water quality still requires further research as results have shown high variability. One promising result from these studies has been the effectiveness of stormwater BMPs in reducing bacteria concentrations as seen in Murrells Inlet and on the Waccamaw River. MST can be a useful tool for developing mitigation efforts in the Grand Strand to maintain good microbial water quality in the coastal ocean.

4.9. References

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