SOURCES, DRIVERS, AND IMPACTS OF FECAL POLLUTION IN COASTAL TEXAS

A Dissertation

by

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This dissertation meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Fecal pollution in marine environments is a leading cause of water impairment in the United States. Millions of waterborne infections occur annually as a result of this pollution, contributing to a multibillion-dollar economic burden. The purpose of this dissertation was to investigate the sources, drivers, and impacts of fecal pollution in the northwestern Gulf of Mexico. This was accomplished by conducting targeted water quality studies in Corpus Christi Bay and Little Bay, Texas, followed by a comprehensive, long-term study of water quality across coastal Texas. Water quality was assessed through a combination of the following methods: quantification of enterococci, measurement of three host-associated fecal markers, characterization of antimicrobial resistance profiles in *Enterococcus* isolates, and analysis of the microbial community composition. Overall, in each of the three independent studies, enterococci frequently exceeded the United States Environmental Protection Agency (USEPA) beach action value. Rainfall often acted as a driver of enterococci, particularly in Corpus Christi Bay, where the mean enterococci concentration after rainfall was nearly 40 times higher than the USEPA beach action value. However, rainfall did not influence the levels of enterococci in Little Bay; rather, enterococci concentrations decreased along the estuarine ecocline, with the highest concentrations detected downstream of a local wastewater treatment plant (WWTP). Regardless of location (i.e., Corpus Christi Bay or Little Bay), enterococci were not correlated with human, canine, or gull fecal pollution markers. In Corpus Christi, elevated levels of the human fecal marker were detected throughout the study, although the concentration of this marker decreased after rainfall, likely due to a dilution effect from rainfall-induced freshwater inflows. In contrast, rainfall acted as a significant driver of human fecal waste in Little Bay. In addition to influencing

fecal bacteria levels, rainfall also acted as a pulse disturbance and altered the microbial diversity in both systems. In terms of long-term water quality trends, enterococci were significantly correlated with population size and sea level throughout coastal Texas. The strongest associations were observed in counties that are currently experiencing rapid population growth and are acutely vulnerable to future sea level rise. Taken together, these findings highlight the dynamic nature of water quality and demonstrate the need for independent and location-specific water quality analyses throughout coastal Texas. Given current and projected rates of population growth and sea level rise, efforts to mitigate and manage coastal water quality should be directed towards the most vulnerable locations, including Harris, Matagorda, and Brazoria Counties. Further, given the lack of correlation between enterococci and human fecal pollution, future water quality assessments should incorporate additional indicators, such as molecular markers of human-associated fecal pollution and assessments of microbial community composition.

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INTRODUCTION

Fecal pollution in the marine environment.

Fecal pollution in marine environments is a major cause of impaired water quality worldwide. The presence of fecal pollution in marine waters can adversely affect human health, as it frequently contains pathogenic microorganisms (e.g., *Giardia*, *Cryptosporidium*, *Campylobacter*, *Salmonella*, noroviruses, enteroviruses, adenoviruses, rotaviruses) that are known to cause gastrointestinal, skin, eye, and ear infections (Lipp et al. 2001a, Griffin et al. 2003, Soller et al. 2010a). Waterborne infections can occur after accidental contact with, or ingestion of, contaminated water in recreational settings (e.g., swimming, surfing, fishing) (Soller et al. 2010a), whereas foodborne illnesses can occur after handling or consuming contaminated aquatic food sources like shellfish (Potasman et al. 2002). The economic burden of these illnesses is significant; up to $3.7 billion is expended annually in the United States due to recreational waterborne illnesses (DeFlorio-Barker et al. 2018), and up to an additional $2.1 billion is expended due to foodborne illnesses from contaminated seafood (Batz et al. 2012).

In addition to harming human health and creating a vast economic burden, microorganisms present in fecal waste can also disrupt the health and stability of local ecosystems. For example, sewage has been identified as a stressor to coral reefs (Wear and Thurber 2015), and a sewage-associated strain of *Serratia marcescens* has been causally linked to a deadly white pox outbreak in Elkhorn corals (Patterson et al. 2002, Sutherland et al. 2010). Other animal species, such as waterfowl and gulls, also experience negative repercussions from sewage and fecal waste. Avian species living in wetlands that receive sewage waste may acquire enteropathogenic bacteria, leading to avian diseases (Benskin et al. 2009). Sewage in the effluent from wastewater treatment plants (WWTPs) contributes to eutrophication, which can further

facilitate the growth of anaerobic enteric pathogens, including *Clostridium botulinum*, the causative agent of deadly avian botulism (Anza et al. 2014).

Residual pharmaceutical compounds are another potentially harmful contaminant of fecal pollution. Examples of these compounds include, but are not limited to, antimicrobials, sterols, endocrine-disrupting compounds, and personal care products (Singh et al. 2010, Rodriguez-Mozaz et al. 2015). The presence of residual antimicrobial compounds can create a selective pressure in the environment and lead to the development of antimicrobial resistance (Amarasiri et al. 2020), and this resistance can be spread further throughout the microbial community via horizontal gene transfer (Karkman et al. 2018). Thus, the presence of antimicrobial compounds in the marine environment can lead to infections that are difficult to treat or even fatal (Leonard et al. 2018). The negative impacts of steroids and endocrine-disrupting compounds, such as estrogens, have also been well-documented in marine environments. These compounds are known to disturb the physiology and reproduction ability of many species, thereby endangering aquatic life (Reviewed by Adeel et al. 2017).

Sources of fecal pollution.

Environmental pollution is often classified based on its origin: point source and nonpoint source pollution. Point source pollution stems from a known point of entry, such as a WWTP outfall. Effluent from WWTPs is often discharged into rivers and nearby waterbodies once the sewage has been treated; however, WWTPs can malfunction and they are not effective in the complete removal of fecal-associated microorganisms, including pathogens (Wang et al. 2020). Point source pollution has historically received the most attention from government and regulatory agencies, partially due to the fact that identifiable sources of pollution are easier to

manage than pollution from unknown sources (League and Corwin 2006). However, nonpoint source pollution has been increasingly recognized as being equally threatening to environmental health and safety, promoting a shift in political and public interest (League and Corwin 2006). As the name suggests, nonpoint source pollution stems from a widespread or unknown point of origin, making it more difficult to manage. Examples of this pollution include stormwater runoff and leaks in sanitary sewer and septic systems that release raw sewage into the environment (Roehrdanz et al. 2017, Sowah et al. 2017).

Rainfall and the resultant stormwater runoff have been identified as significant nonpoint sources of fecal pollution in marine environments (Xue et al. 2018, Economy et al. 2019, Zeki et al. 2020). Hurricanes, tropical storms, and other rainfall events that produce large pulses of runoff have been associated with elevated levels of fecal pollution (Sinigalliano et al. 2007, Roca et al. 2019). For instance, the volume of freshwater that flooded into Galveston Bay, Texas due to Hurricane Harvey (August 2017) was estimated to be approximately three times the original volume of the bay (Du et al. 2019). This massive inflow of freshwater was accompanied by elevated levels of fecal pollution and pathogenic microbes that persisted for months after the hurricane (Yang et al. 2021). Smaller rainfall events have also been shown to result in sizeable volumes of stormwater runoff that result in high levels of fecal pollution (Parker et al. 2010).

During rainfall events, raw and untreated sewage can enter the environment through leaking and overwhelmed sewage systems. Combined sewer systems, in which there is no distinction between sewage and stormwater pipes, serve over 40 million people in the United States (https://www.epa.gov/npdes/combined-sewer-overflow-frequent-questions). Massive volumes of runoff can overwhelm these systems during large rainfall events, resulting in the deposition of combined runoff and raw sewage directly into the receiving environment. In

contrast, sanitary sewer systems are designed to be completely separate from stormwater systems. However, sanitary sewers can also be overwhelmed by excessive runoff, leading to sanitary sewer overflows. The USEPA estimates that up to 75,000 sanitary sewer overflows occur annually in the United States (https://www.epa.gov/npdes/sanitary-sewer-overflows-ssos). Proper maintenance and upkeep of these sewer systems, as well as on-site sewage facilities or septic systems, is imperative for preventing the release of untreated sewage into the environment.

Impact of climate change and urbanization on fecal pollution.

Climate change will undoubtedly influence the loading of fecal pollution in marine environments. The Fourth National Climate Assessment predicted many coastal regions in the United States will experience an increase in extreme storm events due to climate change (USGCRP 2017). Conversely, drought has also been projected throughout certain areas of the United States, particularly in the Southwest (USGCRP 2017). Prolonged drought followed by intense rainfall results in reduced soil-water absorption, which in turn leads to increased flooding and runoff (Rosenzweig et al. 2001). This pattern of intense rainfall following extended periods of dry weather can lead to elevated levels of fecal pollution (Economy et al. 2019).

Another way in which climate change influences coastal water quality is through sea level rise. The Fourth National Climate Assessment predicted that the Gulf Coast region will experience a significant increase in sea level within the next 80 years (USGCRP 2017). Rising sea levels result in rising groundwater, which can infiltrate underground septic systems (Elmir 2018), a known source of fecal pollution (Humphrey et al. 2018). To function properly, septic systems require unsaturated soil; however, rising groundwaters can submerge existing systems, rendering them nonfunctional (Elmir 2018). Even if the systems are not completely submerged,

rising groundwater can still encroach on the drainfields of the systems, limiting the area in which the wastewater would normally be filtered (Elmir 2018). In Florida, thousands of systems in low-lying coastal and inland areas are already compromised, and thousands more are expected to become compromised or fail within the next 20 years (Elmir 2018).

Nearly 40% of the world's population currently reside in or near coastal regions (i.e., within 100 km of the coast) (Muñoz-Sevilla et al. 2019). Many of these coastal regions are projected to experience rapid development, including population growth and urbanization, in the near future (Neumann et al. 2015). This rapid population growth will likely magnify the loading of fecal pollution in urbanized bays and watersheds, as population density has been strongly correlated with high levels of fecal waste (Walters et al. 2011). In addition to population growth, another consequence of coastal development is the destruction of riparian buffers, including the replacement of vegetation-covered surfaces with impervious and industrial surfaces (Chithra et al. 2015). The impervious surfaces prevent water from infiltrating the underlying earth and result in larger volumes of stormwater runoff and anthropogenic pollution (Shuster et al. 2005, Walters et al. 2011, Chithra et al. 2015).

History of fecal pollution monitoring in the United States.

Considerable progress has been made in recent decades in detecting, monitoring, and preventing the occurrence of fecal pollution in drinking and recreational water in the United States. The Federal Water Pollution Control Act (1948) was expanded and reauthorized in 1972 due to the public's collective concern about polluted waters (National Research Council 2004). The amendments included the Clean Water Act (1977) and the Water Quality Act (1987); together, these acts and amendments were subsequently referred to as "The Clean Water Act"

(National Research Council 2004). This regulation was further amended in 2000 with the Beaches Environmental Assessment and Coastal Health (BEACH) Act (USEPA 2000), which required the United States Environmental Protection Agency (USEPA) to monitor recreational water quality based on scientific criteria and communicate to the public when pollution levels are high (USEPA 2000, National Research Council 2004).

For over 150 years, water quality has been monitored through the detection and quantification of fecal indicator bacteria (FIB) (Holcomb and Stewart 2020). FIB are enteric bacteria that originate in the gut or intestines of humans and other animals; thus, their presence in the environment is indicative of fecal waste. Several indicators have been proposed and studied at length in recent decades, including total coliforms, fecal coliforms, *Escherichia coli*, and enterococci. Based on a study performed in Lake Michigan in the mid-twentieth century (Stevenson 1953), total coliforms were adopted as the primary indicator in recreational waters. Not long after, fecal coliforms were recommended in lieu of total coliforms, as this sub-group is capable of withstanding higher temperatures and are more likely to have originated from warm-blooded hosts (National Research Council 2004). However, subsequent studies demonstrated low correlations between total and fecal coliforms and the occurrence of water-acquired gastrointestinal diseases; stronger correlations were detected between disease and *E. coli* in freshwater settings (Dufour 1984) and enterococci in marine settings (Cabelli et al. 1983). As a result of these studies, *E. coli* was prioritized in freshwater monitoring and enterococci were prioritized in marine settings (National Research Council 2004).

Enterococci have long been recommended by the USEPA as a preferred fecal indicator in marine water (USEPA 1986), and they are currently Texas's standard for monitoring recreational water quality (Texas General Land Office 2015). Currently, the Texas Beach Program (managed

by the Texas General Land Office [TGLO]) monitors enterococci at 164 sites (representative of 66 beaches in nine coastal counties) weekly during peak season (i.e., March and May through September) and bi-weekly during nonpeak season. The USEPA mandates there should be no more than 104 colony forming units (CFU) of enterococci per 100 mL of recreational marine water (single-sample standard) or 35 CFU per 100 mL (geometric mean standard) (USEPA 2012b). In Texas, hundreds of sites are currently listed on the state's 303(d) list of impaired water quality due to high bacterial levels (TCEQ 2020a).

Enterococci as traditional indicators of fecal waste.

Enterococci are a group of enteric, Gram-positive bacteria belonging to the *Enterococcus* genus (Byappanahalli et al. 2012). They are often commensal organisms and nearly ubiquitous in the human gut, although they can also be clinically relevant opportunistic pathogens, particularly in nosocomial infections (Byappanahalli et al. 2012). Unlike *E. coli* and other fecal coliforms, enterococci are able to better withstand the higher salinity levels present in marine water, making them a superior indicator in marine settings (Byappanahalli et al. 2012). Although many studies have supported the use of enterococci as an indicator of human health risk (Wade et al. 2005, Colford Jr et al. 2012, Arnold et al. 2017), its reliability has been questioned in systems impacted by nonpoint source pollution (Fleisher et al. 2010, Sinigalliano et al. 2010, Saingam et al. 2020).

The original studies that led to the adoption of enterococci as an indicator of water quality were performed in locations impacted by a known point source of pollution (Cabelli et al. 1983, USEPA 1986). Numerous studies have since reported similar correlations between enterococci and the occurrence of disease (Wade et al. 2005, Colford Jr et al. 2012, Arnold et al. 2017), but this relationship remains complicated, particularly in regions impacted by nonpoint

source pollution. For instance, enterococci are not always indicative of waterborne illness (Fleisher et al. 2010, Sinigalliano et al. 2010) or pathogens (Saingam et al. 2020) in locations impacted by nonpoint source pollution. Furthermore, direct correlations have been observed between enterococci and human fecal waste (Steele et al. 2018), but inverse correlations (Boehm et al. 2009) and non-significant relationships (Sauer et al. 2011) have also been reported.

Additional challenges remain regarding the use of enterococci as a FIB, most notably the fact that these bacteria are not host-specific and many species are able to survive in the environment (reviewed in Devane et al. 2020). Although enterococci are nearly ubiquitous in the gut of humans (Boehm and Sassoubre 2014), they are also prevalent in the gut and feces of many other warm-blooded animals and poikilotherms (Frick et al. 2018). This lack of host-specificity prevents the reliable detection of feces and pathogens from human waste. These bacteria have also been detected in uncontaminated locations (Mote et al. 2012) with evidence of long-term survival in the environment (Badgley et al. 2010). Moreover, two commonly used and USEPA-approved methods for detecting enterococci [i.e., USEPA method 1600 (USEPA 2006) and the IDEXX Enterolert test (https://www.idexx.com/en/water/water-products-services/enterolert/)] are not capable of distinguishing between enteric and environmental enterococci; thus, tests for these FIB may be biased by the presence of naturalized bacteria (Ferguson et al. 2013).

Alternative methods for detecting fecal waste.

Several criteria have been proposed for appointing an "ideal" indicator of fecal waste. These suggested criteria include: 1) the indicator should be reflective of recent fecal waste and the pathogens associated with such waste, 2) the concentration of the indicator should increase as the concentration of the fecal material increases, 3) the concentration of the indicator should be

directly proportional to the health risk associated with the waste, 4) the indicator should not be pathogenic itself, 5) it should be rapidly and easily detected, 6) the survival of the indicator should be similar to the survival of fecal-associated pathogens in the environment, and 7) the indicator should be found in higher numbers than pathogens to ensure small volumes of fecal waste are still detected (Griffin et al. 2001, Byappanahalli et al. 2003). Currently, none of the traditionally used FIB, including enterococci, meet all of these recommended requirements. Thus, the search for an all-encompassing and "ideal" fecal indicator continues.

Alternative methods for detecting fecal waste that do not rely solely on the detection of traditional bacterial indicators have been proposed and well-studied. Namely, several methods have been explored that allow for tracking the original source of the fecal pollution, termed fecal or bacterial source tracking (BST). Examples of these alternative methods include detecting chemicals found in fecal material (Jardé et al. 2018, Devane et al. 2019) and testing for the presence of human viruses and bacteriophage from human feces (Stachler et al. 2018). Alternative methods also include the detection and characterization of changes in microbial community composition (Cao et al. 2013) as well as the detection and quantitation of host-specific genetic markers indicative of fecal waste (Boehm et al. 2013).

Specific chemicals that are excreted in fecal material can be used to detect waste and sewage in the environment. For instance, the presence of caffeine can be indicative of human waste, whereas steroid fingerprints (i.e., ratios of the relative distribution of different steroids) can be used to track fecal material from humans as well as other animals (Jeanneau et al. 2012, Devane et al. 2019). However, the decay rate of these chemicals does not necessarily reflect that of fecal pathogens; a recent microcosm study suggested the rate of decay for fecal stanols was less than half of the rate for human fecal-associated bacteria (Jeanneau et al. 2012). In this case,

the extended persistence of these chemicals may lead to unnecessary health advisories in impacted waters. Additionally, steroid fingerprints can vary greatly between individual humans; therefore, the combination of chemical fecal source tracking with microbiological techniques strengthens the sensitivity and reliability of this method (Jardé et al. 2018).

Several methods involving alternative microbiological indicators have been established in recent years. Viral fecal indicators, including human-associated viruses and bacteriophage (viruses that infect bacterial hosts), have shown promising results in fecal source tracking studies. These indicators may have an advantage over traditional bacterial indicators in the detection of viral pathogens, as FIB are not always correlated with viruses (Harwood et al. 2005). Reliable detection of these pathogens is essential, as viruses are the cause of a large portion of waterborne infections (Boehm and Sassoubre 2014). Studies have also shown that FIB and viruses do not decay at the same rate in the environment (Boehm et al. 2009) and are not removed from wastewater equally (Dias et al. 2018, Wu et al. 2020). While human viruses, including adenoviruses and polyomaviruses, are highly specific to human waste, they are present at an order of magnitude less than other bacterial indicators (Stachler et al. 2018); thus, quantification can be a challenge. Larger volumes of water are needed to concentrate viral particles and circumvent low viral recovery rates (Ahmed et al. 2015). These indicators may not be representative of small volumes of fecal pollution that pose a threat to human health (Staley et al. 2012). Recently, the crAssphage was discovered through metagenomic mining and found to be more abundant than other viral indicators (Dutilh et al. 2014); consequently, crAssphage has since been proposed as a promising indicator of human fecal pollution (Stachler and Bibby 2014).

Community-based approaches for detecting fecal contamination have shown high specificity (i.e., correctly identifying the source of the pollution) but low sensitivity (i.e., successfully detecting low levels of pollution) in bacterial source tracking studies (Cao et al. 2013). Examples of these methods include community fingerprinting (terminal restriction fragment length polymorphism or TRFLP), phylogenetic microarray (PhyloChip), and next-generation sequencing (16S rRNA gene sequencing) (Cao et al. 2013). Analyzing the entire microbial community may be useful in the difficult task of distinguishing differences between human feces, sewage, and septic waste, and this method shows promise in detecting waste from species with no known host-specific marker (Cao et al. 2013). For instance, community-based methods have been able to detect waste from deer and chickens, both of which have traditionally been difficult to distinguish from other animal waste (Boehm et al. 2013).

Host-associated molecular markers.

Host-associated molecular markers have been widely and successfully used in BST studies. A recent 27-lab effort (termed the Source Identification Protocol Project or SIPP) was undertaken in 2013 to compare 41 methods for identifying and tracking fecal waste (Boehm et al. 2013). The results of this collaborative study showed that the quantitative PCR (qPCR) based detection of select host-associated genetic markers was among the most reliable, specific, and sensitive of methods for detecting fecal waste (Boehm et al. 2013). A major advantage of this method is the ability to test for waste from different hosts (e.g., human, canine, avian, bovine) and studies have also supported the use of these host-associated markers in addition to, or in place of, traditional indicators (Flood et al. 2011, Mika et al. 2014). Importantly, knowing the

source or original host of the waste allows for the development of more focused and targeted water quality management and pollution mitigation policies.

Among the recommended molecular markers suggested by the SIPP is the human-associated HF183 marker (Boehm et al. 2013, Layton et al. 2013). This is a marker that belongs to the *Bacteroides* genus and has been used as an indicator of human fecal waste for over 20 years (Bernhard and Field 2000b, Seurinck et al. 2005). *Bacteroides* have exhibited a complex relationship with traditional indicators (Boehm et al. 2009, Sauer et al. 2011, Gordon et al. 2013, Steele et al. 2018) and have been detected even in the absence of enterococci (Ahmed et al. 2008). HF183 has been strongly correlated with genes belonging to several potential pathogens (i.e., *Clostridium perfringens*, total and enteropathogenic *E. coli*, *Campylobacter jejuni*) in beach waters, sand, and sediment (Zhang et al. 2016a). However, HF183 was also detected in the absence of pathogens (i.e., *Giardia*, *Cryptosporidium*, *Salmonella*, norovirus) in a separate study (González-Fernández et al. 2021). These conflicting results are likely due to the detection of different pathogens in each study. Additionally, the studies were conducted in vastly different environments (Montana vs. Costa Rica); the contrary results could also reflect the differential fate and transport of fecal pollution in the separate systems.

Unlike traditional fecal indicators, there are currently no regulatory limitations for an acceptable level of the human-associated HF183 marker in the environment. The USEPA has suggested an estimated illness rate of no more than 32-36 gastrointestinal illnesses per 1,000 people in recreational waters (USEPA 2012). Using this risk benchmark, Boehm et al. recently performed a series of quantitative risk assessments to identify a concentration of HF183 that corresponds to a human health risk (Boehm et al. 2015, Boehm et al. 2018, Boehm and Soller 2020). In the most recent assessment, they determined that the risk-based threshold for HF183 to

be 525 gene copies 100 mL^{-1}, with the assumption that the age of contamination is unknown (Boehm and Soller 2020). This threshold provides the means for a functional interpretation of BST studies, but it should be noted that this threshold is dynamic and may be influenced by several factors. For instance, fecal waste from other animals in combination with humans could have an additive effect on the health risk and therefore require the threshold to be lowered (Boehm and Soller 2020).

Although human waste is of primary concern since it is more likely to carry human pathogens, animal-associated fecal waste can harbor zoonotic pathogens capable of infecting humans (Soller et al. 2014). This waste is nearly omnipresent in the environment and can be attributed to native wildlife, domesticated pets, and agricultural runoff (Walters et al. 2011, Ahmed et al. 2019b). Waste from specific animals (e.g., cattle) may be more likely to cause human illness than waste from other animals (Soller et al. 2010b) and should be prioritized in water quality assessments. Furthermore, the recommended risk-based threshold for fecal waste is lowered drastically when human waste is detected in conjunction with animal waste (Boehm and Soller 2020). Identifying the specific animal source of fecal waste is imperative for mitigating impaired water quality and preventing future waste from being exposed to the environment. Fecal pollution from animals can often be mitigated through proper pet waste disposal and policies focused on animal feeding and loitering habits (Converse et al. 2012, Ervin et al. 2014), as well as regulation of agricultural discharge (USEPA 2001). However, accurately detecting animal-associated fecal waste remains challenging, as a number of species-specific molecular markers have shown cross-reactivity with other hosts (Boehm et al. 2013).

Purpose of this project.

Fecal pollution in marine environments poses a significant threat to human and environmental health. As coastal regions worldwide are experiencing rapid population growth and urbanization, impaired water quality continues to be a major concern. Nearly 100 million waterborne infections occur annually in the Unites States due to polluted waters (DeFlorio-Barker et al. 2018). Segments of the Texas coast in the northwestern Gulf of Mexico have a history of impaired water quality (TCEQ 2020a) and many regions along the coast are acutely vulnerable to climate change (i.e., sea level rise and increased storm frequency and severity) (Strauss et al. 2014). The combination of these factors will undoubtedly contribute to future impairment of coastal waters; yet, the synergistic impact of these factors has not been fully characterized. In consideration of climate change predictions and projections of continued coastal population growth, a comprehensive assessment of fecal pollution will be critical to the development of coastal water quality management priorities and policies. The overall purpose of this research was to investigate the source, fate, and impact of fecal pollution in coastal Texas. We conducted two independent bacterial source tracking studies to investigate fecal pollution in Corpus Christi and Rockport, Texas. In addition to these investigations, we completed a long-term study throughout the entire Texas coastline to identify trends in water quality in the previous decade. We hypothesized that water quality would be impacted by rainfall on a short-term scale, with rainfall acting as a significant driver of enterococci and changes to the microbial community. We also hypothesized that trends in long-term water quality would be influenced by population growth and sea level rise throughout coastal Texas.

CHAPTER I: RELATIONSHIP BETWEEN RAINFALL, FECAL POLLUTION, ANTIMICROBIAL RESISTANCE, AND MICROBIAL DIVERSITY IN AN URBANIZED SUBTROPICAL BAY

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Abstract.

Urbanized bays are vulnerable to fecal bacterial pollution and the extent of this pollution in marine recreational waters is commonly assessed by quantifying enterococci concentrations. Recent reports have questioned the utility of enterococci as an indicator of fecal bacterial pollution in subtropical bays impaired by nonpoint source pollution, and enterococci data alone cannot identify fecal bacterial sources (i.e., hosts). The purpose of this study was to assess relationships between rainfall, fecal bacterial pollution, antimicrobial resistance, and microbial diversity in an urbanized subtropical bay. Thus, a comprehensive bacterial source tracking (BST) study was conducted using a combination of traditional and modern BST methodologies. Findings show that rainfall was directly correlated with elevated enterococci concentrations, including the increased prevalence of *Enterococcus faecium*, although it was not correlated with an increase in the prevalence of antimicrobial-resistant strains. Rainfall was also correlated with decreased microbial diversity. By contrast, neither rainfall nor enterococci concentrations were directly correlated with the concentrations of three omnipresent host-associated fecal markers (i.e., human, canine, and gull). Notably, the human fecal marker (HF183) was inversely correlated with enterococci concentrations, signifying that traditional enterococci data alone is not an accurate proxy for human fecal waste in urbanized subtropical bays.

Introduction.

Fecal waste is a common pollutant in coastal marine environments (Griffin et al. 2001). Previous studies have shown that fecal pollution can increase the prevalence of human enteric pathogens (e.g., *Clostridium perfringens*, enteroviruses, hepatitis A viruses, Norwalk viruses, and adenoviruses) (Lipp et al. 2001a, Griffin et al. 2003) and fundamentally alter the microbial community composition in aquatic environments (Wu et al. 2010, Murphy et al. 2016). The occurrence of fecal pollution poses a serious threat to human and environmental health as the increased prevalence of these pathogens has long been correlated with an increased risk of illness in humans and marine life (Griffin et al. 2003, Wear and Thurber 2015).

Stormwater runoff associated with rainfall events is a significant source of fecal pollution (Parker et al. 2010). In urbanized bays, stormwater can aid in the land-to-sea transport of pollution stemming from leaks in aging sewage and septic infrastructure (Converse et al. 2011, Sauer et al. 2011). As a result of coastal development and the loss of vegetative landscape, even a small rainfall event can create a large pulse of stormwater (Nezlin et al. 2005), whereas extreme weather events such as tropical storms and hurricanes can cause catastrophic flooding and generate massive pulses of stormwater (Torres et al. 2015). Stormwater runoff can also carry excess nutrients, pesticides, residual antimicrobial compounds, petroleum-based pollutants, and heavy metals that may adversely affect coastal systems (USEPA 1999, Brown and Peake 2006, Sidhu et al. 2013a).

Antimicrobial resistance in aquatic environments has been linked to fecal waste, which is a source of antimicrobial-resistant bacteria and residual antimicrobial compounds (Baquero et al. 2008, Karkman et al. 2019). Runoff from landfills and sludge applied to land can also transport antimicrobial-resistant bacteria and residual antimicrobial compounds to coastal systems (Zhang

et al. 2016b). The presence of resistance genes and residual antimicrobial compounds has been shown to select for the evolution and survival of resistant bacteria (Baquero et al. 2008). In turn, the selection and spread of resistance can disturb the structure and function of aquatic microbial communities (Ding and He 2010), and recreational exposure to impacted aquatic environments can lead to harmful and difficult to treat bacterial infections (Leonard et al. 2018).

Fecal pollution is routinely monitored through the measurement of fecal indicator bacteria (FIB) including total coliforms, fecal coliforms, *Escherichia coli*, *C. perfringens,* and enterococci (Griffin et al. 2001, Boehm and Sassoubre 2014). In coastal marine environments, enterococci, a group of enteric Gram-positive bacteria, are commonly used as FIB (Byappanahalli et al. 2012). For example, in Texas (USA), the Texas Beach Watch Program routinely monitors only enterococci as an indicator of marine water quality (Texas General Land Office 2015). However, numerous studies have shown that enterococci are not an ideal indicator as they are often detectable in pristine environments and can persist and multiply long after initial introduction to the environment (Badgley et al. 2010, Mote et al. 2012). Studies have also shown that enterococci are not accurate predictors of human health risks in locations impaired by nonpoint sources of pollution (Fleisher et al. 2010, Sinigalliano et al. 2010). Additionally, enterococci are not a host-specific indicator and thus cannot be used to accurately determine the source of fecal pollution (Boehm et al. 2013).

The Source Identification Protocol Project (SIPP) was conducted to test the suitability of 41 indicators of fecal pollution (Boehm et al. 2013, Layton et al. 2013, Schriewer et al. 2013, Sinigalliano et al. 2013). The authors concluded that the PCR-based quantitation of host-associated molecular markers is the most accurate and informative method for assessing and tracking fecal pollution in the environment (Boehm et al. 2013, Layton et al. 2013, Schriewer et

al. 2013, Sinigalliano et al. 2013). The human-associated *Bacteroides* HF183 marker (Bernhard and Field 2000a, Seurinck et al. 2005), the gull-associated *Catellicoccus* LeeSeaGull marker (Lawson et al. 2006, Lu et al. 2008, Lee et al. 2012, Lee et al. 2013), and the canine-associated Bacteroidales DogBact marker (Dick et al. 2005, Sinigalliano et al. 2010) were among the most specific and sensitive and are, therefore, an improvement over traditional FIB for detecting fecal contamination.

To assess the relationship between rainfall, fecal pollution, antimicrobial resistance, and microbial diversity, we conducted a comprehensive bacterial source tracking (BST) study in an urbanized subtropical bay. The objectives of this study included 1) measuring enterococci concentrations, 2) assessing the antimicrobial resistance profiles among *Enterococcus faecium* isolates, 3) quantifying host-associated markers of human, canine, and gull fecal pollution, and 4) characterizing the microbial diversity. We hypothesized that rainfall would be correlated with increases in enterococci concentrations, the prevalence of antimicrobial resistance, and the abundance of host-associated fecal pollution markers.

Materials and Methods.

Sample collection and environmental parameters.

The city of Corpus Christi (total area 504 mi^2, population 326,554) is located on Corpus Christi Bay along the Texas segment of the northern Gulf of Mexico (nGOM), United States (United States Census Bureau 2019, Corpus Christi Population 2019). Elevated enterococci concentrations have been reported at recreational parks in Corpus Christi Bay (Mott et al. 2010, Center for Coastal Studies 2015, TCEQ 2018) with concentrations frequently exceeding the United States Environmental Protection Agency's (USEPA) single-sample standard of 104 colony forming units (CFU) of enterococci 100 mL^{-1} of water (USEPA 2012). Thus, six stations

in Corpus Christi Bay, including four stations in Cole Park (TX259473) and two stations in Ropes Park (TX821303), were selected as sampling sites for this study (Figure 1). Two-liter surface water samples were collected bi-weekly from May 2017 through January 2018 and additional water samples were collected after major rainfall events. Water samples were collected in autoclave-sterilized polypropylene bottles, stored on ice, and processed within four hours of collection. Sampling events that occurred within 24 hours of rainfall were considered wet-loading events and the remaining events were considered dry-loading.

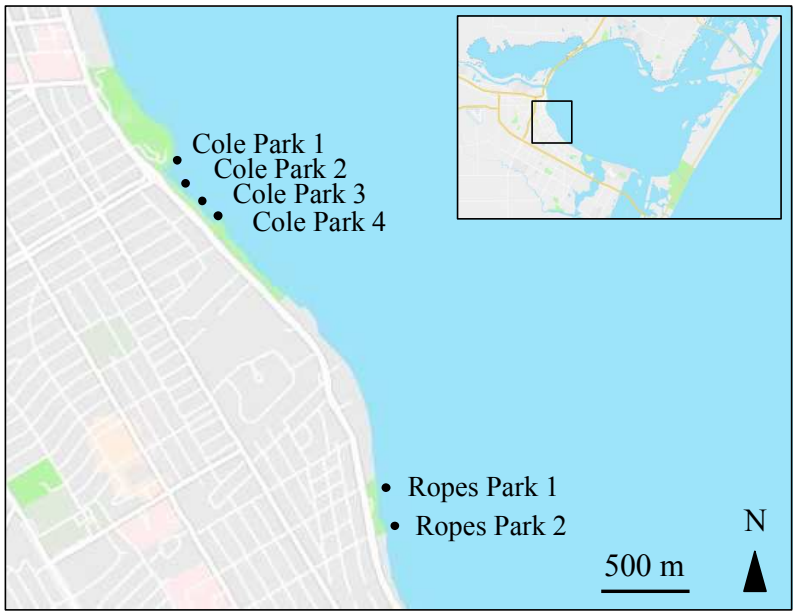

Figure 1. Map of the six sampling sites in Cole and Ropes Parks in Corpus Christi Bay, Texas, US. Ropes Park 1 and Cole Park 1, 3, and 4 were located within five meters of storm drain outfalls.

Physical parameters (water temperature [°C], salinity [ppt], dissolved oxygen [mg mL^{-1}], pH, and specific conductance [µS cm^{-1}]) were measured with a YSI 556 Multi Probe System (YSI Incorporated, Yellow Springs, Ohio, US). A Kestrel wind meter (Kestrel Instruments, Boothwyn, Pennsylvania, US) was used to measure wind speed (mph) and air temperature (°C), and a transparency tube (Ben Meadows, Janesville, Wisconsin, US) was used to measure water transparency (m). Precipitation data (inches day^{-1} and the number of days since precipitation)

was retrieved from the nearest weather station (KTXCORPU268). Additional environmental parameters (e.g., water color, odor, surface conditions, current weather conditions) were observed and recorded as categorical variables. Specifically, the color of the water was classified as colorless, green, tan, or brown, and the odor was classified as odorless, fishy, rotten egg, or sewage. Water surface conditions included calm water, ripples, waves, and white caps, whereas overall water conditions included clear water, debris-, foam-, or scum-laden water. Current weather conditions included rain, overcast, cloudy, or clear sky. Finally, the overall water clarity was classified as clear, cloudy, or turbid. These categorical variables were added in consideration of citizen scientist studies where these and similar variables were significant predictors of water quality (Thornhill et al. 2017, Zheng et al. 2017, Thatoe Nwe Win et al. 2019).

Quantifying enterococci.

Enterococci were quantified using the Enterolert test (IDEXX Laboratories, Westbrook, Maine, US) at the Corpus Christi Nueces County Public Health District Laboratory (CCNCPHDL), which is accredited by the National Environmental Laboratory Accreditation Program (NELAP). Duplicate 100 mL aliquots of each water sample were provided to CCNCPHDL in accordance with the Texas Beach Watch Program testing criteria for marine water (Texas General Land Office 2015). Enterococci concentrations were reported as the most probable number (MPN) of enterococci 100 mL^{-1}. Due to the lower limit of detection (< 10 MPN) in the Enterolert test, statistical correlation tests for censored data were computed on R (version 3.3.1) and RStudio (version 0.99.903) with the use of the NADA package (Lee 2020). Specifically, the cenken test was used to calculate Kendall's tau correlation coefficient of enterococci with continuous environmental variables and the cendiff test was used to test the

association of enterococci with categorical variables. Additionally, enterococci concentrations from wet-loading samples were compared to the concentrations from dry-loading samples using the cendiff test, which served as a censored t-test.

Identifying Enterococcus species.

Presumptive *Enterococcus* colonies were isolated via the USEPA 1600 membrane filtration method (USEPA 2006). Duplicate 100 mL water samples were filtered aseptically through 0.45 µm mixed cellulose ester (MCE) membrane filters (47 mm in diameter; Millipore Sigma, Bedford, Massachusetts, US), placed on sterile membrane-*Enterococcus* Indoxyl β-D-Glucoside (mEI) agar plates (Beckton, Dickinson and Company, Sparks, Maryland, US), and incubated at 41 °C for 24 hours. Up to four CFU with blue halos were randomly selected and streaked for isolation on brain heart infusion agar (BHIA) plates (Beckton, Dickinson and Company, Sparks, Maryland, US) at 37 °C for 24 hours. Isolated colonies (n = 782) were transferred to brain heart infusion broth (BHIB) (Beckton, Dickinson and Company, Sparks, Maryland, US) and grown at 37 °C with shaking (120 rpm) for 24 hours before being cryopreserved at -80 °C in 25 % final concentration glycerol solution. DNA was isolated via a five-minute boil lysis method (Englen and Kelley 2000). PCR primers targeting the species-associated alleles of the *sodA* gene (Table 1) were used to identify *E. faecalis* and *E. faecium* isolates (Jackson et al. 2004) (n = 202/782 randomly selected isolates) with the following cycling conditions (Table 2): five-minute hold at 95 °C, followed by 40 cycles of 96 °C for five seconds, 45 °C for five seconds, and 68 °C for 10 seconds, followed by a one-minute hold at 72 °C. A MANOVA test was used to determine if the presence of either species was correlated with wet- or dry-loading.

Table 1. Primer sequences and gene targets for PCR assays utilized in this experiment.

Target	Primer sequences	Reference
Enterococcus faecalis (*sodA* gene)	Forward: 5'-ACTTATGTGACTAACTTAACC-3' Reverse: 5'-TAATGGTGAATCTTGGTTTGG-3'	Jackson et al. 2004
Enterococcus faecium (*sodA* gene)	Forward: 5'-GAAAAAAACAATAGAAGAATTAT-3' Reverse: 5'-TGCTTTTTTGAATTCTTCTTTA-3'	Jackson et al. 2004
Human-associated *Bacteroides* HF183[1]	Forward primer: 5'-ATCATGAGTTCACATGTCCG-3' Reverse primer: 5'-TACCCCGCCTACTATCTAATG-3'	Bernhard and Field 200; Seurinck et al. 2005
Canine-associated Bacteroidales DogBact[2]	Forward primer: 5'-CGCTTGTATGTACCGGTACG-3' Reverse primer: 5'-CAATCGGAGTTCTTCGTG-3'	Sinigalliano et al. 2010; Dick et al. 2005
Gull-associated *Catellicoccus* LeeSeaGull[3]	Forward primer: 5'- AGGTGCTAATACCGCATAATACAGAG -3' Reverse primer: 5'- GCCGTTACCTCACCGTCTA -3'	Lee et al. 2012; Lee et al. 2013; Lu et al. 2008; Lawson et al. 2006
16S rRNA	Forward: 515fMod 5'-GTGYCAGCMGCCGCGGTAA-3' Reverse: 806rMod 5'-GGACTACNVGGGTWTCTAAT-3'	Walters et al. 2016

[1]Accession number AY618281.1
[2]Accession number AY695700.1
[3]Accession number NR_042357

Table 2. Cycling conditions for PCR assay of the *sodA* gene in *Enterococcus*.

Step	Temp (°C)	Time	Ramp rate	No. of cycles
Enzyme activation	95	5:00		1
Denaturation	96	0:05		40
Annealing	45	0:05	2 ºC s^{-1}	40
Extension	68	0:10		40
Final extension	72	1:00		1

Antimicrobial susceptibility.

Due to funding and resource limitations, 119 of the 133 *E. faecium* isolates were tested in triplicate for antimicrobial susceptibility by disk diffusion in accordance with the Clinical and Laboratory Standards Institute protocol (CLSI 2017). Four antimicrobial compounds belonging to different classes were tested: ampicillin (10 µg), vancomycin (30 µg), chloramphenicol (30 µg), and oxytetracycline (30 µg) (Becton Dickinson, Franklin Lakes, New Jersey, US). The concentration of each compound was chosen based on CLSI recommendations (CLSI 2017). Isolates were grown on BHIA at 37 °C overnight and diluted with a 0.45 % sterile saline solution to approximate a 0.5 McFarland standard. Sterile cotton swabs (Puritan, Guilford, Maine, US) were used to create bacterial lawns on Mueller-Hinton agar plates (Beckton, Dickinson and Company, Sparks, Maryland, US). The plates were divided into equal quadrants and the antimicrobial-infused disks were aseptically placed in the center of each quadrant. A blank disk containing only the saline solution vector was included as a negative control. The plates were incubated at 37 °C (16-20 hours for ampicillin, chloramphenicol, and oxytetracycline; 24 hours for vancomycin). The zones of inhibition were measured in triplicate for each disk and results were recorded and classified as susceptible, intermediate, or resistant. *E. faecalis* ATCC 29212 (susceptible to ampicillin, vancomycin, and high levels of gentamicin and streptomycin) was used as the control strain (Stephen et al. 2005). The results were analyzed with a Wilcoxon-Mann Whitney U test for ordinal categorical data to determine if antimicrobial resistance was correlated with wet- or dry-loading.

Membrane filtration and DNA extraction.

DNA was extracted from water samples (n = 120) for BST and community structure analyses. Duplicate 100 mL water samples were filtered through 0.45 µm MCE filters (47 mm diameter) (Millipore Sigma, Bedford, Massachusetts, US). Previous studies have shown that none of the standard pore sizes (i.e., 0.45, 0.22, and 0.1 µm) are capable of capturing the entire bacterial community (Wang et al. 2007); therefore, this pore size was used as it allowed the volume filtered (100 mL) to be standardized across all analyses (i.e, enterococci, BST, and microbial community analysis). This pore size also allowed for the capture of enterococci as well as *Bacteroides* and *Catellicoccus* species (USEPA 2006, Ahmed et al. 2009, Wu et al. 2017, Gyawali et al. 2020). The filters were placed into sterile 5 mL centrifuge tubes, parafilmed, and stored at -80 °C for no longer than 14 days. Filters were aseptically cut into small strips and DNA was extracted with a DNeasy PowerSoil Kit (Qiagen, Valencia, California, US) following manufacturer's instructions. The DNA was assessed for quality (260/280 nm) and quantity (ng μL^{-1}) using a BioSpectrometer (Eppendorf, Hamburg, Germany) and stored at -20 °C.

Bacterial source tracking.

To determine the most probable sources of fecal pollution, the DNA extracts were tested for the presence of molecular markers of bacterial strains associated with the feces of humans, canines, and gulls (Table 1). The quantity of each marker was assessed individually with a droplet digital PCR (ddPCR) assay following a previously established protocol (Cao et al. 2015). Briefly, a total of 54 water samples, collected from May to January (one sampling event per month), including three wet-loading and six dry-loading events, were tested in triplicate for the presence of the host-associated molecular markers. Each ddPCR reaction consisted of the

following components: 10 µL EvaGreen Supermix (1 X final concentration), 1 µL forward primer (0.25 µM), 1 µL reverse primer (0.25 µM), 3 µL of DNA, and 5 µL PCR-grade, nuclease-free water. Each ddPCR run included positive controls for each marker in the form of synthetic gBlock gene fragments (Integrated DNA Technologies, Skokie, Illinois, US) (accession numbers shown in Table 1) and no template controls (NTCs) that contained sterile, nuclease-free water in place of DNA. Droplets were generated with a QX200 Droplet Generator (BioRad Laboratories, Hercules, California, US) and markers were amplified with a Bio-Rad C1000 Touch thermal cycler (BioRad Laboratories, Hercules, California, US) with the following conditions (Table 3): five-minute hold at 95 °C, followed by 40 cycles of 95 °C for 30 seconds and 59 °C for one minute, followed by a five-minute hold at 4 °C and a final five-minute hold at 90 °C. Following amplification, droplets were transferred to a QX200 Droplet Reader (BioRad Laboratories, Hercules, California, US) and the markers were quantified with QuantaSoft software following the manufacturer's instructions. The peaks from the NTCs were used to manually set the thresholds for positive droplets and wells with fewer than 10,000 reported droplets were removed from the analysis. QuantaSoft reported the number of gene copies 1 μL^{-1} of each ddPCR reaction, which was converted to gene copies 100 mL^{-1} water with Eq. (1):

$$X_{total} = \frac{(X_n)(20\ \mu L)}{(3\ \mu L)(50\ \mu L)} \qquad (1)$$

The final concentration of each host-associated marker (gene copies 100 mL^{-1}) is denoted by X_{total} and X_n is the marker concentration of the PCR reaction (gene copies 1 μL^{-1}); 20 µL is the total PCR reaction volume, 3 µL is the volume of DNA in each PCR reaction, and 50 µL is the total DNA volume from each extraction.

Table 3. Cycling conditions for ddPCR assay of the host-associated molecular markers.

Step	Temp (°C)	Time	Ramp rate	No. of cycles
Enzyme activation	95	5:00		1
Denaturation	95	0:30		40
Annealing/extension	59	1:00	2 ºC s^{-1}	40
Signal stabilization	4	5:00		1
	90	5:00		1

The concentrations of host-associated markers (gene copies 100 mL^{-1} water) and enterococci (MPN 100^{-1} mL water) were log-transformed, and the normality of the transformed data was confirmed through visualization of quantile-quantile plots using the qqnorm function in RStudio. Correlations between the concentrations were tested by calculating Pearson's correlation coefficient and Welch's t-test determined if marker concentrations varied significantly between wet- and dry-loading events. Multiple linear regressions, computed using the lm function in RStudio, were used to test if environmental parameters were correlated with marker concentrations. For this purpose, a full model was generated for each of the host-associated markers to include all of the environmental variables measured during sampling. The full models were then assessed for collinearity with the VIF function from the car package (Fox and Weisberg 2019) and variables with a $GVIF^{(1/(2*DF))} > 2.0$ were removed. Model averaging was then performed using the dredge function from the MuMIn package (Bartoń 2019). The exhaustive lists of potential models were ranked based on AICc values and models with an AICc 2.0 greater than the top model were eliminated. If multiple models remained, they were subsequently ranked based on adjusted R^2 values. The final models were tested for normality using the qqplot function.

Microbial community analysis.

The remaining DNA extracts were used to characterize the microbial communities of 36 wet-loading and 36 dry-loading samples. For this purpose, the V4 region of the 16S rRNA gene was amplified (Walters et al. 2016) (primers shown in Table 1) with a HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, California, US) with the following cycling conditions (Table 4): three-minute hold at 94 °C, followed by 30 cycles of 94 °C for 30 seconds, 53 °C for 40 seconds, and 72 °C for one minute, followed by a five-minute hold at 72 °C. Successful amplification was confirmed through visualization of PCR products in 2 % agarose gel. The samples were then pooled together and purified with Ampure XP beads (Beckman Coulter, Indianapolis, Indiana, US) to create the sequencing library. DNA sequencing was performed on an Illumina HiSeq platform with 250 bp PE chemistry at Molecular Research LP (Shallowater, Texas, US). Barcodes were removed from the raw sequence reads with QIIME (version 1.9), and QIIME2 (version 2018.11) was used for subsequent steps of the analysis (Caporaso et al. 2010, Bolyen et al. 2019). Briefly, the DADA2 plugin (Callahan et al. 2016) was used to demultiplex, denoise, and dereplicate the reads, trim them to a length of 241 bp, and remove chimeric sequences. Next, the sequences were aligned with MAFFT (Katoh et al. 2002) and filtered with default settings. The SILVA 132 release database (Quast et al. 2012) was imported and trained based on the target sequences of the 515 forward and 806 reverse modified primers (Walters et al. 2016) using a Naïve Bayes classifier in QIIME2 (fit-classifier-naïve-bayes command). Taxonomy was assigned based on the database and features mapped to chloroplast or mitochondrial DNA were removed with the taxa filter-table command. A phylogenetic tree was inferred using FastTree (Price et al. 2009) and rooted with default QIIME2 settings for use in the downstream diversity analyses. Alpha diversity (Shannon's diversity index and Faith's phylogenetic diversity [FPD])

and beta diversity (unweighted UniFrac distance values) were calculated using the 'q2-diversity' plugin. The Kruskal-Wallis pairwise H test was used to test for correlation between the wet-loading and dry-loading alpha diversity values. Unweighted UniFrac distance values were used to generate a PCoA to visualize differences in beta diversity, using Phyloseq (version 1.30.0) (McMurdie and Holmes 2013). Community structure differences between wet- and dry-loading samples were analyzed using PERMANOVA. The differential abundance of microorganisms detected in wet- versus dry-loading communities was determined via linear discriminant analysis (LDA) effect size (LEfSe) (Segata et al. 2011). This test was computed using the LEfSe tool on the Galaxy server (https://huttenhower.sph.harvard.edu/galaxy/). Genera that comprised greater than 0.1% of the communities' relative abundance were analyzed using default settings with the significance threshold set to $p < 0.01$.

Table 4. Cycling conditions for PCR assay of the V4 region of the 16S rRNA gene for community analysis.

Step	Temp (°C)	Time	Ramp rate	No. of cycles
Enzyme activation	94	3:00		1
Denaturation	94	0:30		30
Annealing	53	0:40	3.35 ºC s^{-1}	30
Extension	72	1:00		30
Final extension	72	5:00		1

Results.

Sample collection and environmental parameters.

Water sampling (n = 120 total samples) occurred at four sites in Cole Park (TX259473) and two sites Ropes Park (TX821303) (Figure 1). Four sites were located within five meters of stormwater outfalls. A total of 20 sampling events occurred, including six wet-loading events (n = 6 events, n = 6 sites, n = 36 samples) and 14 dry-loading events (n = 14 events, n = 6 sites, n =

84 samples). All wet-loading events were preceded by a minimum of seven days without rainfall. Throughout the study period, water temperature varied seasonally from 10.7 to 32.5 °C, salinity ranged from 28.49 to 72.7 ppt, dissolved oxygen ranged from 4.04 to 11.31 mg mL^{-1}, and pH ranged from 7.35 to 8.27. Water transparency varied from 0.063 to greater than 1.21 m, specific conductance varied from 4,4094 to 5,7627 μS cm^{-1}, and the number of days since precipitation occurred ranged from 0 to 33 days.

Quantifying enterococci.

Enterococci were enumerated from a total of 120 water samples in duplicate via Enterolert testing. Concentrations ranged from fewer than 10 to greater than 24,196 MPN 100 mL^{-1} (lower and upper limits of detection, respectively; Table 5). Samples collected after rainfall had significantly higher levels of enterococci (Figure 2; cendiff test, $p < 0.01$). Based on the cenken test, enterococci concentrations were correlated with the following environmental parameters: the number of days since precipitation occurred (-0.440, $p < 0.001$), water transparency (-0.324, $p < 0.001$), pH (-0.145, $p < 0.05$), specific conductance (-0.142, $p < 0.05$), and salinity (-0.116, $p < 0.1$). Additionally, based on the cendiff test, higher enterococci concentrations were correlated with water that was observed to be turbid rather than cloudy or clear ($p < 0.001$).

Table 5. Minimum, maximum, mean, and median abundances of enterococci (MPN 100 mL^{-1}) and the three host-associated molecular markers (gene copies 100 mL^{-1}) in wet- and dry-loading samples.

Event type	Bacterial target	Minimum	Maximum	Mean	Median
Wet-loading	Enterococci*	15.00	24,196.00	4,062.25	1,080.25
	Human marker**	66.67	3,294.43	385.13	190.00
	Canine marker	17.77	3,681.10	531.62	303.33
	Gull marker**	75.57	3,003.33	465.09	214.73
Dry-loading	Enterococci*	< 10.00	437.50	36.63	10.00
	Human marker**	95.53	3,680.00	771.32	358.33
	Canine marker	59.97	2,036.67	574.32	437.23
	Gull marker**	97.77	3,133.33	801.37	461.65

*Enterococci increased significantly after wet-loading ($p < 0.05$).
**The human and gull-associated markers decreased significantly after wet-loading ($p < 0.05$).

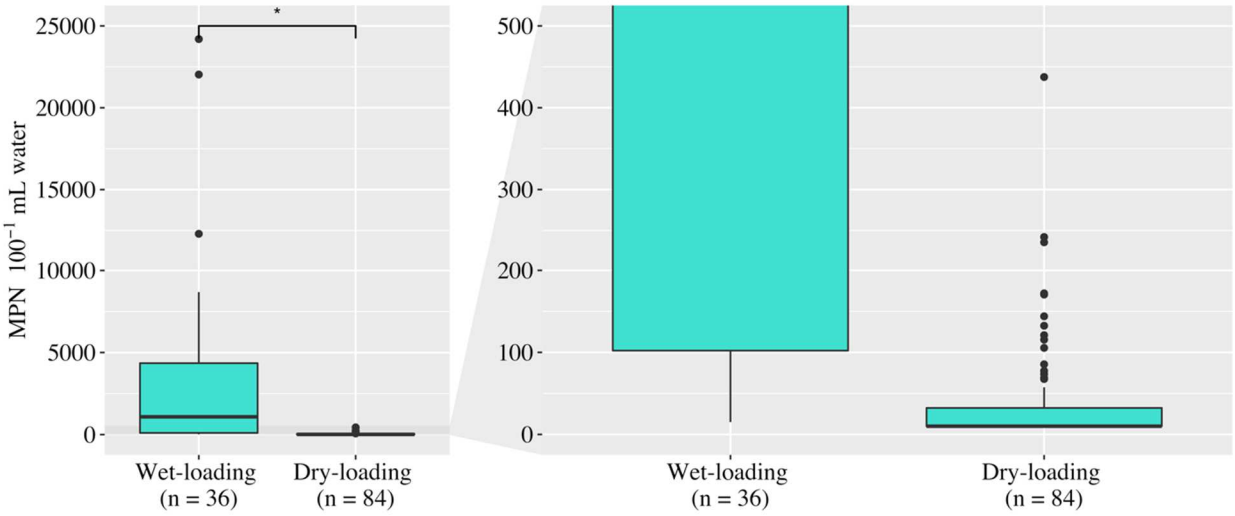

Figure 2. Enterococci concentrations after wet-loading and dry-loading sampling events. The panel on the right shows an expanded view of the dry-loading samples. *Significantly higher concentrations of enterococci were detected after wet-loading events (cendiff test; $p < 0.01$).

Identifying Enterococcus species.

A total of 782 presumptive *Enterococcus* isolates were preserved and 202 isolates were randomly selected for the duplex PCR assay to determine species; 109 isolates were from wet-loading events and 93 isolates were from dry-loading events. In total, 133 were identified as *E. faecium*, 32 were identified as *E. faecalis*, and the remaining 37 could not be identified with this assay. The MANOVA test indicated that the majority of the identifiable *Enterococcus* isolates collected after rainfall were *E. faecium* (87 out of 109; $p < 0.01$), while the occurrence of *E. faecalis* did not change significantly after rainfall. For this reason, the *E. faecium* isolates were chosen for antimicrobial susceptibility testing.

Antimicrobial susceptibility.

All *E. faecium* isolates tested (n = 119; 80 wet-loading; 39 dry-loading) were susceptible to ampicillin and chloramphenicol, and only one wet-loading isolate was intermediately resistant to vancomycin. Eighteen of the wet-loading isolates and four of the dry-loading isolates were intermediately resistant to oxytetracycline, and one dry-loading isolate was fully resistant to this compound. There was no significant increase in the occurrence of antimicrobial resistance after wet-loading in *E. faecium* isolates.

Membrane filtration and DNA extraction.

Successful DNA isolation from all 120 water samples was confirmed with a BioSpectrometer (Eppendorf, Hamburg, Germany), which was used to determine the quality (260/280 nm) and quantity (ng μL^{-1}) of DNA. However, due to funding limitations, all samples were not analyzed. DNA isolated from 54 samples, including one sampling event per month

from May 2017 to January 2018 (n = three wet-loading events with 18 samples and six dry-loading events with 36 samples), was analyzed for the presence of host-associated markers while DNA isolated from 72 samples (n = six wet-loading events with 36 samples and six dry-loading events with 36 samples) was analyzed by 16S rRNA gene sequencing.

Bacterial source tracking.

The three host-associated markers tested in this study (human, canine, and gull) were detected in all of the water samples (n = 54). On average, the gull marker was the most abundant, followed by the human and canine markers (Table 5). The t-tests showed that the human and gull markers were significantly ($p < 0.05$) higher after dry-loading events (Figure 3). By contrast, the canine markers were not correlated with wet- or dry-loading (Figure 3). All host-associated markers were positively correlated with each other, although only the human marker was negatively correlated with enterococci (Pearson's correlation coefficient: -0.313; $p < 0.05$; Table 6). The best fit linear model for human markers included pH and water color (adjusted R^2: 0.158; $p < 0.05$). The canine model included pH, water surface conditions, water odor, and the current weather conditions (adjusted R^2: 0.403; $p < 0.01$). The gull model included water pH, water odor, and current weather conditions (adjusted R^2: 0.292; $p < 0.01$). Despite the negative correlation between enterococci (MPN) and human markers, MPN was not a significant predictor in any of the models.

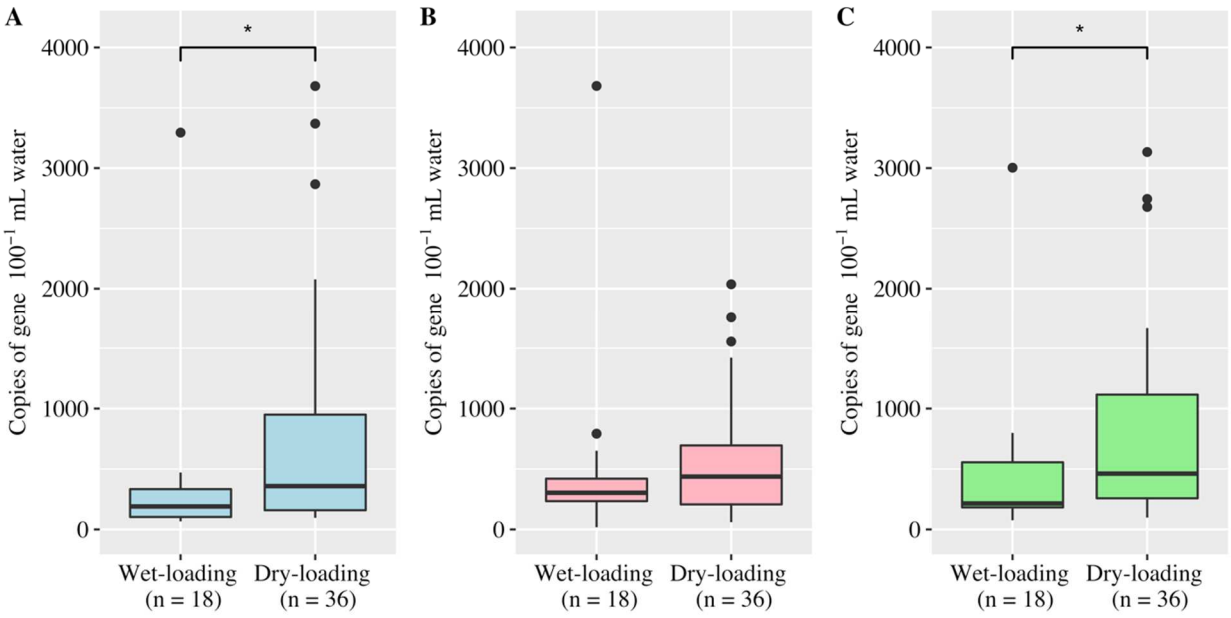

Figure 3. Concentrations of the host-associated markers from wet-loading and dry-loading samples. **A** = human marker (blue), **B** = canine marker (pink), **C** = gull marker (green). *The human and gull markers were significantly higher after dry-loading compared to wet-loading events (Welch's t-test; $p < 0.05$).

Table 6. Pearson's correlation coefficients for enterococci (MPN 100 mL^{-1}) and the abundance of host-associated molecular markers (gene copies 100 mL^{-1}). *$p < 0.05$; **$p < 0.01$. Dashed lines indicate non-significant correlations.

	Gull	**Canine**	**Human**
Enterococci	---	---	-0.313*
Human	0.608**	0.522**	
Canine	0.768**		

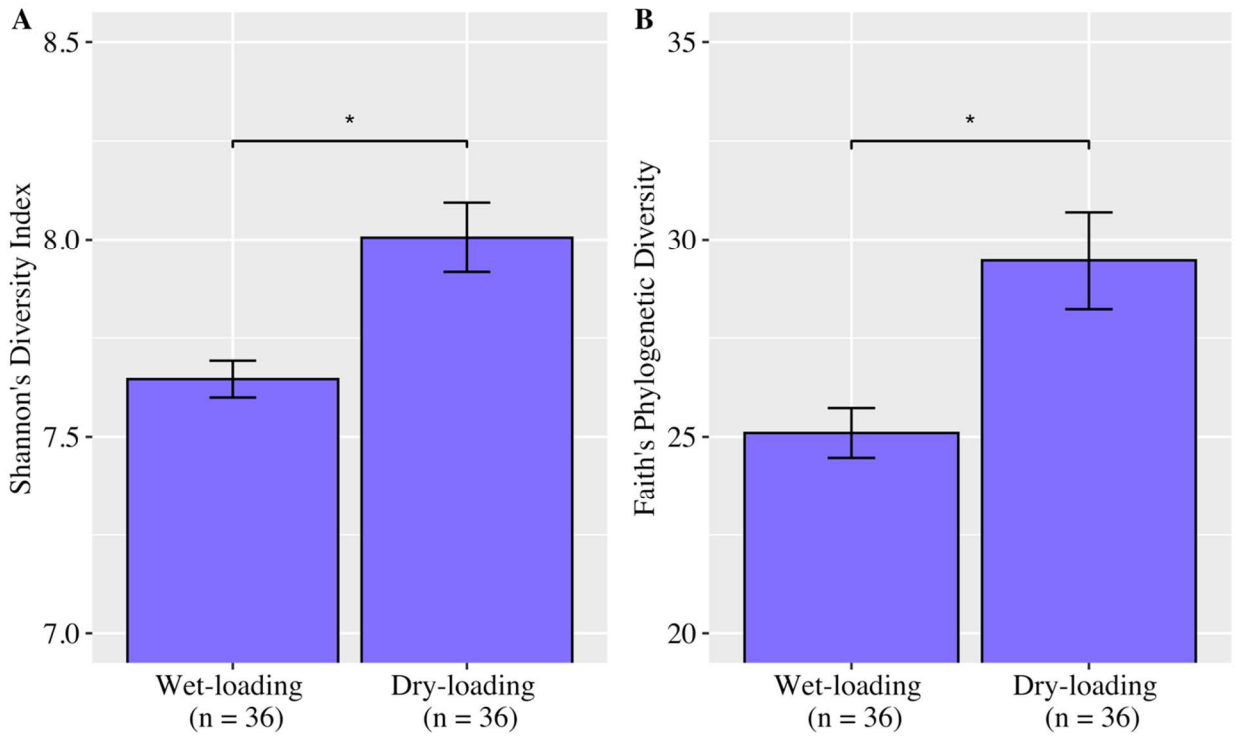

Figure 4. Alpha diversity metrics of wet-loading and dry-loading samples. **A** = Shannon's diversity index, **B** = Faith's phylogenetic diversity. Error bars represent the standard error of the mean. *The dry-loading samples were significantly more diverse than the wet-loading samples (Kruskal-Wallis pairwise H test; $p < 0.01$).

Microbial community analysis.

The Kruskal-Wallis pairwise H tests comparing Shannon's diversity index and Faith's phylogenetic diversity (FPD) between the samples collected after wet- and dry-loading showed that alpha diversity was significantly lower in the wet-loading samples ($p < 0.01$; Figure 4). Similarly, the principal coordinate analysis (PCoA) of unweighted UniFrac distance values (accounting for 18.7% of variance) and subsequent pairwise PERMANOVA test confirmed that wet- and dry-loading communities were significantly different ($p < 0.01$; Figure 5). The linear discriminant analysis (LDA) effect size (LEfSe) further investigated how the wet- and dry-loading communities differed. Results showed that several genera (e.g., Cyanobacteria,

Plantomycetes, Rhodobacterales, Deltaproteobacteria, and *Burkholderiaceae*) were enriched in the dry-loading communities ($p < 0.01$; Figure 6). By contrast, fewer genera (e.g., Actinobacteria, Pseudomonales, and *Alcanivoracaceae*) were enriched in the wet-loading communities.

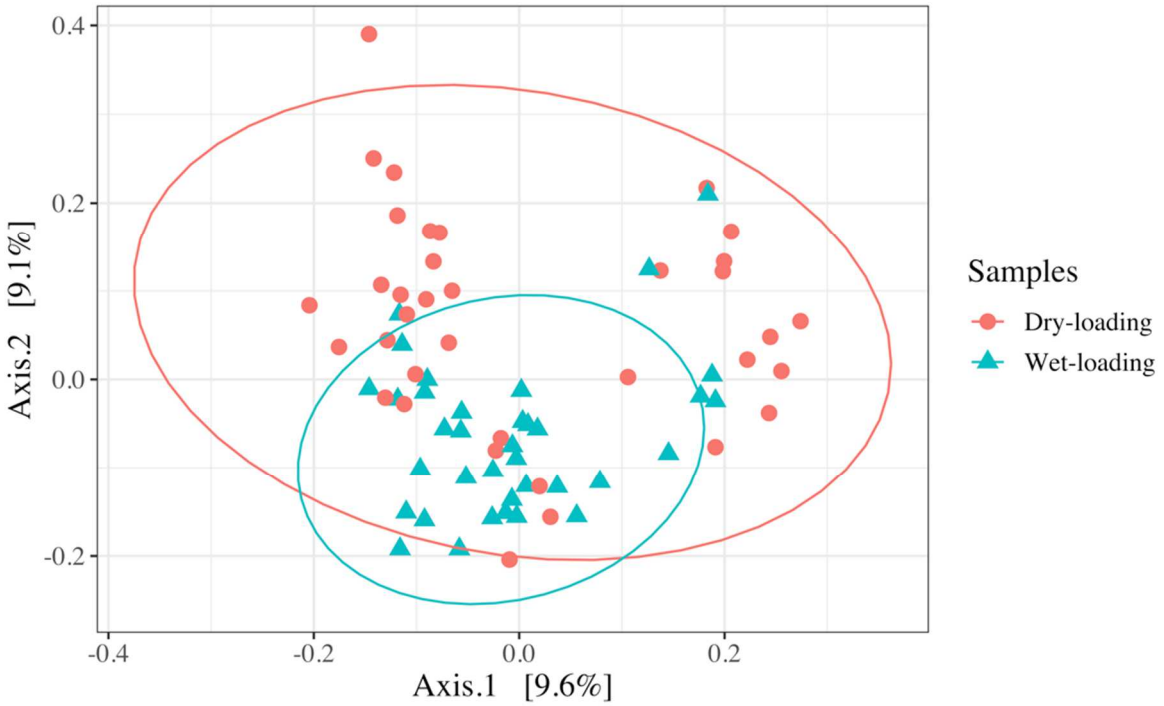

Figure 5. Principal coordinate analysis (PCoA) based on unweighted UniFrac distance values showing beta diversity of dry-loading (red circles) and wet-loading (blue triangles) samples. The ellipses represent 95 % confidence intervals for each sampling type. The community composition of wet-loading and dry-loading samples were significantly different (pairwise PERMANOVA; $p < 0.01$).

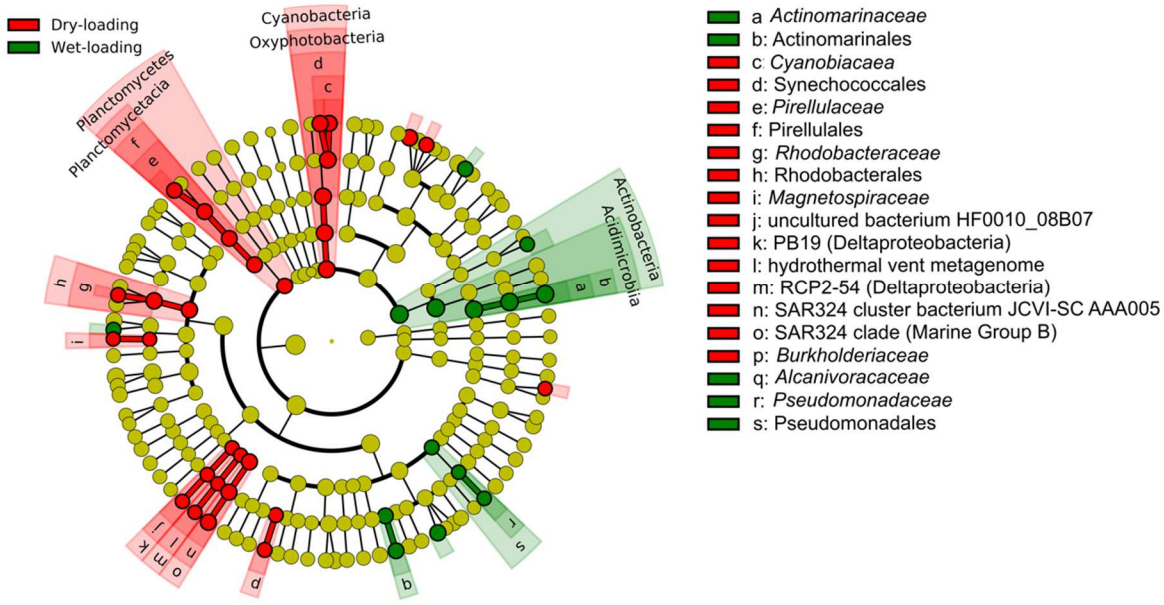

Figure 6. Linear discriminant analysis effect size (LEfSe) cladogram depicting the taxa enriched in the dry-loading (shown in red) and wet-loading (shown in green) samples ($p < 0.01$).

Discussion.

Fecal waste is a widespread pollutant in urbanized bays (Boehm et al. 2003, Noble et al. 2006, Sauer et al. 2011). The extent of fecal pollution in marine surface waters is commonly assessed by measuring FIB including total coliforms, fecal coliforms, *E. coli*, *C. perfringens*, and enterococci (Griffin et al. 2001, Boehm and Sassoubre 2014). However, the detection and quantitation of FIB cannot identify the sources of fecal pollution (Boehm and Sassoubre 2014). Furthermore, traditional FIB indicators cannot explain the larger consequences of fecal pollution such as increases in antimicrobial resistance or decreases in microbial diversity. To address these limitations, this study used a combination of traditional and modern BST methodologies to assess relationships between rainfall, fecal pollution, antimicrobial resistance, and microbial diversity in an urbanized subtropical bay.

Enterococci are routinely monitored throughout coastal Texas as a proxy for water quality by the Texas Beach Watch Program; therefore, this study assessed enterococci in lieu of other commonly used FIB. Enterococci concentrations frequently exceeded the USEPA recreational water quality criterion (104 CFU 100 mL^{-1} single sample standard) by 40-fold and the highest concentrations were measured after rainfall events. In agreement, elevated enterococci concentrations were also inversely correlated with water transparency, pH, and salinity. Conversely, in the absence of rainfall, enterococci concentrations were consistently lower than the USEPA criterion. Rainfall and stormwater runoff have been shown to increase coastal bacteria concentrations and also decrease water transparency, pH, and salinity (Ackerman and Weisberg 2003). The sources of bacteria, however, were unknown as enterococci are not host-specific (Boehm and Sassoubre 2014), have been detected in uncontaminated locations (Mote et al. 2012), and have been shown to persist for long periods without a host (Badgley et al. 2010).

To determine potential sources of fecal pollution, an increasing number of BST studies have included the detection of host-associated molecular markers (Boehm et al. 2013, Mika et al. 2014, Ahmed et al. 2019a). In this study, human-, canine-, and gull-associated markers were ubiquitous and occasionally abundant, but none were positively correlated with enterococci concentrations; by contrast, the human-associated HF183 marker was inversely correlated with enterococci. This lack of direct correlation indicates that enterococci were not an accurate proxy for human-, canine-, or gull-associated fecal pollution in this subtropical bay. Previous studies have reported a direct correlation between enterococci and human-associated *Bacteroides* markers (Steele et al. 2018), an inverse correlation (Boehm et al. 2009), and no correlation (Sauer et al. 2011). Previous studies have also questioned the use of enterococci as the primary

FIB due to the lack of a dose-response relationship between gastroenteritis and enterococci concentrations among recreational bathers in subtropical waters without known point sources of sewage (Fleisher et al. 2010, Sinigalliano et al. 2010).

Rainfall and its accompanying stormwater runoff have been identified as important drivers of fecal pollution and waterborne illness (Parker et al. 2010, Converse et al. 2011, Olds et al. 2018). However, in this study, rainfall and the human-associated marker were inversely related. This finding suggests that stormwater may not be the major source of human fecal waste in this system; rather, the abundance of the human-associated marker during dry-loading may indicate that human fecal waste was omnipresent but diluted during storm events. Abundant fecal waste during dry-loading could result from aging sanitary sewage infrastructure as seepage from leaks and breaks would be a continuous source of pollution (Converse et al. 2011, Sauer et al. 2011). Alternatively, the low concentrations of fecal pollution after wet-loading could also be attributed to reduced dilution that would normally accompany freshwater inputs (Senhorst and Zwolsman 2005, Cann et al. 2013). These results suggest that recreational bathers are at higher risk of exposure to human fecal waste during dry weather conditions. Thus, rainfall-related beach advisories, triggered by increased enterococci concentrations, may not protect recreational bathers from human fecal pollution.

Canines and gulls have been determined to be major sources of fecal pollution in marine surface waters (Riedel et al. 2015). As the presence of animal-associated fecal contamination can indicate the presence of zoonotic pathogens, including these fecal markers in comprehensive BST studies is essential. Rainfall was not correlated with gull fecal pollution in this study; rather, the gull marker was present constantly and was, on average, the most abundant of the markers. While the concentration of the gull marker diminished after rainfall, the canine marker

concentration remained relatively constant. The steady canine pollution could have stemmed from domestic and feral canine waste in coastal neighborhoods and parks that washed into stormwater drains after rainfall. Canine fecal pollution can be mitigated with proper education regarding pet waste disposal (Ervin et al. 2014) and gull abatement programs focused on preventing the loitering of gulls have resulted in significant decreases in gull fecal pollution (Converse et al. 2012).

In addition to fecal waste, rainfall-associated runoff can transport antimicrobial-resistant microbes and residual antimicrobial compounds to surrounding environments (Ahmed et al. 2018), and studies have shown that runoff can increase the occurrence of antimicrobial resistance (Di Cesare et al. 2017, Carney et al. 2019). In this study, rainfall was correlated with an increase in the occurrence of *E. faecium,* but we did not observe a significant increase in antimicrobial resistance in this species. The lack of resistance suggests the enterococci did not stem from a human source and were instead environmental. A previous study detected higher levels of antimicrobial resistance genes in the environment after rainfall; however, this coincided with higher concentrations of both enterococci and the HF183 human-associated fecal marker (Ahmed et al. 2018). A shotgun metagenomic approach could have revealed rainfall-correlated differences in the abundance of antimicrobial resistance genes but that analysis was outside the scope of this study.

Pulses of stormwater have been shown to alter the composition of bacterial (Chaudhary et al. 2018) and viral communities (Williamson et al. 2014). Here, through a combination of four community-based metagenomic analyses (i.e., Shannon's diversity, FPD, PCoA, and LEfSe), we have shown that 1) wet- and dry-loading communities were significantly different, 2) wet-loading communities were less diverse, and 3) specific genera were enriched (i.e., differentially

more abundant) in each community. Higher microbial diversity is thought to promote community stability and functional resilience after disturbance (Girvan et al. 2005). More frequent or more extreme rainfall events could, therefore, impair the stability and resilience of this system. This finding is of particular importance, seeing that the Fourth National Climate Assessment predicted the nGOM will experience increased extreme rainfall events including tropical depressions, tropical storms, and hurricanes (UGSCRP 2017). Urban systems are especially vulnerable to extreme rainfall, which can overwhelm and damage urban infrastructure. Ultimately, per the recommendations of the climate assessment, forward-looking infrastructure designs and practices will be necessary to safeguard urban systems from ongoing and future climate risks.

In conclusion, this study demonstrated that rainfall was correlated with increased enterococci concentrations and decreased microbial diversity. Rainfall was not, however, correlated with the detection of human, canine, or gull fecal pollution. Likewise, rainfall was not correlated with an increase in *E. faecium* antimicrobial resistance. The inverse relationship between enterococci and the HF183 human marker suggests that the presence of elevated enterococci is not an accurate indication of human fecal contamination. Moreover, in agreement with similar BST studies (Flood et al. 2011, Mika et al. 2014), these findings suggest that the utility of fecal pollution indicators may be location-specific and thus, independent analyses should be conducted in areas of concern to identify suitable indicators.

Acknowledgements.

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CHAPTER II: WATER QUALITY DYNAMICS IN RESPONSE TO RAINFALL ALONG AN ESTUARINE ECOCLINE

This chapter under review as Powers NC, Pinnell LJ, Wallgren HR, Marbach S, and Turner JW. (*in review*). Water quality dynamics in response to rainfall along an estuarine ecocline. *ES&T Water*.

Abstract.

Stormwater runoff is an important nonpoint source of fecal pollution in coastal environments. In anticipation of future coastal population growth and urbanization coupled with forecasts of altered weather patterns, it is increasingly important to understand how rainfall impacts coastal water quality. The purpose of this study was to determine how rainfall impacts water quality along an estuarine ecocline in the northwestern Gulf of Mexico (GOM). Decreasing levels of enterococci were detected along the ecocline from land to sea, with the highest concentrations measured immediately downstream of a wastewater treatment plant (WWTP). Human-, canine-, and gull-associated markers were detected consistently throughout the study, but unlike enterococci, they did not experience a significant trend along the ecocline. Surprisingly, enterococci were not correlated with any of the three host-associated markers, indicating that enterococci were not an accurate proxy for the presence of fecal pollution. Rainfall was followed by higher concentrations of the human-specific fecal marker (HF183) and resulted in an altered bacterial community structure. These results question the utility of enterococci as a fecal pollution indicator in the northwestern GOM. Further, these results suggest that increased rainfall frequency or intensity could negatively impact water quality in this region. Taken together, these results clearly demonstrate the need for a data-based approach to water quality management that incorporates modern water quality indicators such as host-associated markers and microbial community composition.

Introduction.

As the bridge between the terrestrial and marine world, coastal regions are acutely vulnerable to anthropogenic impacts, including the loading of harmful pollutants. In particular, coastal regions are vulnerable to fecal pollution introduced by wastewater treatment plant (WWTP) effluent, seepage from impaired sanitary sewers and septic systems, leachate from landfills, and stormwater runoff (Shuval 2003, Sauer et al. 2011, Zhang et al. 2016b, Sowah et al. 2017). Fecal pollution can harbor pathogenic microorganisms (e.g., *Bacteroides*, *Enterobacteriaceae*, Norwalk viruses, adenoviruses, *Cryptosporidium*, and *Giardia*) (Lipp et al. 2001a, Griffin et al. 2003, Soller et al. 2010b) and residual pharmaceutical compounds (e.g., antimicrobials, sterols, estrogens, and endocrine disruptors) (Haack et al. 2009, Singh et al. 2010) that may pose direct or indirect threats to human and environmental health (Bedoux et al. 2012, Lee et al. 2014, Wear and Thurber 2015). Furthermore, fecal pollution can lead to severe economic burdens; recreational contact with polluted waters leads to nearly 100 million infections annually in the United States, costing an estimated $2.2-$3.7 billion (DeFlorio-Barker et al. 2018).

Fecal pollution in recreational waters can be detected by quantifying traditional fecal indicator bacteria (FIB) (Griffin et al. 2001). In marine surface waters, enterococci can tolerate higher levels of salinity compared to other FIB like fecal coliforms and *E. coli* (Byappanahalli et al. 2012), and numerous studies (Wade et al. 2010, Colford Jr et al. 2012, Boehm and Sassoubre 2014) have reported correlations between the presence of enterococci in recreational marine waters and the occurrence of human illnesses (further reviewed by Boehm and Sassoubre 2014). However, conflicting research has shown that enterococci are not ideal FIB as no strains are human-specific and some species do not originate from fecal waste (Byappanahalli et al. 2012).

Further, in subtropical waters without a known point source of pollution, some studies have reported no correlation between enterococci concentrations and the occurrence of human illnesses (Colford Jr et al. 2007, Fleisher et al. 2010, Sinigalliano et al. 2010).

Citing the inadequacies of traditional FIB, alternative indicators have been proposed (Boehm et al. 2013). Namely, the collaborative Source Identification Protocol Project tested several methods for detecting fecal waste and concluded that the quantitative polymerase chain reaction (qPCR) detection of host-associated molecular markers provides the most reliable data (Boehm et al. 2013, Layton et al. 2013, Schriewer et al. 2013, Sinigalliano et al. 2013), and subsequent studies have supported the use of host-associated markers over traditional FIB (Mayer et al. 2016, Ahmed et al. 2019a, Powers et al. 2020). This methods advancement is especially advantageous when attempting to remediate impaired water quality. For instance, fecal pollution stemming from human and cattle waste may pose a greater threat to human health than other animal sources (e.g., gulls, canines, and chickens), and should be prioritized accordingly (Soller et al. 2010a). The detection of human-associated markers may trigger remediation and management practices addressing leaking sewage systems (Dickerson Jr et al. 2007), whereas the detection of other animal markers may trigger remediation and management practices addressing wildlife and pet waste management (Converse et al. 2012, Ervin et al. 2014).

Previous studies have correlated elevated FIB concentrations with urbanization and heavy rainfall in coastal watersheds (Shehand et al. 2005, Sauer et al. 2011, Zhang et al. 2013). Similarly, a strong El Niño associated with increased storm frequency and severity was correlated with elevated FIB concentrations (Lipp et al. 2001b), and massive freshwater inflows associated with Hurricane Irma were correlated with a long-term increase in FIB concentrations

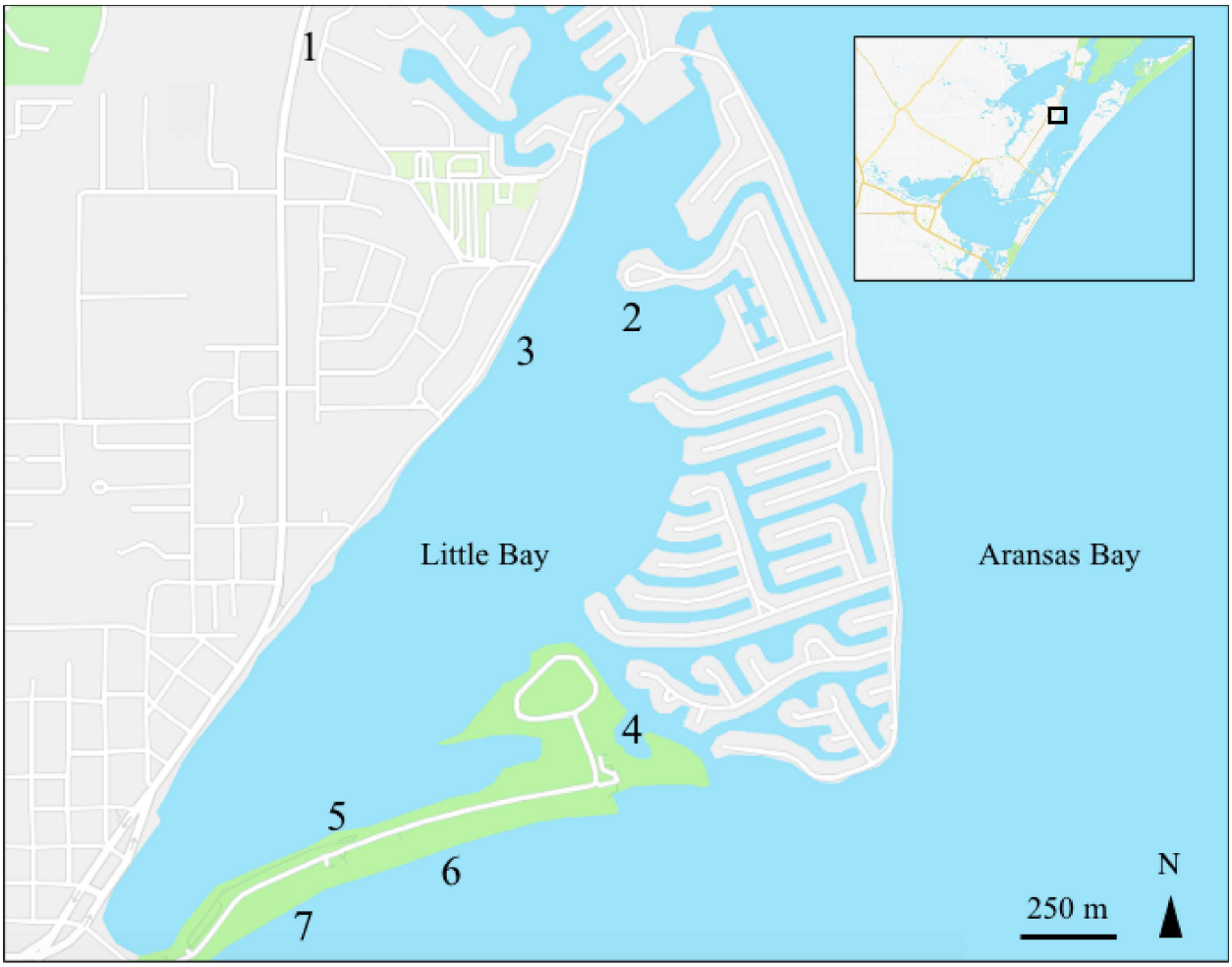
(Roca et al. 2019). Indeed, simple models based on antecedent rainfall can accurately predict FIB concentrations (Coulliette et al. 2009, Stidson et al. 2012). Thus, climate zones predicted to experience an increase in the frequency or severity of rainfall events could experience a corresponding decrease in water quality (Bernstein et al. 2008).

This study assessed how rainfall impacts water quality in a model urbanized nested bay located in the northwestern Gulf of Mexico (GOM). The subtropical, semi-arid region of the northwestern GOM is a natural laboratory for assessing how rainfall and urbanization impact water quality in coastal systems. The region has experienced significant population growth and land-use change while water temperatures and extreme wet-weather inflows are increasing (IPCC 2013). The region is also home to eight barrier islands (encompassing over 215 miles, or 80% of the Texas coastline, from Galveston Island to Brazos Island, Texas, United States) that create nearly three dozen nested and semi-closed bays (e.g., Aransas Bay, Copano Bay, Mesquite Bay, Oso Bay, Redfish Bay) that experience limited flushing and high-residence times (Morton 1994, Solis and Powell 1999, Dunton and Wilson 2010). The objectives of this study included 1) characterizing the bacterial community diversity, 2) measuring enterococci concentrations, and 3) quantifying three host-associated molecular markers (i.e., human, canine, and gull). Sampling locations crossed the estuarine ecocline: 1) a freshwater catchment for WWTP effluent that feeds into 2) a shallow urbanized bay that is nested inside 3) a larger bay that exchanges water with the GOM. We hypothesized that rainfall would be directly correlated with fecal pollution and inversely correlated with microbial diversity. We also hypothesized that the significance of these correlations would diminish along the estuarine ecocline.

Materials and Methods.

Water sampling.

Water samples were collected from the seven sampling sites shown in Figure 7. Site 1 was located in Tule Creek, a small freshwater tributary and riparian buffer downstream of a local WWTP that empties into Little Bay. Sites 2-5 were located inside Little Bay, a shallow nested bay that receives stormwater from the city of Rockport, Texas, United States. Little Bay is separated from the GOM by the much larger Aransas Bay, where sites 6 and 7 were located. Both bays experience limited flushing and high-residence times (Solis and Powell 1999, Dunton and Wilson 2010). Duplicate 1 L water samples were collected at each site on a near-monthly basis from May to November of 2018. Samples were classified as 'dry-loading' if collection was not preceded by rainfall (n = 35) and 'wet-loading' if they were collected within 24 hours of rainfall (n = 14). Water samples were collected in autoclave-sterilized polypropylene bottles at a depth of approximately 0.5 m, immediately stored on ice, and processed within four hours of collection. A YSI 556 Multiprobe System (YSI Incorporated, Yellow Springs, Ohio, US) was used to measure water temperature (°C), dissolved oxygen (mg mL^{-1}), salinity (ppt), and pH. Wind speed (mph) and air temperature (°C) were measured with a Kestrel wind meter (Kestrel Instruments, Boothwyn, Pennsylvania, US), and a transparency tube (Ben Meadows, Janesville, Wisconsin, US) was used to measure the water transparency (m). Precipitation records (inches day^{-1} and the number of days preceding rainfall) were obtained from the Bayshore weather station (ID KTXROCKP71) in Rockport, Texas.



Site No.	Site Name	Latitude (°N)	Longitude (°W)
1	Tule Creek	28.050315	-97.042832
2	Key Allegro Pace Dock	28.043616	-97.032572
3	Tule Creek Outfall	28.043116	-97.035877
4	Rockport Saltwater Pool	28.032564	-97.033296
5	Little Bay Ski Basin	28.030435	-97.039682
6	Rockport Beach Park North	29.030580	-97.034047
7	Rockport Beach Park South	28.026540	-97.045300

Figure 7. Map of the seven sampling sites in this study, including site names and coordinates. Site 1 (Tule Creek) was a freshwater site, located downstream of a wastewater treatment plant. Sites 2-5 were located within Little Bay and Sites 6 and 7 were located in Aransas Bay.

DNA isolation.

The 1 L water samples were mixed via shaking and duplicate 100 mL subsamples were aseptically vacuum-filtered through 0.22 µm polyethersulfone (PES) membrane filters (Millipore Sigma, Bedford, Massachusetts, US). Each filter was placed in a sterile 5 mL centrifuge tube and stored at -80 ºC for up to seven days. Genomic DNA was isolated from the filters with a DNeasy Powersoil Kit (Qiagen, Valencia, California, US) according to manufacturer's instructions. The isolated DNA was assessed for quality (260/280 nm) and quantity (ng μL^{-1}) with a BioSpectrometer (Eppendorf, Hamburg, Germany) and stored at -20 ºC.

Analyzing bacterial community diversity.

To assess the overall microbial community composition, DNA from duplicate water samples (n = 42) was sent to Molecular Research LP (Shallowater, Texas, US) for 16S rRNA gene amplification and sequencing. Due to funding limitations, Site 7 was excluded from this analysis. Briefly, the V4 region of the 16S rRNA gene was amplified using a HotStart Plus Master Mix Kit (Qiagen, Valencia, California, US) with 515 forward and 806 reverse primers (Walters et al. 2016) using the following cycling conditions (ramp rate of 3.35 ºC s^{-1}): enzyme activation for 3 minutes at 94 ºC, followed by 30 cycles of denaturation for 30 seconds at 94 ºC, annealing for 40 seconds at 53 ºC, and extension for 1 minute at 72 ºC, followed by a 5-minute hold at 72 ºC. Amplification was confirmed via gel electrophoresis, and the PCR products were pooled and purified with Ampure XP beads (Beckman Coulter, Indianapolis, Indiana, US). The resulting DNA was sequenced on an Illumina HiSeq platform with paired-end chemistry (2x250 bp). The barcode regions were removed from the raw reads with QIIME version 1.9 (Caporaso et al. 2010). QIIME2 version 2018.6 (Bolyen et al. 2019) was used for subsequent steps, unless

otherwise noted. Briefly, the DADA2 plugin (Callahan et al. 2016) was used to denoise and demultiplex the reads, which were trimmed to a length of 242 bp. Chimeric sequences were removed, and the paired-end reads were merged. The sequences were aligned with MAFFT (Katoh et al. 2002) in the QIIME2 program and filtered with default settings. One wet-loading sample replicate was removed from the analysis due to low-quality reads (n = 83; 60 dry- and 23 wet-loading duplicates) before taxonomy was assigned. A Naïve Bayes classifier was trained on the SILVA release 132 database (Quast et al. 2012) with the 515f/806r 16S rRNA gene primers (Walters et al. 2016), and sequences that mapped to chloroplasts or mitochondria were removed. Finally, a phylogenetic tree was produced with the q2-phylogeny plugin with default settings to calculate diversity metrics.

Faith's phylogenetic diversity and Shannon's diversity index were assigned as alpha diversity metrics, and differences in alpha diversity between each location (i.e., Tule Creek, Little Bay, and Aransas Bay) as well as differences between dry- and wet-loading samples were tested with a Kruskal-Wallis test. Beta diversity was visualized with a principal coordinate analysis (PCoA) using weighted UniFrac values in the phyloseq package (version 1.30.0) in R (McMurdie and Holmes 2013). Differences in the community composition between the three locations and between dry- and wet-loading samples were determined with the PERMANOVA test in QIIME2. Correlations between beta diversity and salinity and water temperature were computed using the metadata distance-matrix and diversity Mantel commands in QIIME2 (version 2019.4). To further visualize differences in bacterial communities under the different rainfall conditions, a linear discriminant analysis (LDA) effect size (LEfSe) was computed using the Galaxy server, with default settings and a significance cutoff of 0.01 (https://huttenhower.sph.harvard.edu/galaxy/) (Segata et al. 2011). As the overall communities in

Little Bay and Aransas Bay were not significantly different, an additional LEfSe was not computed based on location.

Measuring enterococci concentrations.

Additional 100 mL subsamples from each 1 L water sample were allocated for Enterolert testing (IDEXX Laboratories, Westbrook, Maine, US), which was completed by the Corpus Christi Nueces County Public Health District Laboratory (CCNCPHDL) following the procedures developed by the Texas Beach Watch Program (Texas General Land Office 2015). The enterococci concentrations of the duplicate water samples were reported as the mean most probable number (MPN) of enterococci 100 mL^{-1} water. Due to the lower limit of detection of the Enterolert test (< 10 MPN 100 mL^{-1}), the data were analyzed with censored statistical tests from the NADA package (Lee 2020) in R (version 3.6.1) and RStudio (version 1.2.1335). Specifically, the cendiff test was used to compare concentrations of enterococci in each location along the ecocline: Tule Creek (n = 6), Little Bay (n = 28), and Aransas Bay (n = 14). The cendiff test was also used to compare the concentration of enterococci in dry-loading samples (n = 35) versus wet-loading samples (n = 13). Additionally, the cendiff and cenken tests were used to calculate correlations between enterococci concentrations and categorical or continuous environmental variables, respectively. One wet-loading sample in Tule Creek contained an extreme outlier that was greater than 2.5 standard deviations from the mean of all the samples (24,196 MPN); as it greatly skewed the distribution of the data, it was excluded from the analyses.

Quantifying host-associated molecular markers.

Three host-associated markers (human, canine, and gull) were detected and quantified with a droplet digital PCR (ddPCR) assay, as described previously (Powers et al. 2020). Due to funding limitations, samples from Site 7 were excluded from this analysis. Briefly, 42 water samples were tested in technical triplicates for the quantity of each host-associated marker (Table 7): the human-associated *Bacteroides* HF183 marker (Bernhard and Field 2000b, Seurinck et al. 2005), the canine-associated Bacteroidales DogBact marker (Dick et al. 2005, Mayer et al. 2016), and the gull-associated *Catellicoccus* LeeSeaGull marker (Lee et al. 2012, Lee et al. 2013). Each ddPCR reaction had a total volume of 20 µL, including 1X EvaGreen Supermix, 0.25 µM of forward and reverse primers, and 3 µL of DNA. Every ddPCR run included synthetic gBlock gene fragments (Integrated DNA Technologies, Skokie, Illinois, US) as positive controls and no-template controls (NTCs) that contained nuclease-free, PCR-grade water in place of DNA. The positive controls were designed from the sequences listed under the following accession numbers: human marker accession number AY618281.1 (Seurinck et al. 2005), canine marker accession number AY695700.1 (Dick et al. 2005), and gull marker accession number NR_042357 (Lawson et al. 2006, Lu et al. 2008). Droplets were generated manually with the QX200 Droplet Generator (BioRad Laboratories, Hercules, California, US) following the manufacturer's instructions. Target amplification was performed on a Bio-Rad C1000 Touch thermal cycler (BioRad Laboratories, Hercules, California, US) with a ramp rate of 2 ºC s^{-1} as follows: enzyme activation for 5 minutes at 95 ºC, followed by 40 cycles of denaturation for 30 seconds at 95 ºC and annealing/extension for 1 minute at 59 ºC, followed by a 5-minute hold at 4 ºC for signal stabilization and another 5-minute hold at 90 ºC. QuantaSoft software (BioRad Laboratories, Hercules, California, US) was used to determine the concentration of the genetic

markers in each reaction. Importantly, the peak from each NTC was used to manually set the positive threshold for all reactions in its corresponding run. The final concentration of each marker (X_{total}) was converted to gene copies 100 mL^{-1} water with Eq. (1) from Powers et. al 2020:

$$X_{total} = \frac{(X_n)(20\ \mu L)}{(3\ \mu L)(50\ \mu L)} \qquad (1)$$

The concentration of the PCR reaction is denoted by X_n (gene copies 1 μL^{-1}); the 20 µL is the total PCR reaction volume; the 3 µL is the volume of DNA in each PCR reaction; and the 50 µL is the total DNA volume from each extraction.

Table 7. Primer sequences for the 16S rRNA gene and host-associated molecular markers.

Host	**Target**	**Primer sequences**	**Accession number**
--[a]	16S rRNA[b]	Forward: 515fMod 5'-GTGYCAGCMGCCGCGGTAA-3' Reverse: 806rMod 5'-GGACTACNVGGGTWTCTAAT-3'	--[a]
Human	*Bacteroides* HF183[c]	Forward primer: 5'-ATCATGAGTTCACATGTCCG-3' Reverse primer: 5'-TACCCCGCCTACTATCTAATG-3'	AY618281.1
Canine	Bacteroidales DogBact[d]	Forward primer: 5'-CGCTTGTATGTACCGGTACG-3' Reverse primer: 5'-CAATCGGAGTTCTTCGTG-3'	AY695700.1
Gull	*Catellicoccus* LeeSeaGull[e]	Forward primer: 5'- AGGTGCTAATACCGCATAATACAGAG -3' Reverse primer: 5'- GCCGTTACCTCACCGTCTA -3'	NR_042357

[a]The 16S rRNA gene was used for bacterial operational taxonomic unit (OTU) identification; thus, host information and accession numbers are not available.
[b]Walters et al. 2016
[c]Bernhard and Field 2000, Seurinck et al. 2005
[d]Dick et al. 2005, Sinigalliano et al. 2010
[e]Lee et al. 2012, Lee et al. 2013, Lu et al. 2008, Lawson et al. 2006

Host-associated molecular marker analysis.

The technical triplicates of each sample were averaged together. One wet-loading sample from Aransas Bay generated fewer than 10,000 intact droplets in the human marker ddPCR assay and was excluded from analysis. Additionally, one human-associated dry-loading sample from Little Bay was an extreme outlier that was greater than 2.5 standard deviations from the mean of the rest of the samples (736.67 gene copies 100 mL^{-1}); as it greatly skewed the distribution of the data, it was also excluded from the analysis. Due to the non-normal distribution of the data, determined via the qqplot function and Shapiro-Wilk test, and the small sample size of the wet-loading samples, non-parametric tests were utilized for analyses. Specifically, the Kruskal-Wallis test from the coin package in R was used to test for differences between overall locations (i.e., Tule Creek, Little Bay, and Aransas Bay) and the Wilcoxon rank-sum test from the coin package in R (Hothorn et al. 2006) compared the concentrations of dry-loading samples to wet-loading samples. Kendall's tau was calculated to determine the correlations between the three host-associated markers and enterococci concentrations. Generalized linear models (GLMs) were generated for each marker to determine which environmental variables acted as significant predictors. Initial GLMs were computed in R using the glm function. Variables were tested for multicollinearity using the VIF function from the car package (Fox and Weisberg 2019), and those with the highest VIF value were removed individually until all VIF values were less than 5.0 or $GVIF^{(1/(2*DF))}$ values were less than 2.0. The models were then dredged using the dredge function from the MuMIn package (Bartoń 2019) and resulting models with AICc values 2.0 greater than the top model were removed. The final models were chosen based on the highest R^2 values, calculated with the r.squaredGLMM function. Finally, the three models were tested for significance using the ANOVA command against null models with all variables removed.

Results and Discussion.

Bacterial community diversity.

Environmental disturbances can have profound impacts on the community structure and therefore health of an ecosystem (Shade et al. 2012). One example of a disturbance is the occurrence of rainfall, which may introduce harmful pollutants and allochthonous bacteria to marine environments (Sauer et al. 2011), as well as cause fluctuations in salinity and nutrient concentrations (Jeffries et al. 2016). Ecosystem health can be assessed through the measurement of diversity as a proxy for community stability and resiliency in response to disturbance (Girvan et al. 2005, Singh et al. 2014, Feng et al. 2017). In this study, we investigated how microbial diversity varied across the ecocline and we determined how rainfall impacted the overall community diversity. Tule Creek, which is a freshwater catchment located immediately downstream of the local WWTP, had higher alpha diversity (Faith's phylogenetic diversity; Kruskal-Wallis test; $p < 0.01$) and was compositionally distinct from Little Bay and Aransas Bay (Figure 8A; PERMANOVA; $p < 0.01$). These results were unsurprising, given that freshwater and saltwater sites are known to contain markedly different microbial communities (Dupont et al. 2014). In contrast, despite marginal differences between Little Bay and Aransas Bay, these communities were not significantly different.

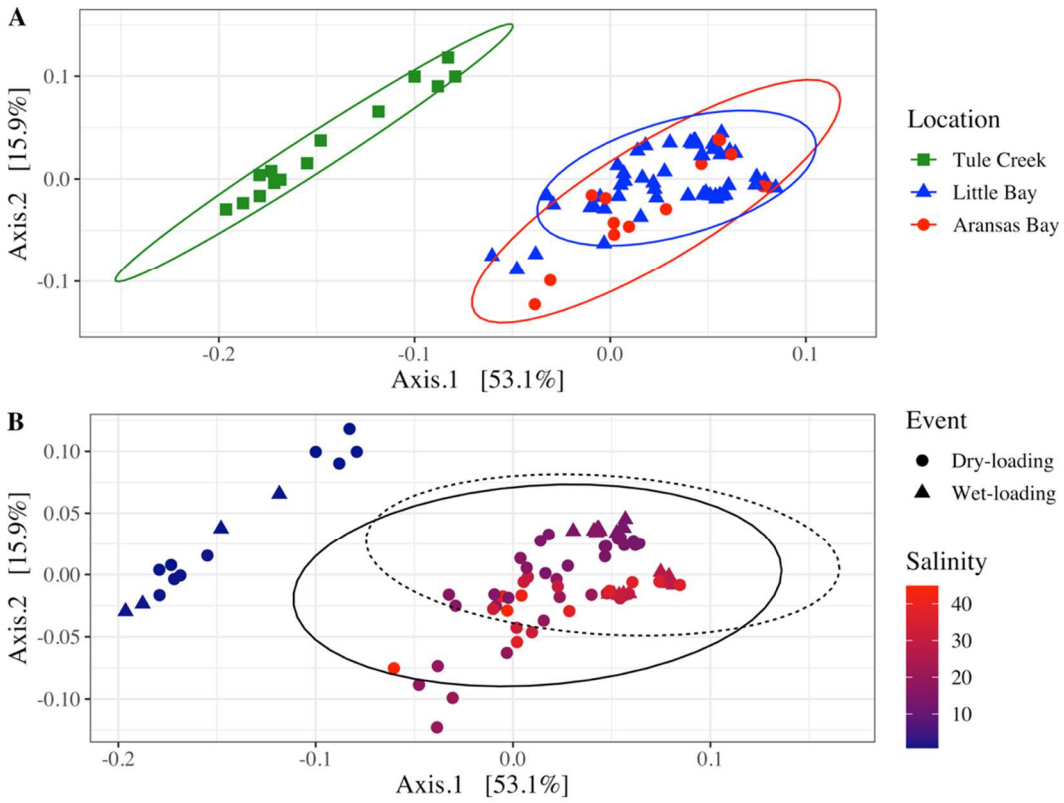


Figure 8. PCoA computed with weighted UniFrac distance values. **A)** Tule Creek samples are shown as green squares, Little Bay samples are shown as blue triangles, and Aransas Bay samples are shown as red circles. 95% confidence ellipses surround each location with their respective colors. Tule Creek was compositionally different from the other locations (PERMANOVA; $p < 0.01$), although Aransas Bay and Little Bay were only marginally different from each other (PERMANOVA; $p < 0.1$). **B)** Dry-loading samples are shown as circles and wet-loading samples are shown as triangles; salinity is shown as a gradient ranging from lower values in blue to higher values in red. The 95% confidence ellipses surround dry-loading samples as a solid line and wet-loading samples as a dotted line. Wet- and dry-loading samples were compositionally distinct (PERMANOVA; $p < 0.05$).

In regard to rainfall, the alpha diversity in dry-loading samples was not significantly different from wet-loading samples. However, the overall bacterial community was compositionally different following rainfall (Figure 8B; PERMANOVA; $p < 0.05$), with salinity acting as a significant driver of those differences (Spearman's rho = 0.42, $p < 0.01$). According to the LEfSe analysis, several taxonomic groups were clearly more prevalent during dry-loading

(e.g., Bacteroidia, Verrucomicrobia, Deltaproteobacteria, Firmicutes), whereas only a few groups were more common during wet-loading (e.g., Alphaproteobacteria and *Methylophilaceae*) (Figure 9; $p < 0.01$). The combination of these results is largely in agreement with a previous study in the nearby Corpus Christi Bay (Powers et al. 2020) and further confirms that rainfall acts as a pulse disturbance to alter microbial community structure (Williamson et al. 2014, Chaudhary et al. 2018, Uritskiy et al. 2019).

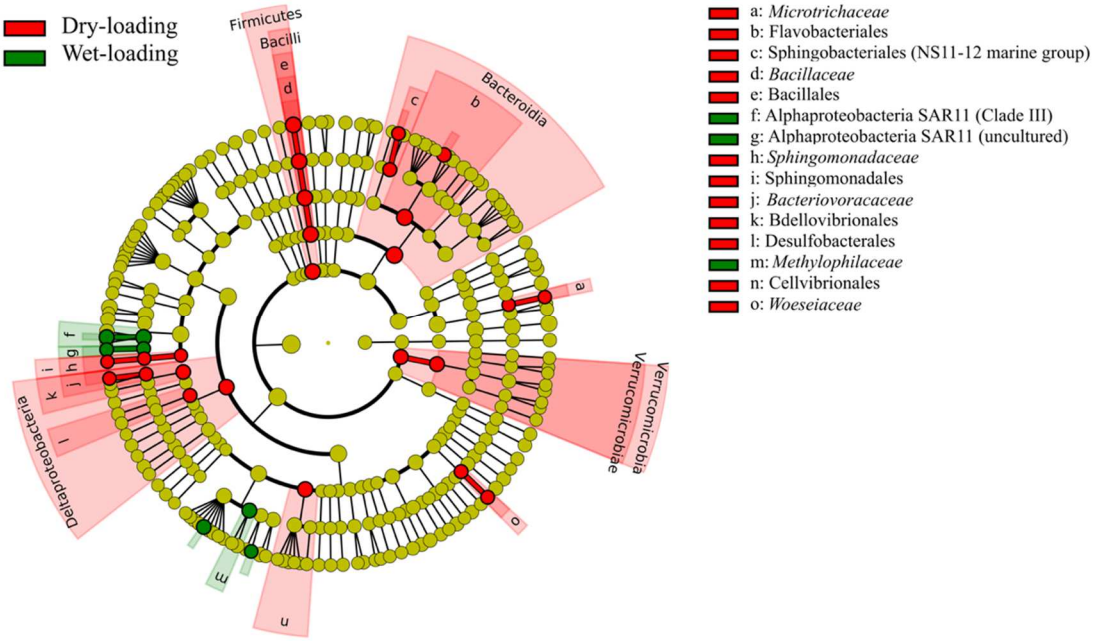


Figure 9. Linear discriminant analysis (LDA) effect size (LEfSe) of the dry-loading (shown in red) and wet-loading (shown in green) samples ($p < 0.01$).

Rainfall-induced changes in microbial community composition could result in a change in overall microbial function (Dupont et al. 2014, Galand et al. 2018). However, it is also possible that the divergent communities remain functionally redundant (Sunagawa et al. 2015,

Louca et al. 2016). Additional research should be focused on functional analyses to test how community function changes in response to rainfall. For instance, previous studies have shown increases in antimicrobial resistance after rainfall and the loading of stormwater runoff into aquatic environments (Cummings et al. 2010, Young et al. 2013, Carney et al. 2019). Rainfall and runoff have also been associated with an increase in the prevalence of virulence genes in bacteria that are known to cause disease in humans and animals (Sidhu et al. 2013b, Abia et al. 2016). Such changes to the microbial community could have cascading and profound effects on ecosystems and human health.

Enterococci concentrations.

The United States Environmental Protection Agency (USEPA) recommends utilizing enterococci as a fecal indicator in marine recreational waters (USEPA 2012). Specifically, the USEPA recommends recreational marine water should maintain a geometric mean standard of fewer than 35 colony forming units (CFU) 100 mL^{-1} or a single-sample standard of fewer than 104 CFU 100 mL^{-1}. In the state of Texas, the Texas Beach Watch Program conducts routine enterococci testing at 164 coastal sampling stations, including four of the seven sampling stations featured in this study (Sites 4-7) (Texas General Land Office 2015). Thus, this study included enterococci in its assessment of water quality.

Impaired water quality is a common occurrence in environments located downstream of WWTPs and other known point sources of fecal pollution (Passerat et al. 2011, Sun et al. 2017). In this study, enterococci concentrations decreased along the coastal ecocline; enterococci were highest in the WWTP catchment (i.e., Tule Creek), followed by Little Bay, and then Aransas Bay (cendiff test; $p < 0.001$; Figure 10 and Table 8). The highest enterococci concentrations were

detected in the two sites closest to the WWTP: Site 1 in Tule Creek and Site 3, which was the outfall of Tule Creek into Little Bay. It is therefore possible that the elevated levels of enterococci originated from WWTP effluent. Alternatively, it is possible that the nutrient-rich effluent from the WWTP stimulated the growth of naturalized (i.e., environmental or non-enteric) enterococci populations. Waters that receive WWTP effluent are often enriched in organic nutrients (Petersen et al. 2005) that stimulate the growth of heterotrophic bacteria, including enterococci (Nogales et al. 2011). Future research that includes the measurement of organic nutrients in conjunction with FIB measurements could offer further support for this hypothesis.

Previous studies have identified rainfall as a driver of fecal pollution in coastal environments (Brownell et al. 2007, Parker et al. 2010, Converse et al. 2011, Cao et al. 2017, Powers et al. 2020). However, the enterococci levels in this study did not increase after rainfall. Rather, the highest enterococci measurement (1,399 MPN 100 mL^{-1}) was detected after dry-loading conditions (Table 9), and the mean enterococci concentrations of both dry- and wet-loading samples (118.46 and 94.73 MPN 100 mL^{-1}, respectively) exceeded or nearly exceeded the USEPA's recommended single-sample standard criterion of fewer than 104 CFU 100 mL^{-1} (USEPA 2012). As previously mentioned, the enterococci could be originating from the WWTP or naturalized enterococci could be enriched by nutrients from its effluent. Alternatively, the enterococci may be originating from leaks in sanitary sewer and septic systems (Sercu et al. 2011, Sowah et al. 2017). It is also possible that rising sea levels in this region may be contributing to elevated enterococci, as sea level rise and saltwater intrusion has been identified as a contributing factor to septic system damage and failure (Elmir 2018). In a recent long-term

assessment of water quality throughout the northwestern GOM coastline, we have shown that sea level is a driver of elevated enterococci concentrations (Powers et al. *in press*).

The reliability of enterococci as a fecal pollution indicator is contingent upon their rapid decline following exposure to the environment (Byappanahalli et al. 2012). However, some enterococci species (e.g., *E. casseliflavus*, *E. mundtii*, *E. hirae*, and *E. aquimarinus*) are well-adapted to life outside of animal hosts, allowing for long-term survival in association with aquatic plants and sediments (Byappanahalli et al. 2012). The presence of naturalized enterococci can therefore confound the results of water quality testing (Ferguson et al. 2013, Devane et al. 2020). In this study, enterococci were inversely correlated with water transparency, water temperature, pH, specific conductance, and salinity (Kendall's tau = -0.21, -0.26, -0.31, -0.34, -0.34, respectively; $p < 0.05$), although no correlations were observed between enterococci and dissolved oxygen or rainfall. The negative correlations presented here and in previous studies (Love et al. 2010, Viau et al. 2011, González-Fernández et al. 2021) could reflect reduced survival after exposure to disparate environmental conditions (i.e., variable temperature, salinity, and UV light); however, weak and nonsignificant correlations (Shibata et al. 2004, Coulliette and Noble 2008) may indicate these tests were biased by the presence of naturalized enterococci that did not originate from animal sources.

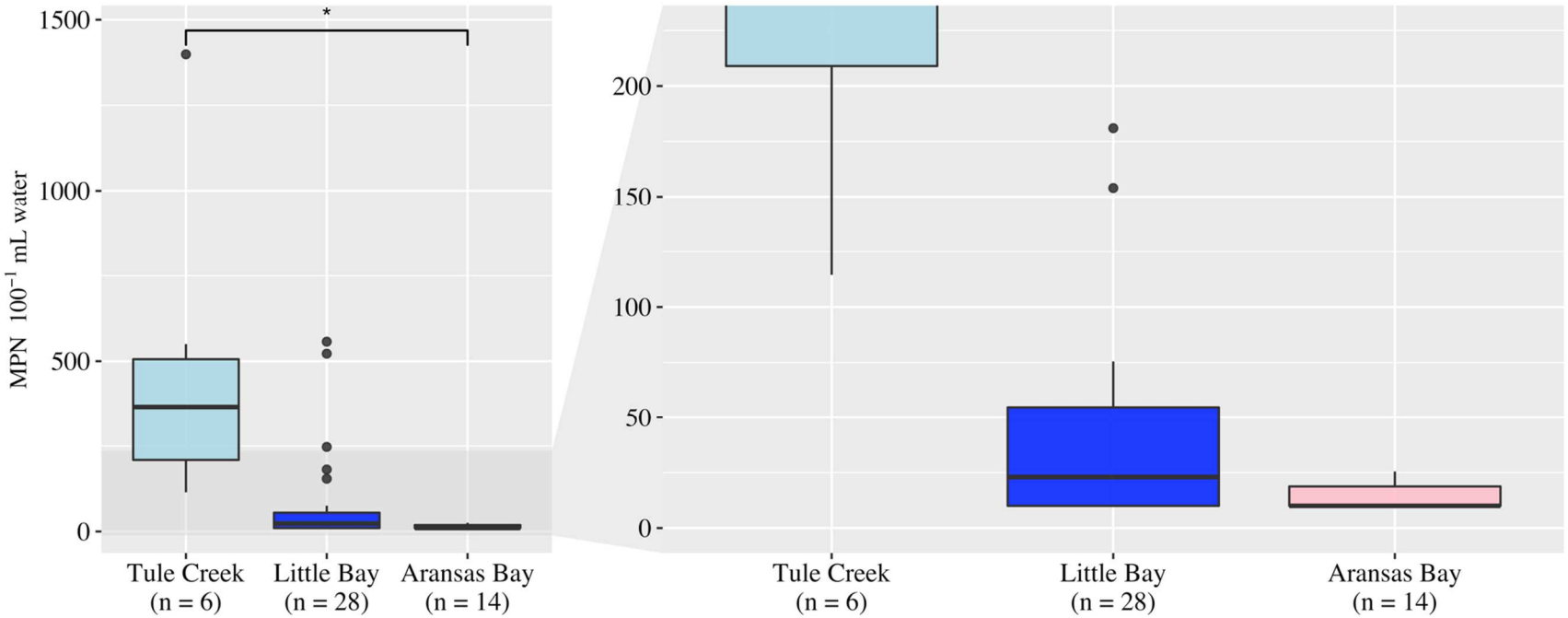


Figure 10. Concentration of enterococci along the coastal ecocline. *Enterococci were significantly higher in Tule Creek (light blue), followed by Little Bay (dark blue), then Aransas Bay (pink) (cendiff test; $p < 0.001$).

Table 8. Concentrations of enterococci (MPN 100 mL^{-1}) and human-, canine-, and gull-associated molecular markers (gene copies 100 mL^{-1}) along the ecocline.

Location	Bacterial target	Min	Max	Mean	Med
	*Enterococci	114.50	1399.00	492.42	366.00
Tule Creek	Human marker	0.00	75.56	29.92	25.56
(WWTP	Canine marker	15.56	96.67	59.21	55.56
catchment)	Gull marker	7.78	78.89	43.18	53.34
	*Enterococci	<10	557.00	79.52	23.00
Little Bay	Human marker	0.00	212.23	41.96	24.45
	Canine marker	0.00	213.34	68.26	54.45
	Gull marker	7.78	198.89	63.65	43.33
	*Enterococci	<10	25.50	14.04	<10
Aransas Bay	Human marker	7.78	76.67	25.38	16.11
	Canine marker	24.45	113.34	62.23	54.45
	Gull marker	7.78	82.23	32.23	16.67

*Enterococci decreased following the coastal ecocline (Tule Creek > Little Bay > Aransas Bay; cendiff test; $p < 0.01$).

Table 9. Concentrations of enterococci (MPN 100 mL^{-1}) and human-, canine-, and gull-associated molecular markers (gene copies 100 mL^{-1}) after wet-loading and dry-loading events.

Event type	Bacterial target	Min	Max	Mean	Med
	Enterococci	< 10.00	557.00	94.73	25.50
Wet-loading	*Human marker	0.00	77.78	43.74	37.78
	Canine marker	33.34	120.00	75.75	77.23
	Gull marker	7.78	138.89	64.36	67.79
	Enterococci	< 10.00	1,399.00	118.46	15.00
Dry-loading	*Human marker	0.00	212.23	34.95	15.56
	Canine marker	0.00	213.34	61.75	49.45
	Gull marker	7.78	198.89	51.26	31.67

*The human-associated markers increased significantly after wet-loading ($p < 0.05$).

Host-associated molecular markers.

Owing to the unreliable nature and lack of host-specificity of traditional FIB (Byappanahalli et al. 2012, Boehm and Sassoubre 2014), the presence of three host-associated markers for humans, canines, and gulls were analyzed in addition to the presence of enterococci in this study. The human and canine genetic markers belong to members of the Bacteroidales,

which, unlike enterococci, are obligate anaerobes that are short-lived outside of their hosts (Roslev and Bukh 2011). Thus, their presence in the marine environment should reflect contamination from recent fecal waste rather than the presence of naturalized fecal-associated bacteria that have persisted for an extended period in the environment. Although the gull marker (*Catellicoccus marimammalium*) is a facultative rather than obligate anaerobe (Lawson et al. 2006), its detection has been strongly associated with gull fecal waste, with the exception of occasional cross-reactivity with similar avian species (i.e., pigeons) (Sinigalliano et al. 2013).

Fecal waste from any source in the environment is cause for concern, but the presence of human fecal waste is particularly worrisome, as it is more likely to contain microorganisms that are known human pathogens (Lipp et al. 2001a, Griffin et al. 2003). Human fecal pollution (HF183) was detected in an overwhelming number of samples (37/40 or 92.5%). However, despite the higher levels of enterococci detected in Sites 1 (Tule Creek) and 3 (Tule Creek Outfall), the human marker was not significantly higher in these sites, implying that the WWTP upstream of Tule Creek is generally effective in removing human-associated fecal bacteria from the effluent. In fact, HF183 concentrations did not follow a significant trend along the ecocline; rather, elevated levels of HF183 were detected throughout the study. The near omnipresence of the HF183 marker is a clear indication of human fecal contamination, and stormwater is one likely source of this pollution, as the human marker was significantly higher after rainfall (Figure 11A; $p < 0.05$). Periodic spikes also occurred under dry-loading conditions (Table 9), indicating that additional sources, such as leaking or aging infrastructure, are also contributing to the pollution. This direct correlation between rainfall and human fecal pollution corroborates the results of previous studies that have identified rainfall as a driver of human fecal pollution in urban regions (Converse et al. 2011, Sauer et al. 2011, Sidhu et al. 2013a).

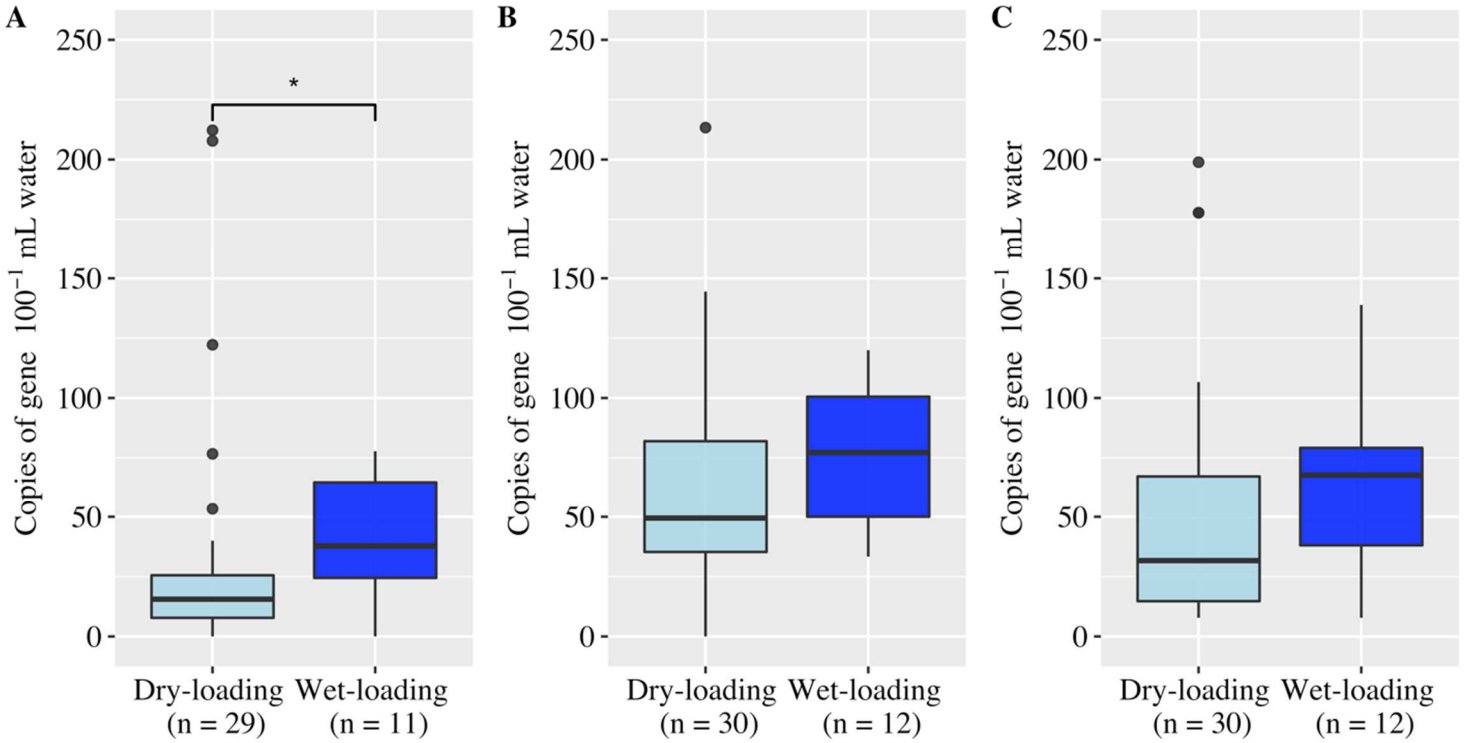


Figure 11. Concentrations of the **(A)** human-, **(B)** canine-, and **(C)** gull-associated molecular markers collected after dry-loading (light blue) and wet-loading (dark blue) events. *The human-associated molecular markers increased significantly after rainfall (Wilcoxon rank-sum test; $p < 0.05$).

Elevated levels of human fecal contamination can result from seepage from impaired sewage and septic systems (Sercu et al. 2011, Sowah et al. 2017), whereas sudden spikes in fecal contamination can result from leaks in sewer force mains (Mallin et al. 2007). For example, prior to this study, in April of 2016, the Texas Commission of Environmental Quality (TCEQ) reported that a leak in the force main under the city of Rockport, Texas resulted in an estimated 12,050-gallon sewage spill (TCEQ 2016). In August of the following year, a break in the force main resulted in a 10,000-gallon spill, followed by another break with an additional 500-gallon spill two weeks later (TCEQ 2017). Although the force main was replaced in 2017 prior to this study (Malcolm Dieckow as Chairperson of the Aransas County Navigation District in Rockport, Texas, personal communication), we did detect a significant spike in the HF183 marker in Site 4 (736.67 gene copies 100 mL^{-1}) during dry-weather conditions. That sample was a unitary extreme outlier in the dataset (i.e., greater than 2.5 standard deviations from the mean) and was therefore excluded from Tables 8 and 9 and Figure 11 for the sake of clarity. This spike, and other smaller spikes, could be explained by a failure in the sanitary collection system or the illegal discharge of sewage from a vessel.

Animal fecal waste can contain zoonotic pathogens, and its risk to human health has been shown to be dose-responsive, meaning that higher levels of this pollution pose a greater health threat to humans (Soller et al. 2014). Throughout this study, the canine marker was detected in every sample except one, and the gull marker was detected in all the samples. Similar to the human marker, neither the canine nor the gull markers followed a significant trend along the coastal ecocline (Table 8). Both the canine and gull markers experienced their highest concentrations after dry-loading conditions (213.34 and 198.89 gene copies 100 mL^{-1}, respectively; Table 9); however, on average, these marker concentrations were slightly elevated,

but not significantly higher, after rainfall (Figure 11B and 11C). This slight increase after wet-loading indicates the human health risk may be higher after rainfall in this location; however, the degree to which any fecal indicator represents a health risk varies extensively, depending on a multitude of factors, including the nature of the indicator, the geographic location, and the degree and age of contamination (Anderson et al. 2005, Soller et al. 2010b, Soller et al. 2014). Elevated levels of canine and avian markers were detected following rainfall in a recent study in Lake Michigan (Shrestha et al. 2020), whereas elevated levels of these markers were detected after dry weather in Corpus Christi Bay, Texas (Powers et al. 2020). The contrasting results presented in all of these studies further supports the need for the development of watershed-specific management plans.

Of the GLMs produced for the three host-associated markers, the only statistically significant model was the gull model ($p < 0.05$). This model included variables for dissolved oxygen (mg mL^{-1}), water clarity (categorical variable characterized as clear, cloudy, or turbid water), and water temperature (°C), and produced an R^2 value of 0.20. The low R^2 value of the gull model and the non-significance of the human and canine models indicate that there are likely other factors not measured in this study that have a stronger impact on the marker concentrations (e.g., occurrence of sewage leaks, urban wildlife and domestic animal behavior, and pet waste disposal habits) (Wright et al. 2009, Sercu et al. 2011, Ervin et al. 2014). That enterococci were not a significant variable in any of the models diminishes its value as a proxy for fecal waste in this system.

Globally, urbanized coastal regions experiencing increased storm frequency or severity are likely to experience a corresponding decrease in water quality (Sauer et al. 2011). The northwestern GOM is already experiencing increases in the frequency and severity of storm

events, including tropical storms and hurricanes, due to climate change (Knight and Davis 2009). Thus, it is imperative to determine the impact of rainfall on coastal water quality. This study characterized water quality along an urbanized estuarine ecocline. The results showed that the traditional FIB, enterococci, decreased along the ecocline, although they were not correlated with the presence of human-, canine-, or gull-associated fecal pollution. The results also demonstrated that rainfall was a driver of human fecal contamination and dramatic changes in the microbial community composition. We hypothesize that prevalence of human fecal pollution and its direct relationship with rainfall will worsen, given predications of increased urbanization and storm activity (Burkett 2008, USGCRP 2017). It would be salient to develop coastal watershed management policies and practices in recognition of these results. Specifically, the findings of this study emphasize the need for 1) reliable fecal pollution monitoring in the form of host-associated molecular markers and 2) effective rainfall and stormwater runoff management, particularly as storm patterns change in response to climate change.

Acknowledgements.

We acknowledge the Aransas County Navigational District and the Coastal Bend Bays and Estuaries Program (CBBEP) for providing funding for this project. We thank Blair Sterba-Boatwright (Professor, Texas A&M University-Corpus Christi) for his support with statistical analyses and we also thank the residents of Rockport, Texas for supporting this project.

CHAPTER III: LONG-TERM WATER QUALITY ANALYSIS REVEALS CORRELATION BETWEEN BACTERIAL POLLUTION AND SEA LEVEL RISE IN THE NORTHWESTERN GULF OF MEXICO

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Abstract.

Long-term data assessments are needed to identify water quality trends and their socio-environmental drivers to guide coastal management and watershed restoration. This study provides the first long-term assessment of fecal bacterial pollution in the northwestern Gulf of Mexico using enterococci data covering the entire Texas coast from 2009 to 2020. The data were representative of 66 beaches, 169 stations, and over 75,000 samples. Findings demonstrate that 22 beaches are 'hotspots' of fecal pollution and experienced enterococci levels that exceeded the USEPA beach action value more than 10% of the time. Further, enterococci concentrations were correlated with time, population size, and sea level. Weak correlations detected in some of the counties highlight the multifactorial nature of coastal water quality; it is likely that additional factors not measured in this study are also influencing enterococci levels. However, the correlation with sea level is concerning, as counties most vulnerable to sea level rise frequently reported enterococci concentrations that exceeded the beach action value. In consideration of sea level rise predictions, targeted studies are needed to pinpoint the sources and drivers of fecal bacterial pollution.

Introduction.

The quality of recreational water is commonly assessed by quantifying the abundance of fecal indicator bacteria (FIB). In the United States (US), recreational water quality was originally assessed by measuring total coliforms based on a study of recreational bather health in Lake Michigan (Stevenson 1953). Subsequent studies in Michigan and Texas led to the adoption of enterococci as a more reliable FIB (Cabelli et al. 1983, Dufour 1984). Enterococci are Gram-positive cocci that belong to the *Enterococcus* genus and are commonly associated with fecal pollution originating from humans and other mammals (Byappanahalli et al. 2012). The enterococci are not presumed to be harmful; rather, the presence of elevated concentrations is viewed as a proxy for fecal pollution that could carry pathogenic disease-causing organisms (Boehm and Sassoubre 2014). Numerous studies have demonstrated a positive correlation between elevated enterococci and the risk of gastrointestinal, respiratory, ear, eye, and skin-related illnesses among recreational bathers (e.g., Prüss 1998, Wade et al. 2003, USEPA 2012, Boehm and Sassoubre 2014).

In US surface waters, outdoor recreational activities such as swimming, boating, and fishing account for an estimated 4 billion recreational events annually (DeFlorio-Barker et al. 2018). These recreational events result in an estimated 90 million gastrointestinal, respiratory, ear, eye, or skin-related illnesses. The primary etiologic agents responsible for these infections are water-borne fecal pathogens such as protozoa (e.g., *Cryptosporidium* and *Giardia*), bacteria (e.g., *Campylobacter* and *Salmonella*), and viruses (e.g., noroviruses and adenoviruses) (Korajkic et al. 2018). The economic burden of these illnesses costs $2.2-3.7 billion annually. In Texas, bacterial pollution is the leading cause of Texas surface water impairments (TCEQ 2020a). The health of recreational bathers is protected when beach advisories, warnings, and

closures are publicly announced in response to elevated enterococci levels that exceed the US Environmental Protection Agency (USEPA) beach action value.

The Beaches Environmental Assessment and Coastal Health (BEACH) Act of 2000 required US states and territories to adopt water quality standards that are protective of human health (USEPA 2000). The BEACH Act is an amendment to the 1977 Clean Water Act that mandates the USEPA to publish scientific criteria for limiting various pollutants. The USEPA beach action value for marine recreational water quality is not more than 104 colony forming units (CFU) of enterococci 100 mL^{-1} water (single-sample standard) or 35 CFU 100 mL^{-1} water (geometric mean standard) (USEPA 2012). The Texas General Land Office (TGLO) manages the Texas Beach Watch Program, which is responsible for monitoring enterococci concentrations to assess marine recreational water quality. Since enterococci were adopted as Texas' standard for assessing marine recreational water quality in 2004, the program has monitored enterococci concentrations through a network of 169 monitoring stations at 66 recreational beaches for the past 16 years. This historical dataset provides a rare opportunity to investigate the long-term spatial-temporal dynamics of enterococci and water quality in marine coastal waters.

Several environmental factors, including rainfall, water temperature, sunlight and UV exposure, and tidal stage, have been identified as short-term drivers of FIB, with strong correlations to water quality (Boehm and Weisberg 2005, Parker et al. 2010, Mattioli et al. 2017). Additional long-term factors, such as sea level rise, population growth, urbanization and infrastructure expansion, and sewage and septic system conditions, have been implicated as drivers of longer-term changes (Walters et al. 2011, Elmir 2018, Humphrey et al. 2018). Characterizing long-term trends in coastal water quality is critical given the current and projected rates of population growth and climate change. Numerous previous studies have focused on

short-term (i.e., diurnal, weekly, or monthly) assessments of water quality and its environmental drivers; however, significant knowledge gaps pertaining to long-term (i.e., yearly or decadal) trends remain.

The purpose of this study was to characterize fecal bacteria pollution in the Texas coastal zone. This characterization involved a comprehensive analysis of enterococci data and environmental metadata to gain insights and improve understanding of the water quality conditions and trends. Long-term trends of enterococci concentrations were predicted to change with population growth, land-use change (i.e., urbanization), weather and climate, and sea level rise. Conversely, short-term variability was predicted to be influenced by extreme rainfall events and sanitary sewer overflows (SSOs).

Materials and Methods.

Summary statistics.

Enterococci data spanning 2009 to 2020 were provided by TGLO. Regular sampling was conducted on a weekly basis during 'peak' season (i.e., March and May through September) and a bi-weekly basis during 'off-peak' season; additional samples were obtained for a higher resolution temporal analysis when exceptionally high levels of FIB were detected (i.e., summer 2019). The Texas Beach Watch Program quantified enterococci using the Enterolert test and the USEPA 1600 membrane filtration method, under a USEPA-approved Quality Assurance Project Plan (QAPP) and reported the enterococci concentration as the most probable number (MPN) or CFU 100 mL^{-1} water (Texas Beach Watch Program, 2015). The data encompassed 169 sampling stations, 66 beaches, and 10 coastal counties (coordinates available at https://cgis.glo.texas.gov/Beachwatch/). Data were inspected and corrected for anomalies and

data entry errors. Samples that contained field replicates were averaged together, and a total of 75,380 samples were analyzed. The mean, median, minimum, and maximum, as well as the number and percentage of exceedances above the USEPA beach action value (104 MPN 100 mL^{-1}) and at the Enterolert test upper limit (24,196 MPN 100 mL^{-1}) were calculated for each county and all individual beaches.

Water quality rankings.

The water quality in the 10 coastal counties and the 66 beaches were ranked based on the percentage of samples that exceeded the USEPA beach action value (104 MPN 100 mL^{-1}). Each location was classified as having 'low' (< 5% exceedances), 'medium' (5-10% exceedances), 'high' (10-20% exceedances), or 'very high' (> 20% exceedances) levels of enterococci. The ranks were chosen according to natural breakpoints in the data, as done in previous studies (Ferretti et al. 2011, Feng et al. 2016). The beaches with 'high' or 'very high' levels of enterococci were considered to be 'hotspots' of bacterial pollution.

Spatial-temporal trends.

The relationships between enterococci and relevant environmental variables were tested. These variables included local hydrodynamic conditions, population size, time, and sea level. Bayside stations representing low flushing environments and Gulfside locations representing higher flushing environments were compared to test the effect of local hydrodynamics. Population size estimates were obtained from the United States Census Bureau (https://www.census.gov/) for nine of the counties from 2010 to 2019. Kleberg County was excluded from this analysis as water samples were only collected in this county through 2011.

Data pertaining to sea level were obtained from NOAA Tides & Currents (https://tidesandcurrents.noaa.gov/) and the Texas Coastal Ocean Observation Network (TCOON) (https://tidesandcurrents.noaa.gov/tcoon.html). Sea level data were assessed based on monthly mean sea level from 2009 to 2020. Due to the censored nature of the enterococci measurements (i.e., lower detection limit of <10 MPN and upper detection limit of >24,196 MPN 100 mL^{-1}), the cenken and cendiff tests from the NADA package (Lee 2020) in R were used to compute Kendall's tau correlation coefficient for the censored data (i.e., enterococci concentrations), and the cor.test in R was used to compute Kendall's tau correlation coefficient when non-censored data were analyzed (i.e., summary statistic data).

A total of 11 interpolated surfaces, one for each year, were generated to effectively visualize the spatial-temporal trends of fecal bacterial pollution in the coastal zone area. The formal delineation of the Texas Coastal Zone Boundary was obtained from TGLO (https://www.glo.texas.gov/land/land-management/gis/). The Inverse Distance Weighted technique (IDW) was used to generate a surface based on the value of the percentage of water samples that exceeded the USEPA's beach action value (>104 MPN 100 mL^{-1}) for each year in each station. To predict the percentage at any location, the IDW uses the percentage value of stations surrounding to the location, gives greater weights to stations that are closest to the location, and the weights decrease with distance. The interpolated surfaces for 2009 to 2011 were generated from the percentage values at all 169 stations. The three Kleberg stations were excluded for 2012 to 2019 due to no sampling in Kleberg after 2011. The stretch method, which spreads the interpolated values along a histogram from the minimum and maximum values, was used to display the interpolated surfaces.

County-specific analyses.

Data obtained from three counties identified as 'hotspots' of bacterial pollution (i.e., Harris, Brazoria, and Matagorda) underwent additional analyses. Namely, elevated concentrations of enterococci, observed in these counties during 2019, were analyzed with respect to rainfall as well as SSOs. Rainfall records corresponding to dates with elevated enterococci were obtained from the nearby weather monitoring stations in each county from the TexMesonet database (https://texmesonet.org/). The USEPA Region 6 office (Robert Cook, personal communication) provided a partial record of known SSOs in Brazoria County from 2018 to 2020. However, this record did not include the estimated 205 failure points in the domestic wastewater collection system in the Village of Surfside Beach, Texas (TCEQ 2019).

Generalized linear models (GLMs).

GLMs were constructed to examine relationships between enterococci and multiple variables in Brazoria, Matagorda, and Harris Counties. The full GLMs included multiple variables (i.e., month, year, time, beach name, bayside versus Gulfside location, sea level, and population size). The models were assessed for multicollinearity using the alias command and the VIF function (from car package in R; Fox and Weisberg 2019); variables with a $GVIF^{(1/(2*DF))} > 2.0$ were removed. The dredge function (from the MuMIn package in R; Bartoń, 2019) was used for model averaging and the potential models were ranked based on AICc values. Only the models with AICc values less than 2.0 from the top model were considered. These models were subsequently ranked based on adjusted R^2 values. The final models were tested for significance against a null model with all of the variables removed.

Results.

Summary statistics.

Table 10 shows the summary statistic values (i.e., mean, median, minimum, and maximum enterococci concentrations and the number and percentage of samples when enterococci concentrations exceeded the USEPA beach action value) for the 10 counties; Appendices 1-4 show the results for the 66 beaches. All beaches experienced minimum enterococci concentrations that were below the Enterolert test limit of detection (< 10 MPN 100 mL^{-1}), and the majority experienced median values equivalent to 10 or < 10 MPN 100 mL^{-1}. However, 65 of the 66 beaches also experienced enterococci concentrations that exceeded the USEPA beach action value multiple times, 21 beaches experienced a mean enterococci value greater than 104 MPN 100 mL^{-1}, and 19 beaches exceeded the upper limit of detection (24,196 MPN 100 mL^{-1}) at least once.

Table 10. Summary metrics for each of the 10 coastal counties. Based on the percentage of samples that exceeded the USEPA beach action value (104 MPN 100 mL^{-1}), water quality was classified as having 'low' (< 5%; shown in green), 'medium' (5-10%; shown in yellow), 'high' (10-20%; shown in orange), or 'very high' (> 20%; shown in red) levels of enterococci. Enterococci concentrations were measured as CFU or MPN 100 mL^{-1} water (Max. = maximum, Med. = median, Avg. = average). Minimum concentrations for every county were equivalent to 10 or < 10 MPN 100 mL^{-1}.

County	Max.	Med.	Avg.	No. beaches	% exceedances	Category
Cameron	2,252.50	10	16.17	9	1.17	Low
Jefferson	1,723.00	10	32.12	2	3.44	Low
Galveston	24,196.00	10	68.16	23	7.09	Medium
Kleberg*	1,995.00	7	49.89	4	7.67	Medium
Aransas	19,863.00	10	107.46	1	8.27	Medium
San Patricio	4,611.00	10	61.98	1	10.55	High
Nueces	24,196.00	10	207.67	18	11.17	High
Brazoria	24,196.00	10	121.15	4	11.93	High
Matagorda	24,196.00	20	235.17	3	21.90	Very high
Harris	24,196.00	30	444.32	1	25.74	Very high

*Kleberg County samples were only recorded from 2009 to 2011.

Water quality rankings.

The 10 counties and 66 beaches were ranked as having 'low', 'medium', 'high', and 'very high' levels of enterococci based on the percentage of samples that exceeded the USEPA beach action value of 104 MPN 100 mL^{-1}. These categories were chosen to reflect natural breakpoints in the data. County-specific trends are shown in Table 10. Overall, Cameron and Jefferson were classified as having 'low' levels of enterococci, as they exceeded the USEPA beach action value less than 5% of the time. Galveston, Kleberg, and Aransas exceeded the USEPA beach action value 5-10% of the time and were classified as having 'medium' levels of enterococci. San Patricio, Nueces, and Brazoria exceeded the beach action value 10-20% of the time and were classified as having 'high' levels of enterococci. Matagorda and Harris were classified as having 'very high' levels of enterococci, as they exceeded the USEPA limit more than 20% of the time. The counties that exceeded the beach action value greater than 10% of the time were also considered to contain 'hotspots' of bacterial pollution (i.e., Harris, Matagorda, Brazoria, Nueces, and San Patricio). Twenty-two of the beaches (33%) had 'low' levels of enterococci, 22 (33%) had 'medium' levels of enterococci, 16 (24%) had 'high' levels of enterococci, and 6 (9%) had 'very high' levels of enterococci (Appendices 1, 2, 3, and 4, respectively).

Spatial-temporal trends.

The sampling stations were classified as either bayside (N = 33) or Gulfside (N = 134), depending on their location relative to the barrier islands. The bayside enterococci concentrations were significantly higher than the Gulfside stations (Figure 12; cendiff test; $p < 0.001$). With respect to time, 60% of the 6,721 samples that exceeded the USEPA beach action value were reported in the last six years, from 2015 to 2020 (Table 11). Similarly, 89% of the 92 samples

that exceeded the upper limit of detection (24,196 MPN 100 mL^{-1}) were reported since 2015 (Table 11). When the beaches were analyzed independently, the majority (N = 41) experienced increasing enterococci concentrations over the 11-year time span, whereas 20 of the beaches did not experience a significant correlation, and five beaches had enterococci concentrations that were inversely correlated with time (Appendices 1-4). In general, the counties with poorer water quality had enterococci levels that were more strongly correlated with time (Table 12).

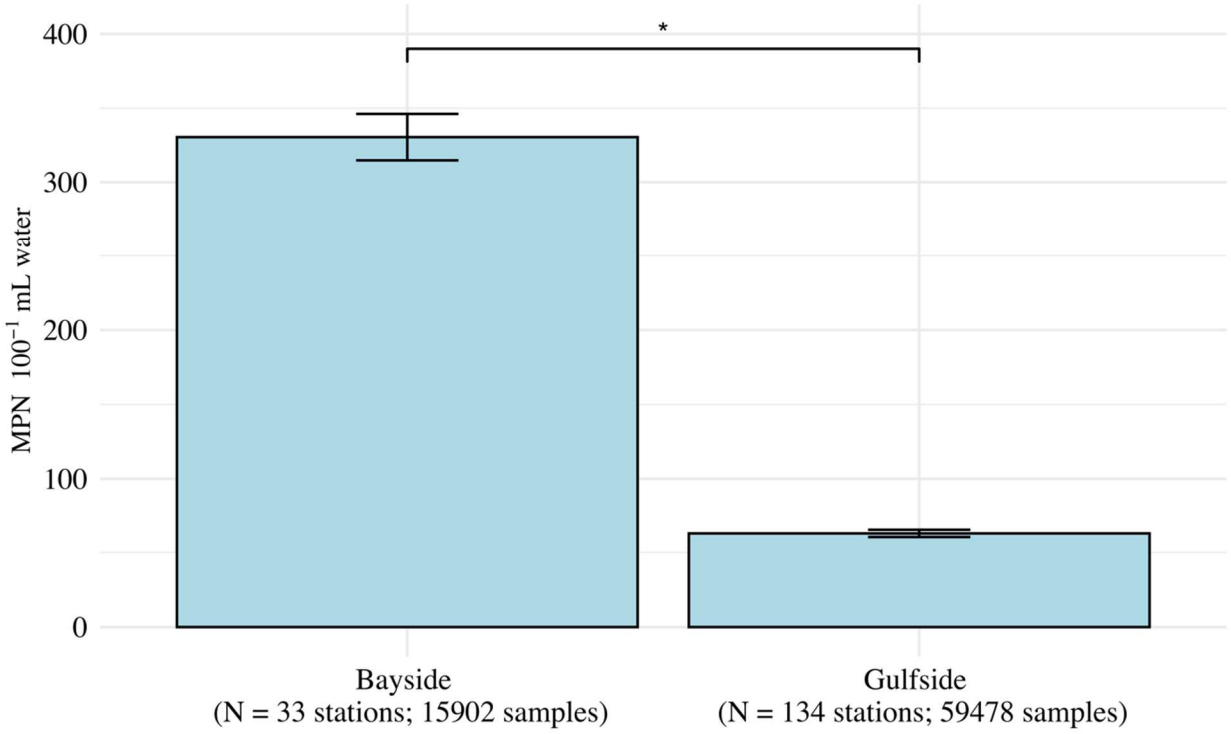

Figure 12. Mean concentrations of enterococci in bayside versus Gulfside water samples. Error bars represent standard error of the mean. Enterococci were significantly higher in bayside sites than Gulfside sites (cendiff test; $p < 0.001$).

Table 11. The number and percentage of enterococci concentration measurements that were above the beach action value (104 MPN 100 mL^{-1}) and the Enterolert test's upper limit of detection (24,196 MPN 100 mL^{-1}) each year. The majority of the exceedances of both values follow an increasing trend over time. *The data from 2020 only included enterococci concentrations from January through mid-May.

	No. and % of exceedances	
Year	**104 MPN 100 mL^{-1}**	**24,196 MPN 100 mL^{-1}**
2009	323 (5.13%)	1 (0.016%)
2010	507 (7.61%)	0 (0%)
2011	327 (4.95%)	0 (0%)
2012	581 (8.98%)	1 (0.015%)
2013	426 (6.77%)	5 (0.079%)
2014	495 (7.76%)	3 (0.047%)
2015	833 (12.31%)	26 (0.074%)
2016	602 (8.75%)	13 (0.189%)
2017	363 (5.74%)	0 (0%)
2018	593 (8.79%)	24 (0.36%)
2019	1,461 (18.84%)	18 (0.23%)
2020*	210 (9.58%)	1 (0.046%)
Total	6,721 (8.91%)	92 (0.122%)

Table 12. Correlations between enterococci concentrations and time (2009-2020), population size (2010-2019), and sea level (2009-2020) in the 10 coastal counties ($p < 0.05$). NA = data not available; ns = not significant.

County	**Time**	**Population size**	**Sea level**
Cameron	-0.0314	-0.0421	0.0237
Jefferson	ns	0.0366	0.0630
Galveston	0.1238	0.1352	0.1294
Kleberg*	ns	NA	ns
Aransas	ns	ns	0.0752
San Patricio	0.0655	0.1025	0.1207
Nueces	0.0383	0.0447	0.0583
Brazoria	0.1581	0.1820	0.1303
Matagorda	0.1754	0.0247	0.1832
Harris	0.2048	0.2137	0.2168

*Data pertaining to Kleberg County was only available from 2009-2011.

Figure 13 shows the temporal and spatial distribution of the total percentage of samples that exceeded the USEPA beach action value throughout the Texas coast. Kendall's tau correlation was computed to test relationships between time and the percentage of samples that exceeded the beach action value (Figure 14A; tau: 0.48, $p < 0.05$) and the percentage of all samples exceeding the upper detection limit (Figure 14B; tau: 0.42; $p < 0.1$). A linear model was generated to show the relationship between the Kendall's tau correlation coefficients (between enterococci and time for each county) and the percentage of samples that exceeded the USEPA beach action value (Figure 15A). Based on this model, counties that did not experience a significant correlation with time (i.e., Kendall's tau correlation coefficient = 0) exceeded the beach action value 5.36% of the time, whereas counties where tau = 0.2 exceeded the beach action value 20.42% of the time.

In general, higher levels of enterococci were correlated with increasing population size in the majority of counties (Table 12). Aransas and Cameron Counties were exceptions, as the former did not experience a significant correlation, and the latter experienced an inverse relationship between the two variables. The correlations between enterococci and population size closely resembled the correlations between enterococci and time, which is unsurprising, given that the population size generally increased over time. A linear model was generated to show the relationship between the Kendall's tau correlation coefficients (between enterococci and population size for each county) and the percentage of samples that exceeded the USEPA beach action value (Figure 15B). Based on this model, counties that did not experience a significant correlation with population size (tau = 0) can be expected to exceed the limit 7.35% of the time, whereas counties where tau = 0.2 can be expected to exceed the limit 17.42% of the time.

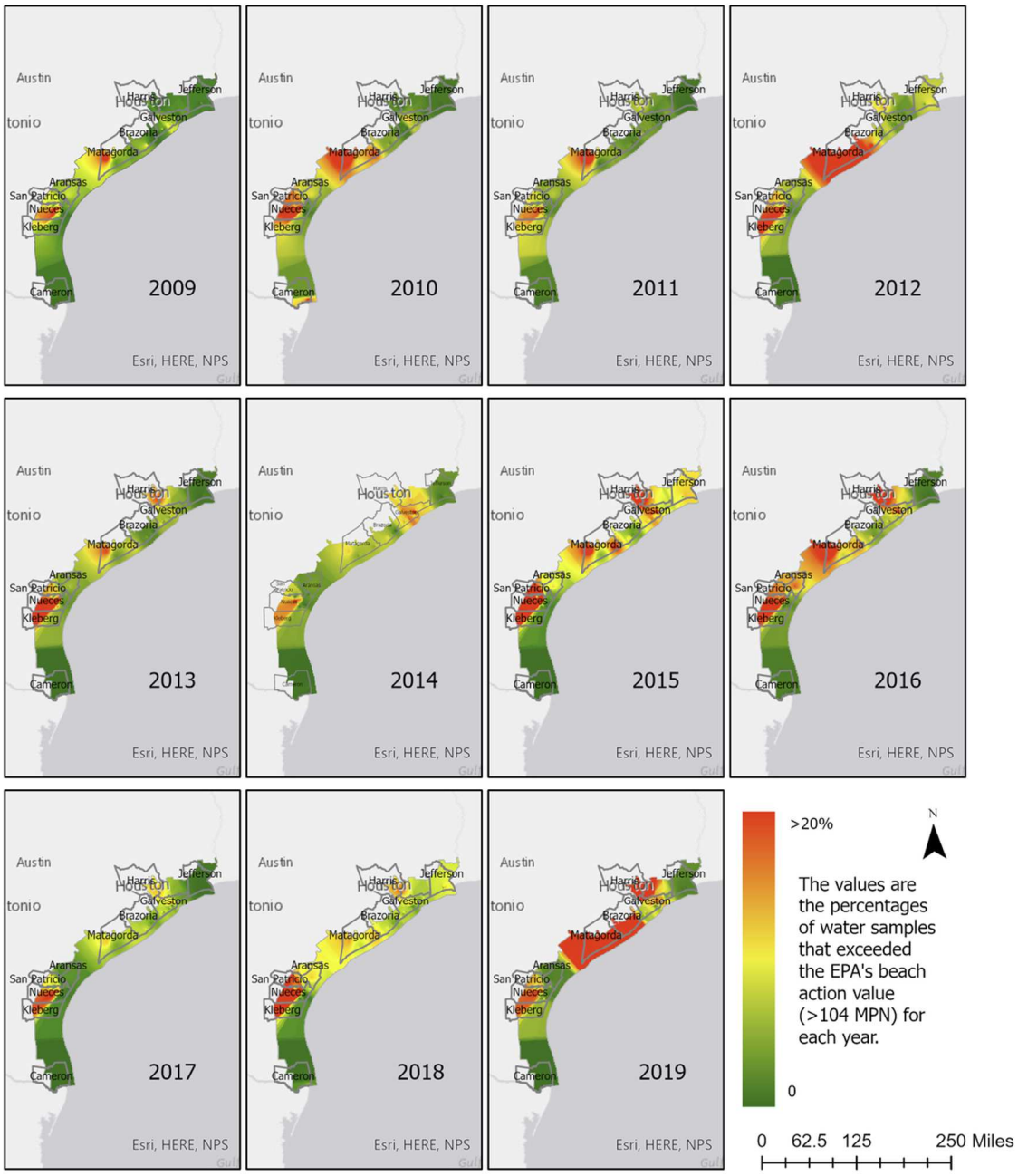

Figure 13. Temporal and spatial trends of water samples that exceeded the USEPA beach action value across coastal Texas from 2009-2019.

Table 12 shows that nine of the 10 counties experienced significant correlations between enterococci concentrations and sea level. Importantly, the only county that did not have significant results was Kleberg County, which only contained data from 2009 to 2011. A linear model was generated to show the relationship between the Kendall's tau correlation coefficients (between enterococci and sea level for each county) and the percentage of samples that exceeded the USEPA beach action value (Figure 15C). Based on this model, counties that do not experience a significant correlation with sea level (tau = 0) can be expected to exceed the limit 1.54% of the time, whereas counties where tau = 0.2 can be expected to exceed the limit 20.23% of the time.

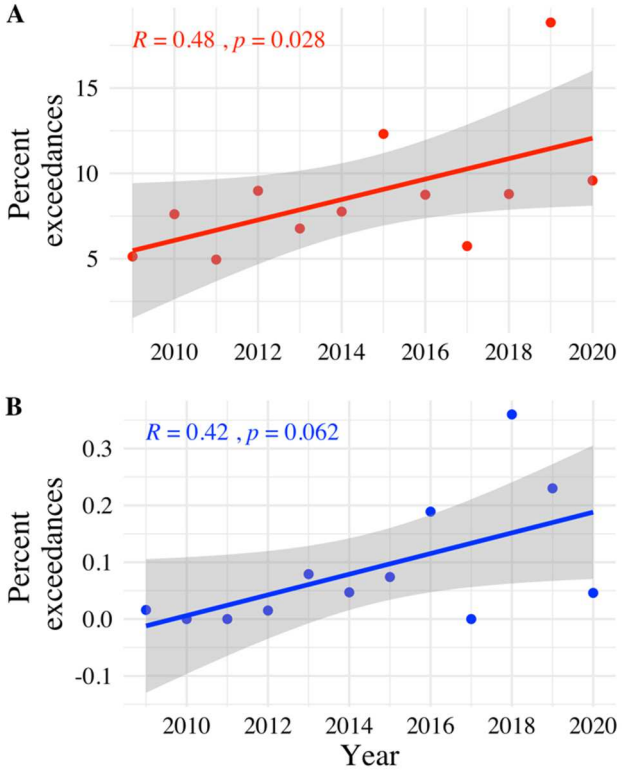

Figure 14. Relationships (Kendall's tau correlation and significance) between the percentage of samples that exceeded the USEPA limit of 104 MPN 100 mL^{-1} and time **(A)** and the percentage of samples that exceeded the Enterolert test's upper limit of detection (24,196 MPN 100 mL^{-1}) and time (**B**) each year.

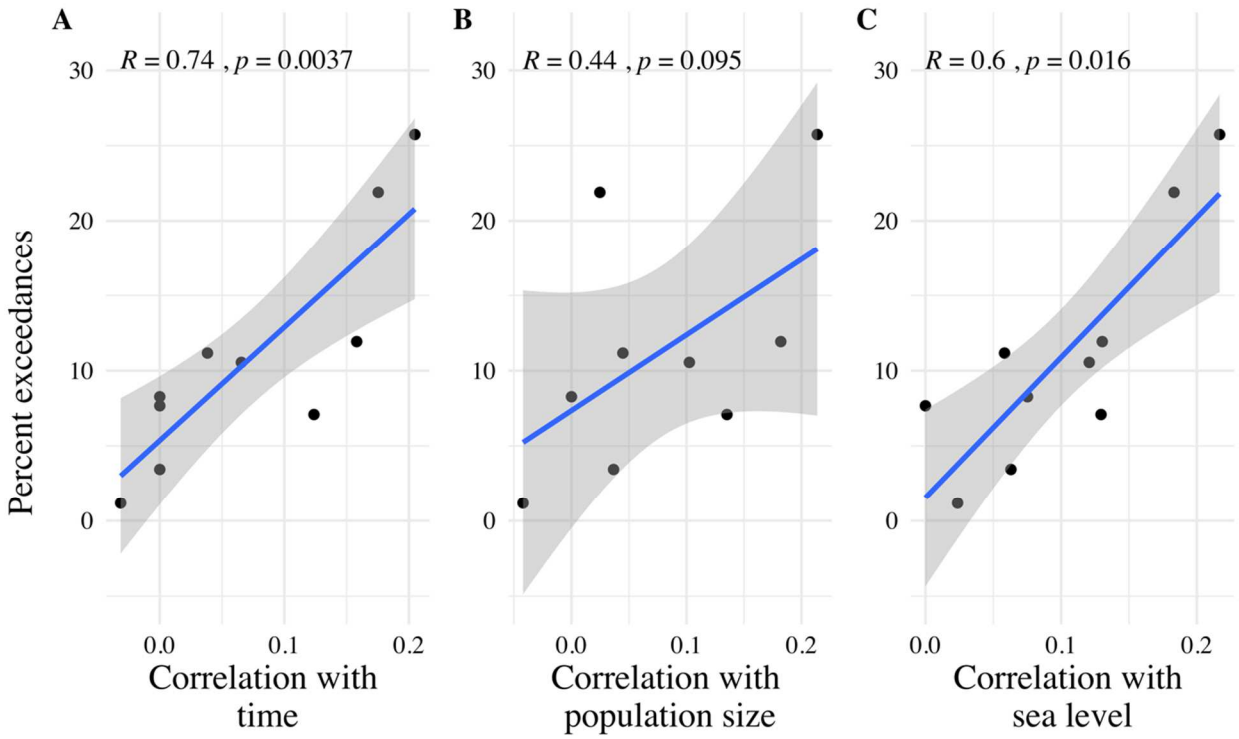

Figure 15. Relationships between the percentage of samples in the 10 counties where enterococci exceeded the USEPA beach action value of 104 MPN 100 mL^{-1} and the Kendall's tau correlation coefficients. The blue lines show the linear relationships between the values and the 95% confidence intervals are shown in gray. **A** = Correlation with time; counties that do not experience a significant correlation with time (tau = 0) can be expected to exceed the limit 5.36% of the time, whereas counties where tau = 0.2 can be expected to exceed the limit 20.42% of the time. **B** = Correlation with population size; counties that do not experience a significant correlation with population size (tau = 0) can be expected to exceed the limit 7.35% of the time, whereas counties where tau = 0.2 can be expected to exceed the limit 17.42% of the time. **C** = Correlation with sea level; counties that do not experience a significant correlation with sea level (tau = 0) can be expected to exceed the limit 1.54% of the time, whereas counties where tau = 0.2 can be expected to exceed the limit 20.23% of the time.

County-specific analyses.

Data from three counties identified as containing 'hotspots' of bacterial pollution (e.g., Harris, Matagorda, and Brazoria) were analyzed more closely due to exceptionally high enterococci values recorded in these regions in 2019. Four beaches (Quintana, Bryan, Sargent, and Palacios) had significantly higher concentrations of enterococci throughout the 11-year

timeframe than the other beaches in these counties (cendiff test; $p < 0.001$). Interestingly, a few occurrences of elevated concentrations in Brazoria County (recorded on 5/11/12, 4/13/15, 3/1/16, and 8/16/19) were preceded or followed by similar increases in neighboring Matagorda County (recorded on 5/23/12, 4/14/15, 3/15/16, and 8/28/19; Figure 16). Three of the four increases in Brazoria were recorded after sporadic rainfall, with the exception of the increase on 3/1/16, whereas the increase recorded days later in Matagorda was not preceded by rainfall. Harris and Galveston Counties also experienced coinciding elevated enterococci levels on three recorded dates (6/02/2016, 9/12/2018, and 10/16/2019) that were preceded by sporadic rainfall.

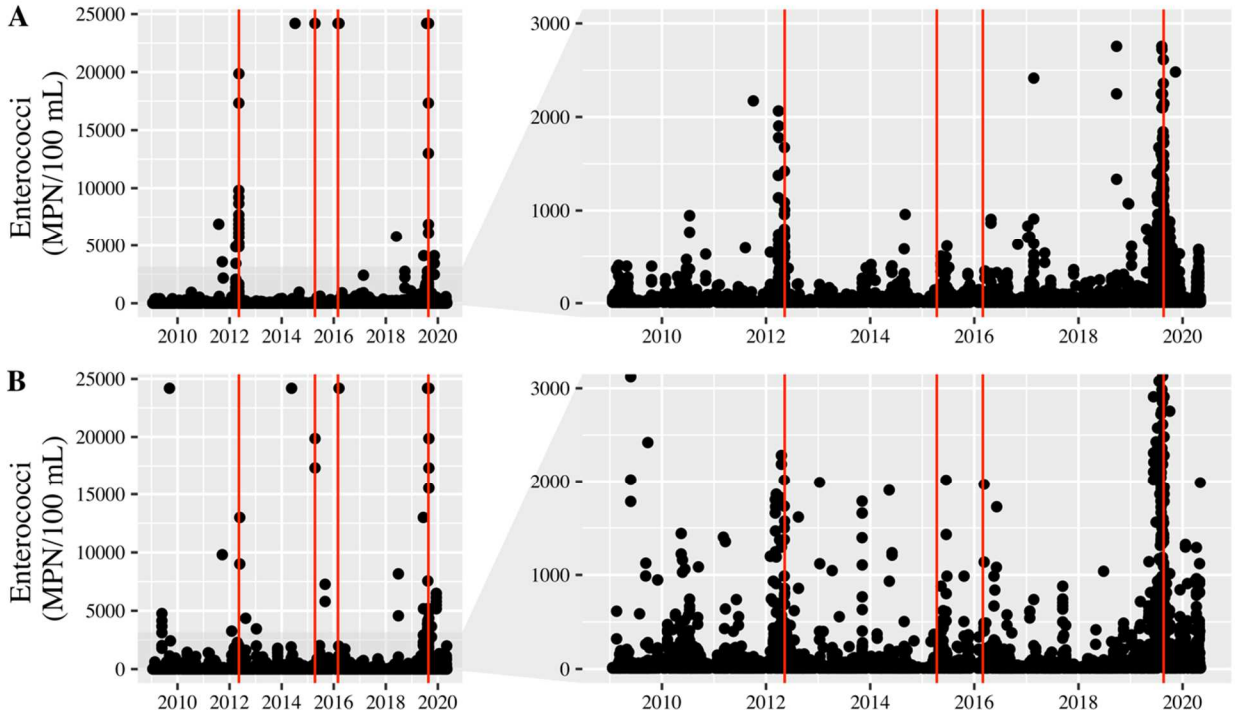

Figure 16. Concentrations of enterococci in Brazoria County (**A**) and Matagorda County (**B**) from 2009-2020. The panels on the right show a closer look at the enterococci concentrations that are under 3,000 MPN 100 mL^{-1}. The red lines coincide with major increases in enterococci.

In 2019, several of the water samples obtained from Brazoria, Matagorda, and Harris Counties had unusually high enterococci levels and were therefore subjected to additional analyses. The concentrations of enterococci in these three counties were significantly higher in 2019 compared to other years (Table 13; cendiff test; $p < 0.001$). All of the beaches in these counties exceeded the beach action value more than 38% of the time, and several beaches (Sargent, Matagorda Jetty Park, Palacios, and Sylvan) exceeded the value more than 50% of the time (Table 13). The vast majority of the elevated enterococci levels were recorded in June, July, and August of 2019. Unusually high levels, approaching or exceeding the Enterolert test upper limit of detection (24,196 MPN 100 mL^{-1}), were recorded at Follet's Island on 8/16/19, Surfside on 8/19/19, Bryan Beach on 8/21/19, Sargent Beach on 8/28/19, and Matagorda Jetty Park the following day. In an attempt to explain the unusually high enterococci levels, episodes of elevated levels in Brazoria from 2018 to 2020 were compared with the partial list of reported SSOs. Although some of the increases coincided with SSOs, the relationship between enterococci concentrations and SSOs was not significant (cendiff test; $p > 0.1$).

Generalized linear models (GLMs).

To identify drivers of enterococci, GLMs were computed for Brazoria, Matagorda, and Harris Counties. The GLM for Brazoria County included beach name, month, population size, and sea level, and only explained 1.3% of the variation between enterococci levels (adjusted R^2 0.013; $p < 0.001$). The GLM for Matagorda County included bayside versus Gulfside site classification, month, population size, sea level, and time. This model explained only 3.9% of the variation between the enterococci levels (adjusted R^2 0.039; $p < 0.001$). The GLM for Harris

County included sea level and population size and explained only 1.1% of the variation between enterococci levels (adjusted R^2 0.011; $p < 0.001$).

Table 13. Comparison of the beaches in Brazoria, Matagorda, and Harris Counties. The data is split by year; the 2019 rows contain data only from the year 2019, whereas the remaining rows contain data from 2009-2020 excluding 2019. The table shows mean, median, minimum, and maximum values of enterococci (MPN) and how often enterococci exceeded the USEPA limit of 104 MPN 100 mL^{-1}. Follet's Island: N = 5 sampling stations; Surfside Beach: N = 8; Quintana Beach: N = 2; Bryan Beach: N = 1; Sargent Beach: N = 3; Matagorda Jetty Park: N = 4; Palacios Beach: N = 2; Sylvan Beach: N = 2. The enterococci concentrations in all three counties were significantly higher in 2019 compared to other years (cendiff test; $p < 0.001$).

County	Beach	Mean	Med.	Min.	Max.	% exceed.
Brazoria	Follet's Island	49.06	10	10	9,208	4.53
	Follet's Island (2019)	361.31	63	10	24,196	44.47
	Surfside Beach	73.33	10	10	24,196	4.87
	Surfside Beach (2019)	201.81	52	10	24,196	38.50
	Quintana Beach	161.09	20	10	24,196	17.99
	Quintana Beach (2019)	802.92	63	10	24,196	46.43
	Bryan Beach	64.94	10	10	3,436	10.60
	Bryan Beach (2019)	512.52	63	10	12,997	41.46
Matagorda	Sargent Beach	120.32	20	10	24,196	15.62
	Sargent Beach (2019)	755.48	164.5	10	24,196	58.93
	Matagorda Jetty Park	76.59	10	10	24,196	9.76
	Matagorda Jetty Park (2019)	826.19	115	10	24,196	51.75
	Palacios Beach	222.34	36	10	24,196	24.71
	Palacios Beach (2019)	744.01	124.5	10	19,860	53.85
Harris	Sylvan Beach	371.35	20	10	24,196	18.36
	Sylvan Beach (2019)	755.53	193	10	8,664	57.23

Discussion.

Since 1960, the Gulf Coast region of the United States has experienced a rapid population increase that is more than double the rate of national population growth. Over the past two decades, coastal watersheds in Texas have also experienced significant human population growth. From 1997 to 2012, the population in Texas coastal counties increased by 29% (Texas Land Trends 2014), and projections suggest that there will be an additional 34% population increase by 2050 (TSDC 2018). In many other regions worldwide, a similar increasing human footprint has led to symptoms of water quality degradation, namely increasing bacterial pollution and symptoms of eutrophication (Cloern 2001, Walters et al. 2011, Wu and Jackson 2016). A recent study of multidecadal trends in the water quality of Texas estuaries found localized evidence of eutrophication, primarily in estuaries with highly urbanized watersheds (e.g., Galveston Bay and Oso Bay) as well as an estuary with a sparsely populated but agriculturally intensive watershed (i.e., Baffin Bay) (Bugica et al. 2020). However, that study did not assess patterns or quantify changes in fecal bacterial pollution, the presence of which can result in severe economic losses and cause serious health burdens in coastal regions across the globe (Malham et al. 2014).

This study provides the first comprehensive decadal assessment of fecal bacteria pollution across coastal Texas. Previous short-term bacteria analyses have targeted specific regions such as impaired segments in Corpus Christi Bay (Nicolau et al. 2011, Nicolau and Hill 2013). Previous short-term studies have also investigated probable sources of fecal pollution in impaired segments (Mott et al. 2010, Turner and Elledge 2018, Turner et al. 2019, Powers et al. 2020). Long-term, coastwide data assessments, such as this study, are needed to identify the drivers of fecal bacteria contamination to guide proactive management and watershed restoration

efforts. For instance, in south Florida, a 10-year analysis of 7,422 historical data points revealed that exceedances were 2,475 times more likely during high tide (Aranda et al. 2016). Another long-term study in California analyzed six years of historical water quality data and determined discharge from a local lagoon to be a significant source of fecal pollution (Riedel et al. 2015).

In this multi-year study, an analysis of 75,380 historical data points (spanning 11 years, 10 coastal counties, 66 beaches, and 169 sampling stations) clearly demonstrates that nearly one-third of Texas beaches (N = 22) and half of coastal Texas counties (N = 5) experienced USEPA beach action value exceedances on a regular basis (i.e., more than 10% of the time). Bayside beaches also experienced higher enterococci concentrations compared to beaches located on the Gulf of Mexico (defined as Gulfside beaches). The higher enterococci concentrations at bayside sites could be attributed to closer proximity to mainland population centers (e.g., Port Arthur, Houston, Port Lavaca, Corpus Christi, and Brownsville) and reduced dilution or flushing as multiple Texas bays and lagoons exhibit limited freshwater inflow and little exchange with the Gulf of Mexico (reviewed by Montagna et al. 2013). A previous long-term study along the Florida coast showed similar results, with geomorphology acting as a strong driver of FIB exceedances; beaches located on the bayside were four times more likely to exceed the USEPA's limit than beaches located on the open coast (Donahue et al. 2017). Nearby rivers and canals were also associated with elevated FIB levels, likely due to the transport of FIB from inland sources (Donahue et al. 2017). Another recent long-term data analysis at the German Baltic coast similarly revealed that bays and lagoons pose higher microbial risks compared to open-water areas (Buer et al. 2018). The authors of that study postulated that bacterial pollution at bayside sites was likely a multifactorial problem owing to the resuspension of bacteria from sediments in

shallow water, prolonged bacterial survival in turbid water, and the heightened mainland impact of stormwater inflows.

A key finding from this study was that enterococci concentrations increased over time. The majority of beach action value exceedances (60%) and the vast majority of the Enterolert test upper limit exceedances (89%) have occurred in the past six years (2015-2020). Additionally, the variability between the samples collected in recent years (i.e., 2016-2019) was nearly twice as high as the variability between samples collected in previous years (i.e., 2009-2015). The high level of variability in these samples coincided with large-scale El Niño-Southern Oscillation (ENSO) events, which occurred in 2014-2016 and 2018-2019 (NOAA; https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php). The increase in enterococci concentrations over time coincided with temporal trends in two important broad-scale environmental drivers: population size and sea level. For the majority of counties, population size was directly correlated with enterococci concentrations. This result is consistent with findings of previous studies; for instance, an analysis of water quality in 14 California coastal sites revealed a direct correlation between FIB levels and urbanization (i.e., population size and impervious surface coverage) (Walters et al. 2011). Cameron was the only county where FIB levels were inversely correlated with time and population size; this county also had the least impaired water, with less than 2% of samples exceeding the USEPA beach action value. Two watershed protection plans in Cameron County (in Arroyo Colorado and the Lower Laguna Madre) could be contributing to the lower levels of fecal bacteria pollution in this region (TCEQ 2020b, TCEQ 2020c). Importantly, Texas' rapid coastal population growth may be predictive of future exceedances along nearly the entire coastline, given that coastal population size is predicted to increase dramatically by 2050 (TSDC 2018).

Throughout the 11-year timespan of this study, enterococci concentrations in eight of the 10 counties were correlated with sea level. Brazoria, Matagorda, and Harris, in particular, exhibited a stronger relationship with sea level, and all three counties have been identified as vulnerable to coastal flooding and rising sea levels (Strauss et al. 2014). Harris County, one of the counties that comprises the Greater Houston region, experienced the strongest relationship between enterococci and sea level. This finding is particularly concerning since this region is also acutely vulnerable to catastrophic flooding and water damage, as evidenced by the recent impacts from Hurricane Harvey (Valle-Levinson et al. 2020). A recent study in South Florida revealed that thousands of septic systems, which are a potential source of fecal pollution (Humphrey et al. 2018), are malfunctioning and contributing to fecal pollution due to sea level rise (Elmir 2018). The Fourth National Climate Assessment predicted sea level rise in the Gulf Coast will be above the global average (i.e., greater than 1-4 feet by 2100) (USGCRP 2017); thus, future research should be focused on the distribution of septic systems in coastal Texas and the impact that rising sea levels have on their integrity.

The water quality in Brazoria, Matagorda, and Harris Counties was of particular concern due to 1) a history of elevated enterococci concentrations and 2) recent episodes of unusually high enterococci levels. During the summer of 2019, the episodes experienced in these counties were unique (i.e., statistical outliers) when compared with other counties across the 11-year dataset. That several beaches in Brazoria and Matagorda experienced similar temporal trends implies that a common set of conditions contributed to the elevated levels. For instance, sea level, water temperature, and precipitation in these counties were higher in June through August of 2019 than in previous years (NOAA; https://ncdc.noaa.gov/temp-and-precip/us-maps/). It is possible that one of these factors or some combination of them were driving these episodes;

however, further study is needed to identify common conditions and drivers. GLMs were constructed to explain the variance in enterococci concentrations observed in Brazoria, Matagorda, and Harris, but the weakness of these models suggests that drivers of these episodes require further characterization. It is possible that SSOs contributed to these elevated FIB levels, but a more complete count of SSOs and their magnitude is needed to test this hypothesis. Evaluating the energetics of the coastal sites could strengthen these models, as wave energy has been identified as a significant driver of FIB levels (Feng et al. 2016). Future studies would benefit from the inclusion of additional metadata (e.g., more robust, daily sea level data, water temperature, salinity, turbidity, recent rainfall, winds, and tide) that may improve the power of these models.

In conclusion, many Texas beaches are negatively impacted by fecal bacteria pollution. Beaches in Harris, Brazoria, Matagorda, Nueces, and San Patricio Counties stand out as 'hotspots' of bacteria pollution. Additionally, enterococci concentrations have been increasing with time, population size, and sea level, with beaches in Harris, Brazoria, and Matagorda exhibiting the strongest correlations. The links between bacterial pollution, population size, and sea level are an indication that seawater inflow and infiltration could be associated with the degradation and failure of sewage collection and treatment systems in coastal regions. However, future evaluations of local sewage collection and treatment systems are needed to further explore this hypothesis. Additional variables should also be explored, since the low correlations detected in many counties implies the water quality impairment is multifactorial. For instance, additional geomorphic factors (i.e., proximity to rivers, canals, and other inland pollution sources) could shed light on water quality drivers, particularly in open coast sites (Donahue et al. 2017). Seeing that the most affected counties (e.g., Harris, Brazoria, and Matagorda) are particularly vulnerable

to future population growth and sea level rise, targeted studies are urgently needed to pinpoint the sources and drivers of fecal bacteria pollution. The findings from this study may be relevant in a global context, as sea level rise and population growth are not limited to the Gulf Coast. Policies in regions with impaired water quality may benefit from focusing on the maintenance and repair of coastal infrastructure that is susceptible to damage from urbanization and rising sea levels.

Acknowledgements.

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SUMMARY

Urbanized coastal regions are acutely vulnerable to fecal pollution. Pathways in which this pollution can reach the marine environment include wastewater treatment plant (WWTP) effluent, leaking and overwhelmed sewage systems, and stormwater runoff (Shuval 2003, Sauer et al. 2011, Sowah et al. 2017). The presence of fecal pollution in the environment is particularly concerning, as it can disrupt natural ecosystems and lead to harmful infections in humans and other animals (Lipp et al. 2001, Benskin et al. 2009, Soller et al. 2010, Wear and Thurber 2015). Over 4 billion recreational aquatic activities are estimated to occur annually in the United States, resulting in 90 million infections caused by contact with polluted waters (DeFlorio-Barker et al. 2018). Due to the vast health and economic burdens caused by fecal pollution, routine and reliable water quality monitoring is necessary to ensure the public is informed when waters are contaminated.

Historically, fecal pollution has been measured through the detection and quantification of fecal indicator bacteria (FIB), such as enterococci. Previous studies have detected a link between elevated enterococci and a human health risk (Wade et al. 2005, Colford Jr et al. 2012, Arnold et al. 2017), although the utility of enterococci is not straightforward in locations impacted by nonpoint source pollution (Fleisher et al. 2010, Sinigalliano et al. 2010, Saingam et al. 2020). Moreover, enterococci are not an ideal indicator of human fecal waste as they are not human-specific and some strains can survive and multiply in the environment (Badgley et al. 2010, Mote et al. 2012, Boehm and Sassoubre 2014). Several studies support the quantitative detection of host-associated molecular markers in addition to the detection of traditional FIB (Flood et al. 2011, Boehm et al. 2013, Mika et al. 2014). The accurate determination of the

pollution's original source or host is critical to the design and implementation of mitigation and management strategies.

The purpose of this dissertation was to investigate the sources, drivers, and impacts of fecal pollution in coastal Texas. The first objective of this study was to conduct a multi-dimensional water quality assessment in Corpus Christi Bay. To detect fecal waste, we quantified enterococci and three host-associated molecular markers indicative of waste from humans, canines, and gulls. We also investigated the relationship between these fecal indicators and relevant environmental variables (e.g., salinity, turbidity, temperature, DO, pH, antecedent rainfall), as well as the occurrence of antimicrobial resistant *Enterococcus* isolates. Additionally, we assessed the structure and diversity of the microbial community at large as an indicator of water quality. The second objective was to conduct a similar targeted study of the water quality in Little Bay. Specifically, we investigated if a WWTP catchment was a source of fecal pollution and if rainfall was a driver of fecal pollution (i.e., enterococci and three host-associated markers), and if that pollution decreased along an estuarine ecocline. We also assessed how the microbial community diversity shifted along the ecocline and in response to rainfall. Finally, our third objective was to identify long-term trends and drivers of elevated enterococci levels throughout coastal Texas. We investigated the role of local hydrodynamic conditions (i.e., bayside or Gulfside sites), sanitary sewer overflows (SSOs), rainfall, population size, and sea level as potential drivers of enterococci.

Our results showed that antecedent rainfall was a driver of fecal pollution in Corpus Christi Bay, where elevated levels of enterococci were detected immediately following rainfall events. However, higher levels of enterococci were not correlated with an increase in antimicrobial-resistant *Enterococcus* isolates, nor were they correlated with human, canine, or

gull waste. Fecal waste from all three of these hosts was omnipresent throughout the study, although the human and gull markers were detected in significantly higher concentrations under dry-loading conditions. The reduction in these markers after rainfall could be attributed to dilution from large volumes of freshwater during storm events, which has been observed previously in other highly urbanized settings (Santiago-Rodriguez et al. 2012). The continuously elevated level of the human marker (HF183) is the greatest concern for human health, as it is the most likely to be accompanied by known human pathogens (Lipp et al. 2001a, Griffin et al. 2003). The constant presence of this marker during wet- and dry-loading conditions suggests it may be originating from leaking sanitary sewer systems.

Contrasting results were observed in Little Bay, where enterococci were not impacted by rainfall; rather, enterococci decreased in concentration along the estuarine ecocline (i.e, Tule Creek, Little Bay, Aransas Bay). Unlike the results in Corpus Christi Bay, rainfall was a significant driver of human fecal pollution, evidenced by the increase in the concentration of HF183 following rainfall events. Interestingly, the human marker was not higher in locations immediately downstream of a local WWTP, and it did not decrease along the ecocline, suggesting that human waste originated from leaks in underground sewage collection systems rather than WWTP effluent. In both systems (i.e., Little Bay and Corpus Christi Bay), none of the environmental variables (e.g., salinity, turbidity, temperature, DO, pH) acted as a strong predictor for the host-associated markers, indicating that additional factors not measured in these studies could be influencing the levels of these fecal indicators.

Although enterococci and all three of the fecal markers were detected in nearly every sample from both Corpus Christi Bay and Little Bay, the concentrations detected in Corpus Christi Bay were nearly an order of magnitude higher than the concentrations detected in Little

Bay. Enterococci frequently exceeded the USEPA's beach action value (104 MPN 100 mL^{-1}) (USEPA 2012) in both locations, and the human marker frequently exceeded a suggested risk-based threshold of 525 gene copies 100 mL^{-1} (determined by Boehm and Soller 2020) in Corpus Christi Bay. It is worth noting that this threshold does not account for combined fecal waste from humans and additional sources. Since canine and gull waste were also detected in conjunction with human waste in the majority of samples in both systems, the risk-based threshold may need to be more stringent in these locations to accurately represent the human health risks associated with this fecal pollution.

In both Corpus Christi Bay and Little Bay, microbial diversity (i.e., beta diversity) shifted in response to rainfall. In Corpus Christi Bay, this compositional change was also accompanied by a significant decrease in alpha diversity. Together, these results suggest that rainfall was acting as a pulse disturbance; future research that further characterizes the extent of this disturbance may determine the resiliency and recovery rates of the communities. As higher levels of diversity have been linked with stability and resiliency, the reduction in diversity observed in this study could negatively affect the health of the overall systems, including the higher trophic organisms dependent on the microbial communities (Girvan et al. 2005, Singh et al. 2014, Feng et al. 2017).

The long-term analysis of water quality throughout coastal Texas revealed that one-third of the beaches experienced enterococci concentrations that exceed the USEPA's beach action value on a regular basis. In the vast majority of counties, enterococci concentrations have been increasing over time and were correlated with sea level and population size. These direct relationships suggest that rising sea levels may be contributing to the degradation of sewage collection systems and septic systems and leading to elevated levels of fecal pollution. Although

significant, the weak correlations between enterococci, population size, and sea level suggest other factors that were not measured are also influencing enterococci levels. For instance, enterococci have been correlated with variables such as water temperature, turbidity, salinity, and UV exposure on a short-term scale (Shibata et al. 2004, Coulliette and Noble 2008); relationships with these or similar variables could be masking larger-scale trends between enterococci and sea level.

Although our results indicate that enterococci are not accurate in representing human, canine, or gull fecal waste in Corpus Christi Bay and Little Bay, these results may be system specific. Thus, enterococci should not be dismissed as an indicator of fecal pollution in other coastal environments without further study. Rather, future water quality analyses would benefit from the inclusion of host-associated fecal waste markers as part of a larger, multivariate assessment. Several studies have supported the use of enterococci as a human health risk indicator (Wade et al. 2005, Colford Jr et al. 2012, Arnold et al. 2017); therefore, independent studies should be conducted to determine the accuracy of enterococci in different watersheds throughout coastal Texas. This study identified several regions with exceptionally high enterococci levels that may indicate a health concern, where future studies should be focused (i.e., Harris, Matagorda, Brazoria). Importantly, these regions with the most impaired water quality are all vulnerable to climate change, including sea level rise and increased storm events, both of which may contribute to increased fecal pollution loading in the future.

The results presented here indicate that several regions throughout coastal Texas experience impaired water quality due to fecal pollution. These results also identify specific fecal sources that are impacting Corpus Christi Bay and Little Bay, and highlight that enterococci are not an accurate indicator of fecal waste from humans, canines, or gulls in these systems. The

need for accurate assessments of water quality monitoring and source tracking is essential, given the projections of climate change (i.e., increased storm events and sea level rise) and coastal population growth and urbanization, all of which can contribute to deteriorating water quality.

Coastal management stands to benefit from data that describes the drivers of poor water quality in specific bays and watersheds. Long-term data that is descriptive of entire coastlines could be instrumental in guiding coastal management policy in collaboration with state agencies and initiatives, such as the Texas Clean Coast Program. Given the results presented throughout this dissertation, we recommend that coastal managers consider vulnerability to population growth and sea level rise when developing future policy. In particular, it would be prudent to direct resources toward the repair, maintenance, and climate-readiness of sewage collection and septic systems in densely populated, low-lying coastal regions. Furthermore, routine water quality monitoring could be strengthened by increasing the spatial and temporal resolution of sample collection, particularly in regions that experience high levels of human interaction. Measurement of not only enterococci, but also host-associated molecular markers, the presence of pathogens, and relevant environmental variables could facilitate the creation of a fecal pollution index that provides information regarding health risks. Currently, beach closures are recommended based on occurrences of enterococci exceeding the beach action value; however, these occurrences do not always coincide with a health risk.

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Appendix 1. Summary statistics for the beaches classified as having "low" concentrations of enterococci (< 5% of samples exceeded the USEPA beach action value of 104 MPN 100 mL^{-1} water) and Kendall's tau correlation coefficient between enterococci concentrations and time (R, $p < 0.05$; ns = not significant). Enterococci concentrations were measured as CFU or MPN 100 mL^{-1} water (Max. = maximum, Med. = median, Avg. = average). Minimum concentrations for every beach were equivalent to < 10 MPN. *Sites that had enterococci concentrations exceeding the upper limit of detection for Enterolert testing (24,196 MPN 100 mL^{-1}).

Beach name	Max.	Med.	Avg.	No. exceedances	No. samples	% exceedances	R
Andy Bowie Park	344.00	10	12.30	1	848	0.12	ns
Park Road 100 Access Point #4	239.00	10	12.03	2	424	0.47	ns
Park Road 100 Access Point #3	478.00	10	12.86	2	424	0.47	ns
City of South Padre Island	560.00	10	13.20	24	3,852	0.62	-0.017
Park Road 100 Access Point #6	945.00	10	14.65	6	850	0.71	ns
Isla Blanca Park	222.00	10	13.42	7	855	0.82	ns
Mustang Island State Park	341.00	10	12.60	18	2,120	0.85	0.071
Atwood Park	764.00	10	16.38	9	853	1.06	-0.049
Mustang Island	340.00	10	13.04	5	428	1.17	0.097
Park Road 100 Bay Access #2	2,252.50	10	21.24	7	427	1.64	-0.053
JP Luby Park	2,392.00	10	23.60	41	1,733	2.37	0.032
Boca Chica State Park	1,645.00	10	23.51	72	2,609	2.76	-0.065
McFaddin NWR	987.00	10	27.55	71	2,429	2.92	ns
*Port Aransas Park	24,196.00	10	37.88	53	1,744	3.04	ns
Rollover Pass East	1,990.00	10	27.46	48	1,273	3.77	0.164
Port Aransas – South	2,224.00	10	25.42	36	882	4.08	0.056
Padre Balli Park	5,475.00	10	32.61	150	3,536	4.24	0.096
Apffel Park	1,480.00	10	28.03	39	868	4.49	0.053
Sea Rim State Park	1,723.00	10	41.52	53	1,179	4.50	ns
Packery Channel Park	14,136.00	10	81.84	19	421	4.51	0.115
Lighthouse Lake	12,033.00	10	57.62	18	382	4.71	ns
West End Galveston – Sea Isle	13,400.00	10	56.68	43	894	4.81	0.121

Appendix 2. Summary statistics for the beaches classified as having "medium" concentrations of enterococci (5.1 – 10% of samples exceeded the USEPA beach action value of 104 MPN 100 mL^{-1} water) and Kendall's tau correlation coefficient between enterococci concentrations and time (R, $p < 0.05$; ns = not significant). Enterococci concentrations were measured as CFU or MPN 100 mL^{-1} water (Max. = maximum, Med. = median, Avg. = average). Minimum concentrations for every beach were equivalent to < 10 MPN. *Sites that had enterococci concentrations exceeding the upper limit of detection for Enterolert testing (24,196 MPN 100 mL^{-1}).

Beach name	Max.	Med.	Avg.	No. exceedances	No. samples	% exceedances	R
Galveston Island State Park – Beach	1,900.00	10	27.4	45	896	5.02	0.097
West End Galveston – San Luis Pass	4,000.00	10	31.28	45	890	5.06	0.157
West End Galveston – SG/B Beach	1,700.00	10	31.04	71	1,344	5.28	0.122
Kaufer-Hubert Memorial Park #3	1,995.00	9	45.38	6	113	5.31	ns
Crystal Beach – Clara St.	2,420.00	10	27.95	96	1,765	5.44	0.204
West End Galveston – Pirates Beach	9,678.00	10	421.86	101	1,791	5.64	0.113
West End Galveston – Jamaica Beach	8,200.00	10	58.93	26	451	5.76	0.112
Crystal Beach – Gulf Shores	19,863.00	10	62.94	56	890	6.29	0.209
Crystal Beach – Seadrift	2,909.00	10	42.2	28	443	6.32	0.206
*North Beach	24,196.00	10	108.53	111	1,736	6.39	ns
Stewart Beach	5,000.00	10	54.45	91	1,358	6.70	0.042
West End Galveston – Indian Beach	5,000.00	10	69.02	32	457	7.00	0.097
Kaufer-Hubert Memorial Park #2	905.00	6	33.54	8	113	7.08	ns
*West End Galveston – Dellanera Park	24,196.00	10	126.56	64	893	7.17	0.117
Galveston Seawall – 25 St.	5,790.00	10	59.3	133	1,823	7.30	0.063
Crystal Beach – O'Neill Road	3,080.00	10	63.99	33	449	7.35	0.224
*Galveston Seawall – 45 St.	24,196.00	10	89.16	224	2,746	8.16	0.075
Rockport Beach Park	19,863.00	10	107.46	145	1,754	8.27	ns
Riviera Beach Pier	1,820.00	5	61.5	9	104	8.65	ns
Rollover Pass West	2,910.00	10	52.46	38	438	8.68	0.183
Galveston Seawall – 61 St.	10,500.00	11	72.2	129	1,386	9.31	0.054
Kaufer-Hubert Memorial Park #1	1,571.50	9	60.08	11	113	9.73	ns

Appendix 3. Summary statistics for the beaches classified as having "high" concentrations of enterococci (10.1 – 20% of samples exceeded the USEPA beach action value of 104 MPN 100 mL^{-1} water) and Kendall's tau correlation coefficient between enterococci concentrations and time (R, $p < 0.05$; ns = not significant). Enterococci concentrations were measured as CFU or MPN 100 mL^{-1} water (Max. = maximum, Med. = median, Avg. = average). Minimum concentrations for every beach were equivalent to < 10 MPN. *Sites that had enterococci concentrations exceeding the upper limit of detection for Enterolert testing (24,196 MPN 100 mL^{-1}).

Beach name	Max.	Med.	Avg.	No. exceedances	No. samples	% exceedances	R
JFK Causeway – SW	5,400	10	81.32	45	449	10.02	-0.206
*Surfside	24,196	10	93.63	395	3,879	10.18	0.177
Highway 35 – Nueces Bay #3	4,611	10	61.98	48	455	10.55	0.066
Crystal Beach – West End	17,000	10	118.99	103	932	11.05	0.226
*Follet's Island	24,196	10	100.22	269	2,429	11.07	0.170
Laguna Shores	4,106	15.5	79.85	27	220	12.27	ns
*McGee Beach	24,196	10	151.99	113	917	12.32	ns
*Corpus Christi Marina	24,196	10	332.87	184	1,397	13.17	0.055
*TAMUCC – University Beach	24,196	10	216.49	63	467	13.49	ns
*Galveston Island State Park	24,196	10	248.96	64	441	14.51	0.155
Bryan Beach	12,997	20	135.82	80	517	15.47	0.107
*Texas City Dike	24,196	20	249.00	61	397	15.37	0.124
Port Bolivar – Rettilon Road	20,700	16	201.22	84	495	16.97	0.224
*Matagorda County Jetty Park	24,196	10	201.46	344	2,053	16.76	0.245
Emerald Beach	17,329	15.5	202.78	91	490	18.57	0.086
*Quintana	24,196	20	263.59	196	1,052	18.63	0.093

Appendix 4. Summary statistics for the beaches classified as having "very high" concentrations of enterococci (> 20% of samples exceeded the USEPA beach action value of 104 MPN 100 mL^{-1} water) and Kendall's tau correlation coefficient between enterococci concentrations and time (R, $p < 0.05$; ns = not significant). Enterococci concentrations were measured as CFU or MPN 100 mL^{-1} water (Max. = maximum, Med. = median, Avg. = average). Minimum concentrations for every beach were equivalent to < 10 MPN. *Sites that had enterococci concentrations exceeding the upper limit of detection for Enterolert testing (24,196 MPN 100 mL^{-1}).

Beach name	Max.	Med.	Avg.	No. exceedances	No. samples	% exceedances	R
*Sargent Beach	24,196	20	228.96	377	1,637	23.03	0.206
*Poenisch Park	24,196	20	383.26	133	533	24.95	ns
*Sylvan Beach Park	24,196	30	444.32	225	874	25.74	0.205
*Palacios Pavilion	24,196	41	300.55	353	1,214	29.08	ns
*Cole Park	24,196	31	744.67	750	2,345	31.98	0.066
*Ropes Park	24,196	63	856.55	490	1,203	40.73	0.044