



**A Bacterial Source Tracking Project to Identify Sources of Fecal Pollution at Little Bay:
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Abbreviations

ACND	Aransas County Navigational District
ANOVA	analysis of variance
BMPs	best management practices
CBBEP	Coastal Bend Bays and Estuaries Program
CCNCPHDL	Corpus Christi Nueces County Public Health District Laboratory
cm	centimeter
ddPCR	droplet digital polymerase chain reaction
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FIB	fecal indicator bacteria
in	inch
L	liter
m	meter
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeter mercury
mph	miles per hour
MPN	most probably number
NELAP	National Environmental Laboratory Accreditation Program
NTC	no template control
OTU	operational taxonomic unit
PCoA	principle component analysis
PERMANOVA	permutational multivariate analysis of variance
rRNA	ribosomal ribonucleic acid
SILVA	from Latin silva meaning forest
SIPP	Source Identification Protocol Project
TAMU-CC	Texas A&M University-Corpus Christi
TCEQ	Texas Commission on Environmental Quality
TGLO	Texas General Land Office
USA	United States of America
WWTP	wastewater treatment plant
µm	micrometer
µM	micromolar
µS	micro seconds

Summary

Fecal contamination poses a serious threat to marine environments, and consequently can impair recreational water quality. In response to stakeholder concerns of elevated levels of enterococci in Little Bay, this bacterial source-tracking project was designed to identify probable sources of fecal contamination. The main objectives of this study include 1) conducting an analysis of historic enterococci data, 2) monitoring enterococci concentrations for a period of seven months, 3) quantifying host-associated molecular markers to determine probable sources of fecal pollution (i.e., humans, canines, or gulls), and 4) characterizing the overall bacterial community composition through 16S rRNA gene sequence analysis. Findings indicated that enterococci concentrations were historically higher inside Little Bay compared to Aransas Bay. Tule Lake (the site located closest to a wastewater treatment plant) continuously exceeded the EPA's recreational water quality limit, while the Little Bay and Aransas Bay sampling sites exceeded the limit occasionally. Importantly, the maximum enterococci concentration at Tule Lake (24,196 MPN 100 mL⁻¹) exceeded the EPA single-sample standard criteria by ~233-fold. The findings of this study also showed that the canine and gull molecular markers were detected consistently, although spikes in the abundance of the human marker (gene copies 100 mL⁻¹) far exceeded the maximum abundance of the canine and gull markers. The abundance of these markers was not correlated with enterococci concentrations, indicating that the sources of enterococci pollution remain unknown, but these sources do contribute to bacterial pollution in Little Bay. Rainfall events were not correlated with the increased detection of enterococci or host-associated markers, but only two sampling events followed rainfall. Regardless, salinity was a driver of shifts in the overall bacterial community composition. We recommend that future efforts to remediate the bacterial pollution in Little Bay focus on four best-management practices

(BMPs). First, wastewater effluent flowing into Tule Lake should be diverted through a larger riparian buffer that terminates into Aransas Bay. Second, a pet waste outreach program should aim to limit canine fecal waste in surrounding areas. Third, the loitering of gulls should be addressed through policies that prohibit the feeding of gulls and limit improper food waste disposal. Fourth, the repair and maintenance of sanitary sewer systems and septic systems should be ensured to limit episodic leaks that contributed to spikes in human fecal pollution.

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Introduction

Marine environments associated with urbanized bays can suffer from impaired water quality stemming from accidental fecal pollution from unknown point and nonpoint sources (Gronewold et al. 2008). To assess this contamination, the EPA has approved the measurement of enterococci as a proxy for other fecal-associated microbes (USEPA 2004). Enterococci are a group of Gram-positive enteric bacteria that are commonly found in the gut and intestines of humans and other animals (Boehm and Sassoubre 2014). Studies have shown that enterococci concentrations are positively correlated with reported incidences of human illness after interaction with contaminated water (Byappanahalli et al. 2012). For this reason, as well as the ease and speed of detection, the EPA chose enterococci as the federal standard for measuring marine water quality. Specifically, the EPA's suggested limit for healthy recreational water quality is not more than 35 colony forming units (CFU) 100 mL^{-1} (geometric-mean standard) and not more than 104 CFU 100 mL^{-1} (single-sample standard) (USEPA 2011). Enterococci concentrations are routinely measured throughout Texas via the Texas Beach Watch program, which is under the administration of the Texas General Land Office (TGLO).

Despite correlations between enterococci and human illness, recent studies have questioned its use as a measure of water quality, as enterococci can persist in the environment and do not always originate from fecal waste (Mote et al. 2015; Signoretto et al. 2005). The results of the Source Identification Protocol Project (SIPP) indicated that the measurement of specific, host-associated genetic markers is a more reliable method (Boehm et al. 2013; Harwood et al. 2013; Stewart et al. 2013). This method allows for the detection of potentially small concentrations of fecal contamination, which can still pose a large threat to the environment. Additional studies

have also shown that characterizing the entire bacterial community via 16S rRNA gene sequencing can also help monitor changes in response to fecal contamination (Tan et al. 2015; Vierheilig et al. 2015). Therefore, the combination of these methods can provide a stronger tool to detect fecal pollution than one method alone.

Little Bay is a shallow lagoon that experiences limited exchange with Aransas Bay and the Gulf of Mexico and consequently has a high residence time. Due to its close proximity to a wastewater treatment plant and the city of Rockport, residents have become concerned that Little Bay may act as a sink for wastewater effluent and stormwater runoff, which can contribute to the loading of harmful pollutants and fecal-associated microorganisms. The associated environmental repercussions and health risks are expected to increase with urbanization, growing populations, and climate change (Lipp et al. 2002). Remediation efforts have included the diversion of WWTP effluent through an engineered riparian buffer and the construction of oyster reefs to filter the water of excess particulate organism matter and bacteria. However, two of the routinely monitored Texas Beach Watch stations in Little Bay have shown elevated levels of fecal indicator bacteria (FIB), thus prompting this seven-month bacterial-source tracking project. The main objectives of this study include 1) conducting an analysis of historic enterococci data, 2) monitoring enterococci concentrations for a period of seven months, 3) quantifying host-associated molecular markers to determine probable sources of fecal pollution (i.e., humans, canines, or gulls), and 4) characterizing the overall bacterial community composition through 16S rRNA gene sequence analysis.

Methods

Water Sampling. Water samples (1 L) were collected at 0.5 m depth in duplicate at seven sites in Little Bay in Rockport, Texas (Figure 1). The sampling locations included Tule Lake #1 (Latitude 28.050315, Longitude -97.042832), Key Allegro Pace Dock #2 (Latitude 28.043616, Longitude -97.032572), Tule Creek Outfall #3 (Latitude 28.043116, Longitude -97.035877), Rockport Saltwater Pool #4 (Latitude 28.032564, Longitude -97.033296), Rockport Beach Park North #5 (Latitude 29.030580, Longitude -97.034047), Little Bay Ski Basin #6 (Latitude 28.030435, Longitude -97.039682), and Rockport Beach Park South #7 (Latitude 28.026540, Longitude -97.045300). The samples were collected monthly, from May 31, 2018 to November 1, 2018, and included an additional sampling event in the month of August. Two sampling events (in August and October) corresponded with rain events. In accordance to the TCEQ Surface Water Quality Monitoring Procedures Manual (TCEQ, 2012) guidelines, water samples were collected in sterile bottles and stored on ice for no longer than four hours before they were processed at the lab.



Figure 1. Map of the seven sampling stations in Little Bay and Aransas Bay.

Environmental Parameters. Environmental parameters [water temperature ($^{\circ}\text{C}$), specific conductance ($\mu\text{S cm}^{-1}$), dissolved oxygen (mg mL^{-1}), barometric pressure (mmHg), salinity, and pH] were measured using a YSI 556 Multi Probe System (YSI Incorporated, Yellow Springs, Ohio, USA). Wind speed (mph) and air temperature ($^{\circ}\text{F}$) were measured with a Kestrel wind meter (Kestrel Instruments, Boothwyn, Pennsylvania, USA). Weather conditions [air temperature ($^{\circ}\text{C}$), days since last precipitation, amount of precipitation (in), barometric pressure (mmHg) and wind direction] were obtained from <https://www.wunderground.com/>. Water transparency (m) was determined using a 120 cm transparency tube (Ben Meadows, Janesville, Wisconsin, USA).

Enterococci Concentrations. Water samples (100 mL) were collected in duplicate and delivered to the National Environmental Laboratory Accreditation Program (NELAP)-accredited Corpus Christi Nueces County Public Health District Laboratory (CCNCPHDL) for Enterolert testing (IDEXX Laboratories, Westbrook, Maine, USA). The CCNCPHDL followed procedures for enterococci sampling that have been developed by the Texas Beach Watch Program (<https://cgis.glo.texas.gov/Beachwatch/docs/QAPP2016-2017.pdf>). The Enterolert test is EPA certified and quantifies enterococci as the most probable number (MPN) of viable cells per 100 mL which can be interpreted as an approximation of the number of colony forming units (CFU) per 100 mL. The relationship between enterococci concentrations and storm events was determined by comparing wet- and dry-loading concentrations with a cendiff test, using the NADA package for censored data in R (Lee and Helsel, 2005; Lee 2017). Additionally, the cenken test, which calculates the Kendall's tau correlation coefficient, was used to determine

relationships between enterococci concentrations and physical parameters (Lee and Helsel, 2005; Lee 2017).

DNA Isolation. Duplicate 100 mL samples from each sampling site were homogenized and vacuum filtered onto two 0.22 μm PES membrane filters of 47 mm diameter (Millipore Sigma, Bedford, Massachusetts, USA). The filters were stored at -80°C for a maximum of seven days before DNA isolation. The DNA was extracted from the filters with a DNeasy Power Soil Kit (Qiagen, Valencia, California, USA). The isolated DNA was quantified ($\text{ng } \mu\text{m}^{-1}$) and tested for quality (260/280 nm) using a Biospectrometer (Eppendorf, Hamburg, Germany). The isolated DNA was stored at -20°C . The DNA from the first filter was used for the quantitation of host-associated markers while the DNA from the second filter was used for the bacterial community composition analysis (i.e., 16S rRNA gene sequencing).

Host-Associated Markers. Droplet digital PCR (ddPCR) was performed to determine the concentration of each host-associated genetic marker (i.e., human, canine, and gull), as described previously (Cao et al. 2013). Table 1 shows the primer sequences for the bacterial strains, which were chosen based on the recommendations of the Source Identification Protocol Project (SIPP) (Boehm et al. 2013; Harwood et al. 2013; Stewart et al. 2013). Positive controls were designed for each primer set in the form of synthetic gBlock gene fragments (Integrated DNA Technologies, Skokie, Illinois, USA). Each of the ddPCR reactions were run in triplicate and each run included positive DNA, sample DNA, or a no template control (NTC) without DNA. The composition of each run had a total volume of 20 μL and included the following: 10 μL EvaGreen Supermix (1X), 1 μL forward primer (0.25 μM), 1 μL reverse primer (0.25 μM), and 3

μL of DNA (or water, in the case of the NTC). Following the manufacturer's instructions, droplets were generated with the QX200 Droplet Generator (BioRad Laboratories, Hercules, California, USA). After droplet generation, the samples were transferred to a thermal cycler (conditions listed in Table 2). Following the completion of the cycling, the droplets were read with the QX200 Droplet Reader (BioRad Laboratories, Hercules, California, USA) and analyzed with QuantaSoft software. The peaks from the NTC runs were used to determine the positive threshold of each run, which was set manually. The results of each reaction were then reported in copies μL^{-1} of the PCR reaction, and subsequently converted to the final concentration of gene copies 100 mL^{-1} water sample with the following equation: $(\text{copies } \mu\text{L}^{-1}) * (20 \mu\text{L ddPCR reaction} / 3 \mu\text{L DNA per reaction}) * (50 \mu\text{L of DNA obtained from each DNA extraction})$. The results from each triplicate were averaged together and used for subsequent statistical analyses. The Pearson's product moment correlation and the Wilcoxon signed rank test were used to test relationships between the abundance of host-associated markers (gene copies 100 mL^{-1}), physical parameters, and enterococci concentrations. ANOVA and Wilcoxon signed rank tests were conducted to test if the abundance of host-associated markers were related to storm events (i.e., periods of wet-loading, dry-loading, or neither). To manage analysis costs, one sampling site from Aransas Bay (site #7 Rockport Beach Park South) was excluded from the ddPCR analysis. Additionally, only one of the two sampling events in the month of August was included in this analysis.

Table 1. Primer sequences used for the detection of host-associated molecular markers.

Target	Primer and probe sequences	Reference
Human-associated <i>Bacteroidales</i> HF183	Forward primer: 5'-ATCATGAGTTCACATGTCCG-3' Reverse primer: 5'-TACCCCGCCTACTATCTAATG-3'	Bernhard and Field 2000 (F); Seurinck et al. 2005 (R)
Canine-associated <i>Bacteroidales</i> DogBac	Forward primer: 5'-CGCTTGTATGTACCGGTACG-3' Reverse primer: 5'-CAATCGGAGTTCTTCGTG-3'	Sinigalliano et al. 2010 (F); Dick et al. 2005 (R)
Gull-associated <i>Catelliboccus</i> LeeSeaGull	Forward primer: 5'- AGGTGCTAATACCGCATAATACAGAG -3' Reverse primer: 5'- GCCGTTACCTCACCGTCTA -3'	Lee et al. 2013 (F); Lee et al. 2012 (R)
16S rRNA	Forward: 515fMod 5'-GTGYCAGCMGCCGCGGTAA-3' Reverse: 806rMod 5'-GGACTACNVGGGTWTCTAAT-3'	Walters et al. 2016

Table 2. ddPCR thermal cycler conditions used to quantify host-associated fecal markers.

Step	Temp (°C)	Time	Ramp rate	Number of cycles
Enzyme activation	95	5:00		1
Denaturation	95	0:30	2°C s ⁻¹	40
Annealing/extension	59	1:00		40
Signal stabilization	4	5:00		1
	90	5:00		1

Bacterial Community Composition. Overall trends in bacterial community composition were determined using 16S rRNA gene sequencing. To manage sequencing costs, only the duplicates of the samples that were used in the ddPCR analysis were sent for sequencing. Samples from wet-loading events (N = 50) and dry-loading events (N = 19) were sequenced at Molecular Research LP (Shallowater, Texas, USA). Briefly, the V4 region of the 16S rRNA gene was

amplified using a HotStart Plus Master Mix Kit (QIAGEN) as described previously (Walters et al. 2016). Amplification was checked for success visually using gel electrophoresis, and samples were pooled in equal proportions based on their molecular weight and DNA concentrations. Pooled PCR products were then purified using calibrated Ampure XP beads (Beckman Coulter, Indianapolis, Indiana, USA), and the resulting pooled library was sequenced on an Illumina HiSeq instrument using paired-end chemistry (2 x 250 bp). Raw sequence reads were processed using a combination of QIIME version 1.9 (Caporaso et al. 2010) and QIIME2 version 2018.6 (Bolyen et al. 2018). Briefly, reads were demultiplexed and denoised using DADA2 (Callahan et al. 2016) using a trim length of 242 bp on both the forward and reverse reads. In addition to denoising the data, DADA2 filtered the sequences for quality, removed chimeric sequences, and merged paired-end reads. A phylogenetic tree was then generated using the ‘q2-phylogeny’ pipeline with default settings, which was used to calculate phylogeny-based diversity metrics. Both alpha and beta diversity were calculated using the ‘q2-diversity’ plugin. Alpha diversity metrics included Shannon’s diversity index, and weighted UniFrac values were used as a beta diversity metric. All diversity analyses were carried out using a sequencing depth of 10,364. Principal coordinate analysis (PCoA) of weighted UniFrac values was also carried out using the ‘q2-diversity’ plugin. Taxonomy was assigned using a Naïve Bays classifier trained on the SILVA release 132 99% OTUs database (Quast et al. 2013), where sequences had been trimmed to include only the 250 bases from the 16S region that was sequenced (V4 region bound by the 515F/806R primer pair). Taxonomic barplots were generated using ‘taxa barplot’ tool to visualize taxonomic differences between samples. Statistical analyses were carried out using the ‘q2-diversity’ plugin, and differences between sites and moisture levels were tested using a

pairwise Kruskal-Wallis test for alpha diversity metrics, while a pairwise PERMANOVA was used for beta diversity.

Data Analysis. All of the statistical analyses were completed with R and R Studio. Results were considered significant if $p < 0.05$, unless noted otherwise. Historical data of enterococci concentrations were obtained from the Texas Beach Watch Program and the Texas General Land Office.

Results and Discussion

Historical Enterococci Concentrations. Historical data were obtained from the Texas Beach Watch Program and the Texas General Land Office regarding enterococci concentrations at sites #4 through #7 (Rockport Saltwater Pool, Rockport Beach Park North, Little Bay Ski Basin, and Rockport Beach Park South) from August, 2004 to December, 2017. Two sites were located in Little Bay (Rockport Saltwater Pool and Little Bay Ski Basin), while the other two sites were located in Aransas Bay (Rockport Beach Park North and Rockport Beach Park South). A one-way ANOVA test was used to determine that the enterococci concentrations in Little Bay were higher ($0.002, p < 0.05$) than those found in Aransas Bay, although the small correlation coefficient implies that the difference is not biologically relevant. This history of elevated concentrations in Little Bay suggests that increased flushing and exchange with Aransas Bay could remediate bacterial loading. Alternately, rerouting Little Bay inflows to Aransas Bay could also remediate bacterial loading.

Water Sampling. A total of 112 water samples were obtained from eight sampling trips ($N = 8$ sampling events, $N = 7$ sampling sites, $N = 2$ replicates, $N = 112$ total water samples). Sampling trips spanned May, 2018 to November, 2018. During that time frame, two trips were conducted after major storm events occurred (August 2 and October 10). Post-storm samples were collected since stormwater runoff has been shown to be stressor in aquatic ecosystems, potentially loading the environment with fecal contamination, microbes, and residual pharmaceuticals (Williamson et al. 2014).

Physical Parameters. Over the seven-month sampling period (May 2018 to November 2018), water temperature ($^{\circ}\text{C}$), dissolved oxygen (mg L^{-1}), salinity, Specific conductance ($\mu\text{S cm}^{-1}$), and pH varied seasonally: water temperature ranged from 20.5 to 31.66 $^{\circ}\text{C}$, dissolved oxygen ranged from 1.28 to 8.04 mg mL^{-1} , salinity ranged from 0.73 to 44.89, specific conductance ranged from 1,474 to 65,978 $\mu\text{S cm}^{-1}$, and pH ranged from 7.21 to 8.39 (Figure 2). Water transparency ranged from 19.6 to 63.2 m. Air temperature ($^{\circ}\text{C}$), wind speed (mph), and barometric pressure (mmHg) ranged from 16.83 to 36.1 $^{\circ}\text{C}$, 0.0 to 12.5 mph, and 758.5 to 770 mmHg, respectively.

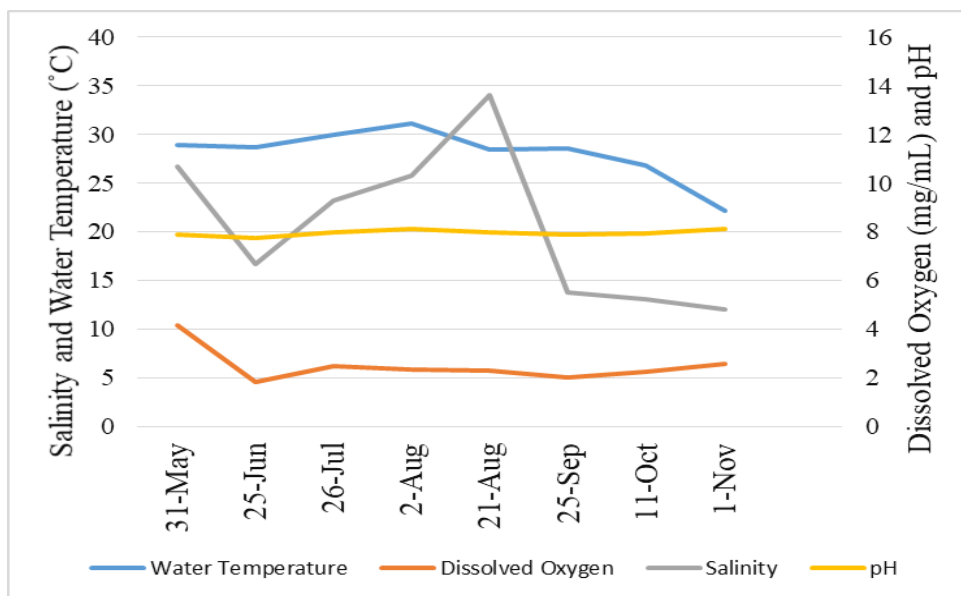


Figure 2. Seasonal variation in averaged water temperature (blue), salinity (gray), dissolved oxygen (orange), and pH (yellow) over the seven-month sampling period (May 2018 to November 2018, N = 8 sites per sampling trip).

Enterococci Concentrations. Enterococci concentrations ranged from < 10 to 24,196 MPN 100 mL^{-1} (Figure 3). Concentrations were not correlated with seasonal variation in air temperature or the number of days since the event of rainfall. Concentrations were significantly correlated with

water temperature (-0.2, $p < 0.05$), dissolved oxygen (-0.183, $p < 0.05$), salinity (-0.139, $p < 0.001$), specific conductance (-0.337, $p < 0.001$), pH (-0.333, $p < 0.001$), and water transparency (-0.199, $p < 0.05$). Sampling site #1 (Tule Lake) continually exhibited freshwater conditions and enterococci concentrations were orders of magnitude higher than those found at other sites. When identical statistical analyses were run on data from the six marine sampling stations (excluding data from Tule Lake), water temperature (-0.286, $p < 0.05$), water color (tan water, $p < 0.05$), water conditions (calm water, $p < 0.05$), were found to be significantly correlated with enterococci concentrations. The exceptionally high bacterial concentrations in Tule Lake (maximum 24,196 MPN 100 mL⁻¹) may constitute a public health risk but estimates of risk should be assessed in consideration of enterococci concentrations and the abundance of host-associated markers (below).

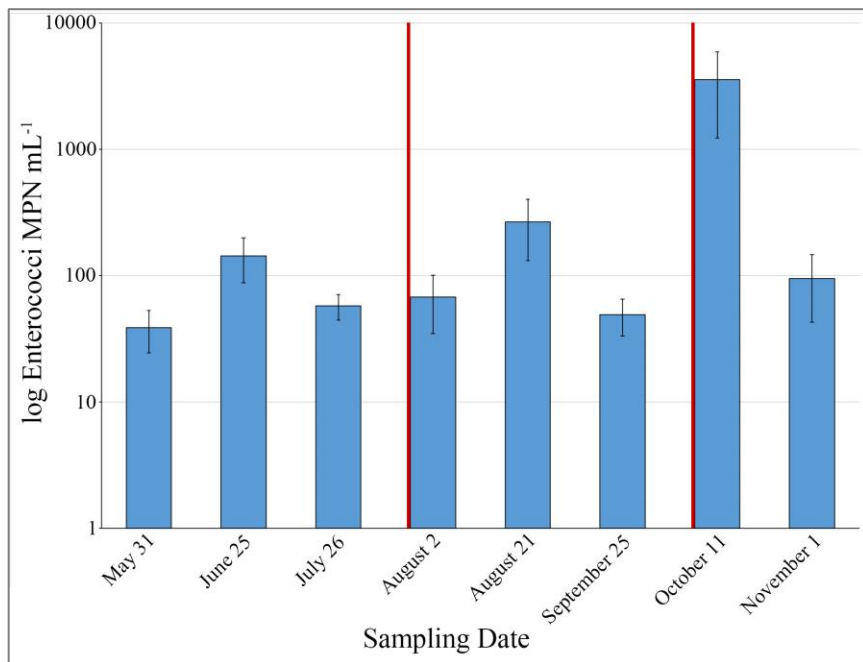


Figure 3. Variation in averaged enterococci concentrations (log MPN 100 mL⁻¹) in surface water samples collected around Little Bay in Rockport, TX. Vertical red lines mark storm events (N = 8 sites per sampling trip).

DNA Isolation. The DNA isolation with the DNeasy Power Soil method yielded approximately $10 \text{ ng } \mu\text{l}^{-1}$ of DNA per 100 mL sample. The quality of the DNA was determined by the 260/280 nm ratio and was approximately 1.96.

Host-Associated Markers. Table 3 shows the results of the minimum, maximum, and average concentration of each host-associated marker (i.e., human, canine, and gull), as determined by ddPCR. Figure 4 shows the concentration of each marker detected in sites #1-6 during each sampling trip. Only the gull marker was detected in each sample, while the human and canine markers were not always present (Figure 4). On average, the canine marker was the most abundant followed by the gull and human markers, although spikes in the abundance of the human marker far exceeded the maximum abundance of the canine and gull markers (Table 3). The gull marker was the only marker correlated with water temperature ($0.286, p < 0.001$) and only the human marker was correlated with specific conductance ($0.190, p < 0.05$). None of the host-associated markers were significantly correlated with enterococci or the occurrence of rainfall. The lack of correlation between enterococci and host-associated markers suggests that humans, canines, and gulls contribute minorly to enterococci concentrations in Little Bay. However, the presence of these markers do contribute to bacterial loading and could be indicative of a public health risk. For instance, there was a large increase in the abundance of the human marker in site #4 (Rockport Saltwater Pool) following a rain event that occurred in August (Figure 4). Spikes in human marker abundance could be the result of unknown breaks or overflows in stormwater or sanitary sewer systems. The Tule Lake site, which is located closest to the WWTP, did not experience high levels of the human-associated marker. The low abundance of the human marker at Tule Lake indicates that wastewater treatment or a

combination of wastewater treatment and riparian buffer were effective in removing human-associated *Bacteroidales*.

Table 3. Minimum, maximum, and average abundance (gene copies 100 mL⁻¹) of the three host-associated markers.

Marker	Minimum	Maximum	Average
Human	0.0	706.6	53.1
Canine	0.0	213.3	62.9
Gull	7.7	198.8	57.7

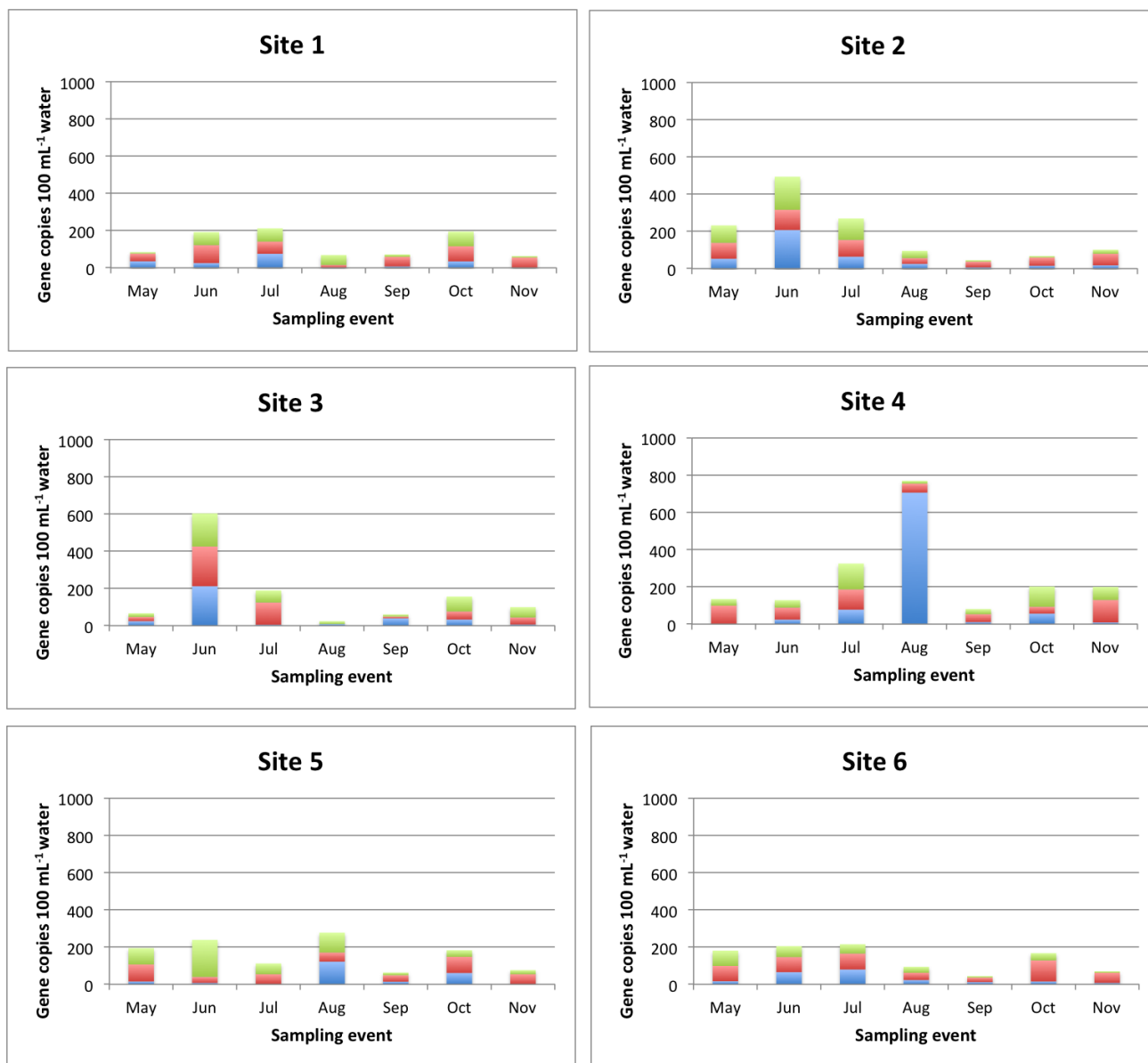


Figure 4. Averaged abundance (gene copies 100 mL⁻¹ water) of gull (green), canine (red), and human (blue) markers in surface water samples collected in Little Bay. Each graph represents a different sampling site; the site numbers correspond to the sites in Figure 1.

Bacterial Community Composition. Trends in community composition between sample sites were visualized using PCoA. Site #1 (Tule Lake) samples clustered together while the five other sites were interspersed with each other (Figure 5). Similarly, a pairwise PERMANOVA on

unweighted Unifrac values indicated that the Tule Lake community was significantly different compared to the six other sites (Figure 6). As Tule Lake was freshwater and the other six sites were marine, the observed difference in community structure is likely the result of differences in salinity. Due to the profound shift in community from freshwater to marine, further analyses of Tule Lake and the six marine sites were conducted separately. At Tule Lake, no significant differences in alpha or beta diversity were detected between wet- and dry-loading samples. In the six saltwater sites, alpha diversity analyses of the Shannon diversity index also demonstrated no significant differences between dry-loading samples and wet-loading samples (Kruskal-Wallis H test; $p > 0.05$; Figure 7). While stormwater-associated pulses have been demonstrated to function as a disturbance event that reduces biodiversity in marine systems (Williamson et al. 2014), bacterial community diversity in Little Bay was not significantly impacted by rain events. Similarly, the PCoA of unweighted UniFrac values at the marine sites did not exhibit differences in bacterial community composition between wet-loading and dry-loading events. However, salinity was a driver of bacterial community composition at the marine sites (Figure 8).

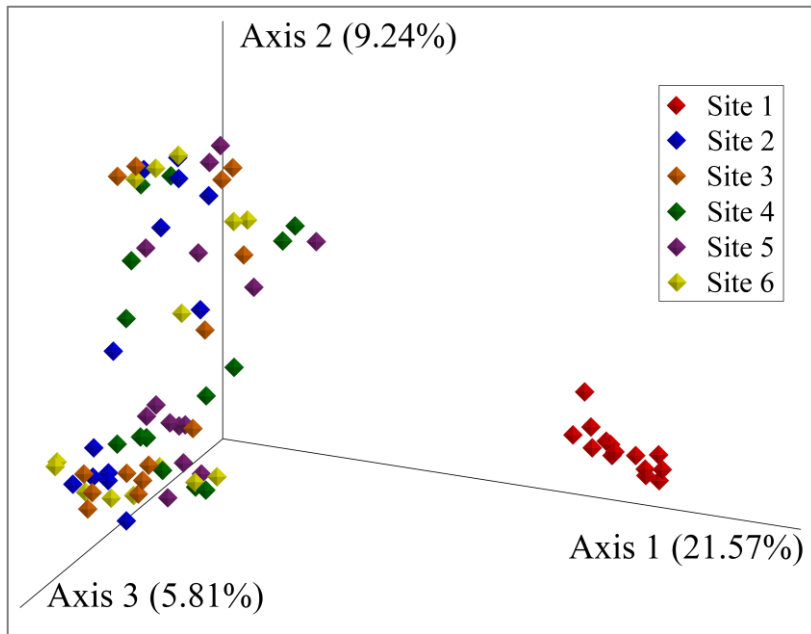


Figure 5. Principal coordinate analysis (PCoA) of beta diversity computed using unweighted UniFrac distance values. Site one was a freshwater site, while the other six sites were saltwater. The percent variation explained is given in parentheses beside each axis name.

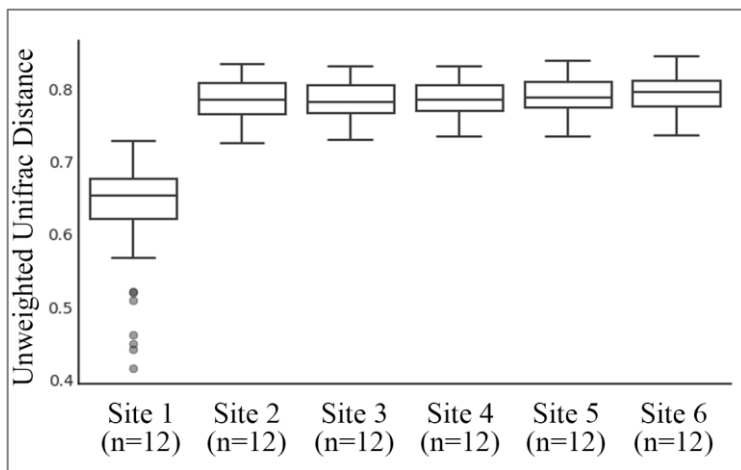


Figure 6. Results of the pairwise PERMANOVA showing the beta diversity of all seven sampling sites. Site one (freshwater) was significantly different from all other sites (saltwater) ($p < 0.001$).

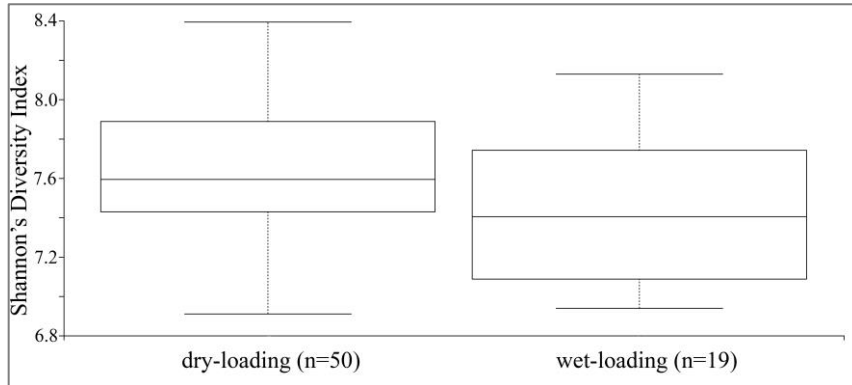


Figure 7. Results of the Kruskal-Wallis pairwise H test showing the diversity of wet- versus dry-loading samples. There was no significant difference between dry-loading and wet-loading samples ($p > 0.05$).

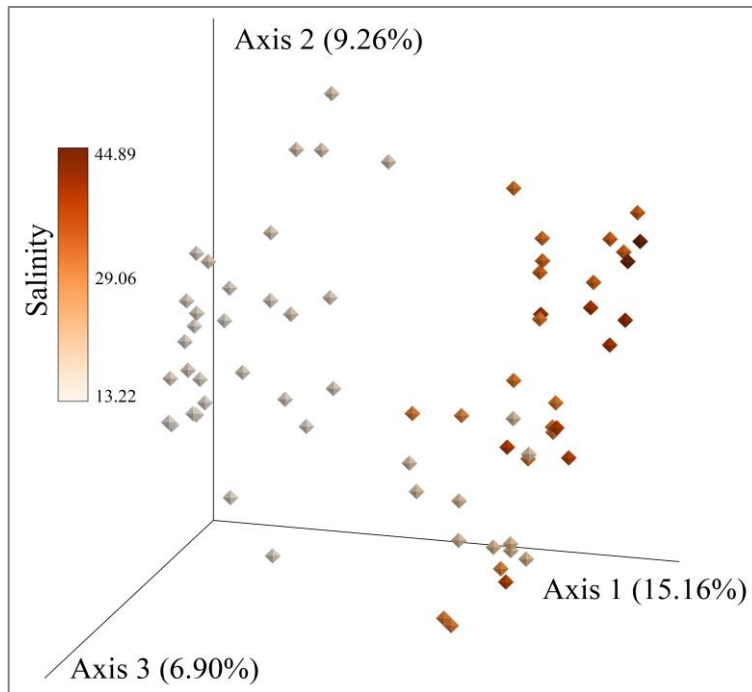


Figure 8. PCoA of beta diversity from the six saltwater sampling sites computed using unweighted UniFrac distance values. A color gradient was used to show changes in salinity, with the highest salinities (44.89) represented by red dots and the lowest salinities (13.22) represented by white dots. The percent variation explained is given in parentheses beside each axis name.

Conclusions

This project utilized a traditional culture-dependent method of detecting fecal contamination (i.e., quantifying enterococci) as well as newly-recommended culture-independent methods (i.e., detection of host-associated genetic markers and 16S rRNA gene sequencing) to determine sources of bacterial pollution in Little Bay. Analysis of historical Texas Beach Watch enterococci data showed that concentrations in Little Bay were higher than concentrations in Aransas Bay. Analysis of current data show that enterococci concentrations often exceeded the EPA's water quality criterion of 104 CFU 100 mL⁻¹. In particular, the Tule Lake site frequently exhibited enterococci concentrations that far exceeded the EPA water quality criterion. Enterococci concentrations were significantly correlated with water temperature, dissolved oxygen, salinity, pH, specific conductance, and water transparency. However, enterococci concentrations were not correlated with the abundance of rainfall events or the concentration of host-associated genetic markers. Testing for the abundance of host-associated genetic markers revealed that the average abundance of the canine and gull markers exceeded that of the human marker, but episodic spikes in the abundance of the human marker far exceeded the abundance of the canine and gull markers. The abundance of the gull marker was correlated with water temperature and the abundance of the human marker was correlated with specific conductance. The lack of a correlation between enterococci concentrations and host-associated markers suggests that humans, canines, and gulls were not significant sources of enterococci pollution. The lack of a correlation, however, does not suggest that humans, canines, and gulls are not a significant source of bacterial pollution. Rather, the culture-dependent and culture-independent methods for assessing bacterial pollution lack concurrency and further research is needed to determine which method is most representative of a public health risk. For instance, the detection

of a spike in human-associated marker abundance at the Rockport Saltwater Pool in August could have been indicative of a recent break or leak in a stormwater or sanitary sewer system. Characterization of the bacterial community, via 16S rRNA gene sequencing, provided a more complete view of the Little Bay system. Changes in salinity were correlated with taxonomic shifts in bacterial community composition but rainfall events were not a significant driver of community diversity. The lack of correlation with rainfall may be explained by the small number of rainfall events or the absence of a high-intensity rainfall event. Together, results show that certain segments of Little Bay experience elevated enterococci concentrations but those elevated concentrations could not be linked to a specific host, nor could those elevated concentrations be linked to rainfall events. Tule Lake, in particular, showed continuously elevated enterococci concentrations, likely due to the stimulation of bacterial growth by high nutrient levels in the WWTP effluent. We recommend that future efforts to remediate the bacterial pollution in Little Bay focus on four best-management practices (BMPs). First, wastewater effluent should be diverted through a larger riparian buffer that terminates into Aransas Bay. Second, a pet waste outreach program should aim to limit canine fecal waste. Third, the loitering of gulls should be addressed through policies that prohibit feeding of gulls and limit improper food waste disposal. Fourth, the repair and maintenance of sanitary sewer systems and septic systems should be ensured to limit episodic leaks that may contribute to spikes in human fecal pollution.

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