Assessment of Water Quality in a Model Coastal Bend Canal Community Quality

July 2022 - December 2023

Publication - 177
Project Number - 2236
December 2023

Prepared by:
Nicole C. Powers, Data Manager
Nora Bleth, Laboratory Manager
Andrew Johnson, Field Supervisor
Jacqueline Nicolay, Laboratory Technician
Ray Ayers, Citizen Scientist
Jeffrey W. Turner, Principal Investigator
Texas A&M University-Corpus Christi
6300 Ocean Drive, TH240
Corpus Christi, Texas 78412
Phone: 361-825-6206
Email: jeffrey.turner@tamucc.edu

Submitted to:
Coastal Bend Bays & Estuaries Program
615 N. Upper Broadway, Ste 1200
Corpus Christi, Texas 78401

This project has been funded wholly or in part by the United States Environmental Protection Agency under assistance agreement CE-98685818-1 to the Coastal Bend Bays & Estuaries Program. The contents of this document do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does the EPA endorse trade names or recommend the use of commercial products mentioned in this document.

Table of Contents

Abbreviations

CBBEP	Coastal Bend Bays & Estuaries Program
CBTS	City By The Sea
CFU	Colony forming units
ddPCR	Droplet digital PCR
USEPA	United States Environmental Protection Agency
FIB	Fecal indicator bacteria
IDT	Integrated DNA Technologies
LOD	Limit of detection
MCE	Mixed-cellulose ester
MPN	Most probable number
NEP	National Estuary Program
NTC	No-template control
PCA	Principal Component Analysis
PCR	Polymerase chain reaction
PES	Polyethersulfone
POA	Property Owners' Association
RBT	Risk-based threshold
SIPP	Source Identification Protocol Project
TAMUCC	Texas A&M University-Corpus Christi
TCB	Texas Coastal Bend
WUL	Water Utilities Lab
WQX	Water Quality Exchange

Summary

City By The Sea (CBTS) is a model canal community within the Texas Coastal Bend (TCB) vulnerable to bacterial pollution due to decreased flushing within the canal system and terrestrial runoff. The CBTS has conducted bacterial testing for the past 25 years ($16,290 past contribution), and they have observed a recent increase in bacteria (i.e., enterococci) levels. Enterococci are often measured as a proxy for fecal contamination, although enterococci are not host-specific and cannot accurately determine the source of pollution. This project aimed to assess the sources and environmental drivers of bacterial pollution in the CBTS. The objectives included 1) measuring enterococci concentrations, 2) quantifying three host-associated markers indicative of fecal contamination (i.e., human, canine, gull), and 3) establishing a bacterial baseline against which future changes in water quality can be measured with respect to population growth and watershed development.

The results showed that enterococci were detected in 56.4% of samples, ranging from <10 to 175 most probable number (MPN), and were significantly higher after rainfall. In contrast, the human-associated HF183 fecal marker was detected in 43.6% of samples, ranging from 0 to 146.67 gene copies 100 mL^{-1} water, and was significantly higher during dry-weather conditions. The lack of a direct correlation between enterococci and HF183 raises questions about the utility of enterococci as a fecal indicator. The lack of correlation also suggests enterococci may not be an accurate proxy of health risks associated with human fecal pollution in this system.

Both enterococci and the human marker were detected below the United States Environmental Protection Agency's (USEPA) risk-based threshold (RBT) of 32 illnesses per 1,000 primary recreation events (enterococci: geometric mean < 35 MPN and fewer than 10% of samples exceeded the statistical threshold value (STV) of 130 MPN; HF183: < 525 gene copies 100 mL^{-1} water). This baseline should be considered in the context of future population growth, watershed development, and aging infrastructure, which may contribute to future RBT exceedances.

Enterococci levels at the Highway 35 drainage ditch (C10) were significantly higher than all other sites within the canals (i.e., C1-C9). Additionally, sites C1 and C5, which receive drainage through culverts off Highway 35, had the highest recorded enterococci levels following rainfall events. In contrast, HF183 was detected consistently throughout the canals under dry weather conditions.

Based on the results presented in this report, we recommend the continued preventative maintenance of CBTS septic systems and the diversion of drainage from Highway 35 to Redfish Bay rather than the canals. Future development west of Highway 35 would likely increase stormwater runoff and exacerbate bacterial pollution; hence, we also recommend that future developments include plans to divert drainage to Redfish Bay.

Acknowledgements

The Coastal Bend Bays and Estuaries Program (CBBEP) (Contract No. 2236) and the United States Environmental Protection Agency (USEPA) for provided funding for this research. Funding for an Undergraduate Research Fellow (Andrew Johnson) was provided by the National Science Foundation Louis Stokes Alliance for Minority Participation (NSF LSAMP Award No. HRD-1911375). We thank the CBTS Property Owners' Association (POA) for assistance with the conception and design of the research. In particular, we thank Chairperson Randy Grobe for his assistance in organizing CBTS resources and facilitating POA communication.

Introduction

Fecal bacterial pollution poses a serious threat to environmental and human health. A meta-analysis of 216 studies clearly demonstrated that anthropogenic contamination, including sewage pollution, reduces diversity and resilience in coastal marine systems (Johnston and Roberts, 2009). Threats to diversity and resilience disrupt ecosystem services and endanger the sustainability of coastal ecosystems and economies (Levin and Lubchenco, 2008). Moreover, human pathogens associated with sewage contamination can negatively impact human health and deter recreation and tourism (Malham et al., 2014). Nationwide, an estimated 90 million surface water recreational illnesses occur annually, and the estimated economic burden of those illnesses ranges from $2.2 to 3.7 billion (DeFlorio-Barker et al., 2018).

Fecal bacterial pollution is increasing along the Texas coast. A recent decadal analysis of historical Texas Beach Watch data (75,000 water samples, 169 stations, 66 recreational beaches) showed enterococci concentrations are increasing with time, population growth, and sea rise (Powers et al., 2021). An independent report revealed that 90% of Texas recreational beaches were unsafe for swimming on at least one occasion in 2022 (Lewis and Berman, 2022). Yet the 66 recreational beaches monitored by Texas Beach Watch account for a fraction of the coast. Data describing water quality in residential canal communities is a conspicuous knowledge gap.

Residential canal communities are ubiquitous along urbanized coasts. The CBTS is a model Texas residential canal community. The community was founded in the late 1960s and includes 145 lots (131 homes and 12 condominiums, with additional houses planned or under construction) divided across three canals off Redfish Bay (Judith Vlasek, personal communication, 27 December 2023). Residential wastewater is treated by on-site septic systems.

The CBTS community is centrally located in the Texas Coastal Bend (TCB). The Nueces Estuary and the Mission-Aransas Estuary are the two main TCB estuaries; they are recognized together as an estuarine system of national significance under the USEPA and National Estuary Program (NEP). The TCB boundaries encompass the Aransas National Wildlife Refuge and the northern segment of the Padre Island National Seashore. Connectivity and flushing between the TCB and the Gulf of Mexico is limited by several barrier islands.

This study quantified bacterial pollution and identified its sources and physiochemical drivers in the CBTS. This community and similar canal communities across the TCB are vulnerable to bacterial pollution resulting from runoff, septic system malfunction, and poor flushing. At the regional scale, the data collected here will advance understanding of canal water quality throughout the TCB. At the local scale, data will inform planning to manage stormwater runoff, primarily through engagement with the local jurisdiction, to improve water quality.

Methods

Water Sampling.

Surface water samples (1 L) were collected in duplicate during monthly or bi-monthly sampling events (N = 12 collection events) from 2/14/2023 until 9/13/2023. Samples were collected from 10 stations in the CBTS canal community (see Table 1 and Figure 1). Site C10 was a Highway 35 drainage ditch that connects with the canals through culverts. Samples were only collected twice from C10 following rain events. Water samples were collected in sterile propylene bottles, stored on ice, and processed within six hours of collection.

Table 1. Locations and coordinates of sampling sites.

Station	Description	Latitude	Longitude
C1	City By The Sea	27º57'08"N	97º06'24"W
C2	City By The Sea	27º57'08"N	97º06'13"W
C3	City By The Sea	27º57'10"N	97º06'04"W
C4	City By The Sea	27º57'15"N	97º06'00"W
C5	City By The Sea	27º57'05"N	97º06'26"W
C6	City By The Sea	27º57'05"N	97º06'15"W
C7	City By The Sea	27º57'01"N	97º06'14"W
C8	City By The Sea	27º57'05"N	97º06'05"W
C9	Estes Flats	27º57'02"N	97º05'58"W
C10	Drainage Ditch	27º57'09"N	97º06'30"W

Figure 1. Locations of sampling sites from an aerial view of CBTS. The community currently includes 131 homes and 12 condominiums. Just north of CBTS, new homes are under construction in the La Buena Vida canal community.

Environmental Parameters.

Water temperature, salinity, specific conductance, dissolved oxygen, and pH were measured at each sample site using a YSI Pro Plus (Yellow Springs, OH). Water transparency was measured with a Carolina transparency tube (Carolina Biological, Burlington, NC). Wind speed and air temperature were recorded using a Kestrel 3000 meter (Kestrel Instruments, Boothwyn, PA) and relevant notes about weather and sampling site conditions were recorded for each event. Environmental variable data were deposited in the USEPA Water Quality Exchange (WQX) database.

Rainfall data was obtained from the TexMesonet database (https://www.texmesonet.org/) using the closest weather monitoring station: KRKP (coordinates: 28.08371, -97.04664). Samples were classified as dry-loading if no rainfall was recorded in the week preceding sample collection. Samples were classified as wet-loading if collected within six days of a rainfall event. The volume of rainfall was determined by calculating a weighted average (Shahin et al., 2022) from the preceding six days with the following equation:

$$Preceding\ rainfall\ = \frac{(R1 * 6) + (R2 * 5) + (R3 * 4) + (R4 * 3) + (R5 * 2) + (R6 * 1)}{(6 + 5 + 4 + 3 + 2 + 1)}$$

R1 = rainfall volume from one day prior to sample collection; R2 = rainfall volume from two days prior to sample collection; R3 = rainfall volume from three days prior to sample collection, etc.

Nutrient Concentrations.
Nitrate, nitrite, and ammonia concentrations were measured via the USEPA 300.0 method at the NELAP-accredited Corpus Christi Water Utilities Lab (WUL). Nutrient concentration data were deposited in the USEPA WQX database.

Enterococci Concentrations.
Enterococci were quantified using the Enterolert Test (IDEXX, Westbrook, ME) at the WUL. Enterococci concentration data were deposited in the USEPA WQX database.

DNA Isolation.
Duplicate water samples (100 mL) were filtered aseptically through 0.45µm mixed-cellulose ester (MCE) filters (Millipore, Burlington, MA). Visually turbid water samples were filtered through low-binding 0.45µm polyethersulfone (PES) filters (Millipore, Millipore, Burlington, MA). Filters were stored at -80°C prior to DNA extraction, which was completed with a DNeasy PowerSoil Pro extraction kit (Qiagen, Hilden, Germany). The DNA was assessed for quality (A_{260}/A_{280} values) and quantity (ng/µL) using a BioSpectrometer (Eppendorf, Hamburg, Germany) and stored at -80°C until the host-associated markers were measured.

Host-Associated Markers.
Three host-associated markers were chosen based on the results of the Source Identification Protocol Project (SIPP) (Boehm et al., 2013; Layton et al., 2013; Schriewer et al., 2013; Sinigalliano et al., 2013): the human marker HF183, the gull marker LeeSeaGull (a modified version of the Gull-2 marker that produces a shorter PCR product suitable for quantitative PCR), and the canine marker DogBact. Primer sequences are shown in Table 2.

Table 2. Sequences of primers and positive control accession numbers for the quantitation of host-associated fecal markers.

Target	Primer and Probe sequences	Reference
Human-associated Bacteroidales HF183[a]	Forward primer: 5'-ATCATGAGTTCACATGTCCG-3' Reverse primer: 5'-TACCCCGCCTACTATCTAATG-3'	(Bernhard et al., 2000; Seurinck et al., 2005)
Gull-associated *Catellicoccus* LeeSeaGull[b]	Forward primer: 5'-AGGTGCTAATACCGCATAATAC AGAG-3' Reverse primer: 5'-GCCGTTACCTCACCGTCTA-3'	(Lee et al., 2012; Lee et al., 2013; Lu et al., 2008; Lawson et al., 2006)
Canine-associated Bacteroidales DogBact[c]	Forward primer: 5'-CGCTTGTATGTACCGGTACG-3' Reverse primer: 5'-CAATCGGAGTTCTTCGTG-3'	(Sinigalliano et al., 2010; Dick et al., 2005)

[a]Accession number AY618281.1

[b]Accession number NR_042357.1

[c]Accession number AY695700.1

The host-associated markers were quantified with a droplet digital PCR (ddPCR) assay. Each sample was analyzed in duplicate, along with positive and no-template controls (NTC), following the EvaGreen supermix protocol with the QX200 Droplet Digital PCR System (Bio-Rad Laboratories, Hercules, CA). The positive controls were synthetic gBlocks (Integrated DNA Technologies; IDT, Coralville, IA) of the target DNA sequences (accession numbers listed in Table 2). The DNA targets were amplified in an Eppendorf Mastercyler nexus (Eppendorf, Hamburg, Germany) with the thermal cycling conditions listed in Table 3. Droplets were analyzed with the QuantaSoft software (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's protocol. Every ddPCR run included a positive control and a NTC, which were used to manually set the fluorescent thresholds for classifying droplets as positive or negative. If a sample contained fewer than 10,000 accepted droplets, it was excluded from the analysis. Duplicate sample results were averaged together, and the initial concentration of each marker (gene copies μL^{-1}) was used to calculate the total concentration (gene copies 100 mL^{-1} water) with the following equation (Powers et al., 2020):

$$Total\ concentration = \left(\frac{X\ gene\ copies}{1\ \mu L\ PCR\ volume}\right) * \left(\frac{20\ \mu L\ PCR\ volume}{3\ \mu L\ DNA}\right) * \left(\frac{50\ \mu L\ extracted\ DNA}{water\ sample\ [100\ mL]}\right)$$

The host-associated marker data were deposited in the USEPA Water Quality Exchange (WQX) database.

Table 3. Cycling conditions for the EvaGreen ddPCR assay.

Step	Temp (^{O}C)	Time	Ramp rate	No. of cycles
Enzyme activation	95	5:00	3^{O}C s^{-1}	1
Denaturation	95	0:30	3^{O}C s^{-1}	40
Annealing/extension	59	1:00	3^{O}C s^{-1}	40
Signal stabilization	4	5:00	3^{O}C s^{-1}	1
	90	5:00	3^{O}C s^{-1}	1

Data Analysis.

Enterococci and host-associated marker data were analyzed using R (v4.1.2) and RStudio (v2023.06.1+524). Due to the censored nature of the enterococci data, censored tests were used for all tests involving enterococci. Enterococci and the human-associated HF183 marker were quantified and analyzed in all samples (n=110). The canine- and gull-associated markers were quantified in a subset of samples, representing approximately 15% of samples, including one wet-loading and one dry-loading event (n=18). A subsequent peer-reviewed publication will report canine- and gull-associated marker data for all samples (Powers et al., *In preparation*).

A censored Kendall's tau (cenken test) was computed using the NADA package (v1.6-1.1; Lee, 2020) to test for correlations between enterococci and the host-associated markers; the variables were also visualized through scatter plots to visually assess potential non-linear trends.

To determine the effect that site and weather conditions (i.e., wet-loading or dry-loading) had on enterococci concentrations, a censored version of a two-way ANOVA (cen2way command; parametric two-factor fixed effects ANOVA for censored data) from the NADA2 package (v1.1.5; Julian and Helsel, 2023) was used. An interaction term between site and weather conditions was included in the model, although the interaction was not significant, so the model was refit, excluding the interaction term (Engqvist, 2005). The drainage ditch at site C10 was only sampled twice (both times after rainfall); due to the small sample size and wet-loading bias, this site was excluded from analysis unless otherwise noted. A cendiff test (NADA) was used to test for 1) differences between enterococci concentrations in sites C1 and C5, which received drainage from the ditch running along Highway 35, compared to C2, 3, 4, 6, 7, 8, and 9, and 2) differences in enterococci at site C5 (located closest to the oldest homes in the community, which were built on 50-foot-wide lots) compared to C1, 2, 3, 4, 6, 7, and 8 (which were located near newer homes built on 60-foot-wide lots).

A two-way ANOVA was used to determine the effect that site (i.e., C1-C9) and weather conditions (i.e., wet-loading or dry-loading) had on HF183 concentration. An interaction term between site and weather conditions was included in the model, although the interaction was not significant, so the model was refit, excluding the interaction term (Engqvist, 2005). Similar two-way ANOVAs were used to determine the effect of site and weather conditions on the gull and canine markers; however, due to the smaller sample sizes, which included one wet-loading and one dry-loading event, interaction terms could not be included. Two additional ANOVAs were used to test for 1) differences between HF183 concentrations in sites C1 and C5, which receive drainage from the ditch running along Highway 35, compared to C2, 3, 4, 6, 7, 8, and 9, and 2) differences in HF183 at site C5 (located closest to the oldest homes in the community, which were built on 50-foot-wide lots) compared to C1, 2, 3, 4, 6, 7, and 8 (which were located near newer homes built on 60-foot-wide lots). All ANOVAs with significant results were followed by a Tukey multiple comparisons of means posthoc test.

To test for correlations between enterococci and relevant environmental variables (i.e., salinity, water temperature, pH, dissolved oxygen, nitrate, and the volume of rainfall (weighted average in the preceding week), a linear model for censored data was generated using the cencorreg test from the NADA2 package in R. A linear model (for non-censored data) was generated using the lm function in R to test for correlations between host-associated markers and relevant environmental variables. The distribution of residuals for every model was observed visually through Q-Q plots and assessed for normality through skewness values, kurtosis values, and Shapiro tests. Variance inflation factors (VIF) were assessed for each model; VIF values greater than 5.0 were removed stepwise from the models.

To visualize the relationship between environmental variables, a principal component analysis (PCA) was computed using the autoplot function from the ggfortify package in R (Tang et al., 2016; Horikoshi and Tang, 2018). Due to the smaller sample sizes, the gull and canine markers were excluded from this analysis.

Results and Discussion

Water Sampling.

A total of 110 water samples were processed from twelve sampling events (2/14/2023 to 9/13/2023). Seven of the events were considered wet-loading, as samples were collected within six days of rainfall; the other five events were considered dry-loading, as they were collected more than seven days after a rainfall event. Two water samples were collected at a nearby drainage ditch (i.e., site C10) after rainfall events in May and June.

Environmental Parameters.

Salinity ranged from 5.83 to 39.1 ppt, with a median value of 30.5 ppt. The pH ranged from 5.09 to 8.7, with a median value of 8.06. Dissolved oxygen ranged from 22.6 to 108%, with a median value of 78.2%. Water temperature ranged from 16.9 to 32.2°C, with a median value of 27.6°C. Water transparency values ranged from 20.2 to 104 cm, with a median value of 59 cm. Specific conductance ranged from 11,876 to 66,648 µS/cm, with a median value of 45,417 µS/cm. Water depth ranged from 0.5 to 9.5 ft, with a median value of 6.9 ft.

Nutrient Concentrations.

Ammonia and nitrite in all samples were measured at or below the limit of detection. Nitrate ranged from < 0.025 to 11.9 mg/L, with a median value of 0.29 mg/L.

Enterococci Concentrations.

Table 4 shows a summary of the enterococci concentrations detected in this study. Enterococci were detected in 56.4% of samples (n=110), ranging from <10 to 175 MPN, and a median value of <10 MPN.

The USEPA has established a two-tier RBT for enterococci levels in recreational marine waters, based on the occurrence of 32 or fewer illnesses from every 1,000 primary contact recreation events. First, the geometric mean of enterococci measurements should not exceed 35 colony-forming units (CFU) 100 mL^{-1} of water. Second, enterococci should not exceed the statistical threshold value (STV) of 130 CFU in more than 10% of samples (USEPA, 2012). In this study, the geometric mean of enterococci was 18.74 MPN, below the EPA's RBT, and only four samples (4.8%) exceeded the STV of 130 CFU or MPN.

DNA Isolation.

DNA was successfully isolated from 110 samples. The average concentration of DNA was 55.0 ng $\mu L^{-1,}$ and the average A_{260}/A_{280} ratio was 1.61.

Host-Associated Markers.

Enterococci and HF183 were measured in every sample (n=110), although the results from 27 enterococci samples were excluded from analysis due to improper dilution by the WUL. To ensure the timely completion of this report, the gull and canine markers were reported for a subset of samples: one wet-loading event (n=9) and one dry-loading event (n=9), representing 15% of all samples.

Table 4 summarizes the host-associated marker concentrations detected in this study. The human marker was detected in 43.6% of samples (n=110), ranging from 0 to 146.67 gene copies 100 mL^{-1} water. The gull marker was detected in 11.11% of samples, ranging from 0 to 288.34 gene copies 100 mL^{-1} water, and the canine marker was detected in 33.33% of samples, ranging from 0 to 60 gene copies 100 mL^{-1} water.

Similar to enterococci, a RBT has been proposed for the human marker HF183 that equates to the USEPA's threshold of 0.032. Without considering the age of the sewage, the RBT for HF183 is 525 gene copies 100 mL^{-1} of water (Boehm and Soller, 2020). However, this threshold is based solely on HF183; if any fecal contamination from other sources is present, this concentration is reduced to remain under the RBT of 0.032. Although human waste poses the greatest risk to human health due to the presence of known human pathogens, fecal waste from other sources can also pose a risk. For instance, if the gull marker is present at a concentration of 100 gene copies, the RBT for HF183 is reduced to 175 gene copies to account for pathogens present in both sources (Boehm and Soller, 2020). To our knowledge, an equivalent RBT has not been proposed for the canine marker.

All of the HF183 measurements in our study were below the RBT of 525 gene copies 100 mL^{-1}, but several samples also contained gull and canine markers. HF183 was not detected in the sample with the highest gull marker (288.34 gene copies), so the risk from human and gull waste combined was below the 0.032 RBT in this sample. Even in the sample with the highest combination of HF183 (81.67 gene copies) and gull (186.67 gene copies), the risk remained below the RBT of 0.032 for these two markers.

Table 4. Concentrations of enterococci (MPN 100 mL^{-1} water) and the host-associated markers (gene copies 100 mL^{-1} water). *Geometric mean was calculated with the EnvStats package in R (Millard, 2013) by replacing all 0 values with 0.01.

Marker	Min	Max	Median	Mean	Geometric mean*	% of samples = 0 or below LOD
Enterococci (n=83)	< 10	175	< 10	30.71	18.74	49.4%
HF183 (n=110)	0	146.67	0	14.79	0.30	56.4%
LeeSeaGull (n=18)	0	288.34	49.17	67.23	19.80	11.11%
DogBact (n=18)	0	60.00	13.34	20.28	1.89	33.33%

Data Analysis.

No linear relationships between enterococci and the host-associated markers were visualized through scatter plots, although enterococci and HF183 as well as enterococci and LeeSeaGull showed a slight inverse relationship. Due to the potential non-linear relationship, a censored rank-based Kendall's tau was used to assess correlations between the variables. However, the results were not significant for any of the host-associated markers, suggesting the enterococci originated from the environment or a different fecal source that was not tested in this study. Alternately, the enterococci results could be unreliable, seeing that studies have reported the Enterolert test is prone to contamination (i.e., false positives) (Peperzak and van Bleijswijk, 2021).

Enterococci were significantly higher after wet-loading events (Figure 2A; censored two-way ANOVA; $p<0.05$). In contrast, HF183 was significantly lower after wet-loading events (Figure 2B; two-way ANOVA; $p<0.05$). The gull marker was marginally lower after wet-loading events, although the results were not statistically significant (Figure 3). Previous studies have shown similar results, with omnipresent pollution and fecal markers decreasing in concentration after wet-loading events due to a dilution effect from the runoff and freshwater inputs (Powers et al., 2020; Cann et al., 2013; Senhorst and Zwolsman, 2005). Unlike the human and gull markers, the canine marker was not influenced by wet- or dry-loading.

The increase in enterococci under wet-loading conditions suggests these fecal indicator bacteria (FIB) may have originated from inland or terrestrial sources and were transported to the canal system with runoff. In contrast, the higher levels of HF183 detected during dry-loading conditions suggest this marker did not originate from the same inland source as enterococci; rather, this marker may have originated from a more local source, such as CBTS septic system malfunction or leaks in the sewage collection infrastructure outside CBTS.

Neither enterococci, HF183, nor the gull marker differed significantly between sites C1-C9. When the drainage ditch at site C10 was sampled (twice after rainfall), the concentration of enterococci was significantly higher than sites C1-C9, although the results for HF183 were not significant. In contrast, the canine marker was influenced by location; sites 4 and 5 had significantly higher DogBact than sites 1 and 2 (Figure 3; two-way ANOVA; $p<0.01$).

Notably, sites C1 and C5, which receive drainage through culverts off Highway 35, had the highest levels of enterococci following rainfall, although the higher levels were not statistically significant. Drainage from Highway 35 culverts could have contributed to the elevated enterococci levels. Home density could be an additional contributor, as homes proximal to C1 and C5 are older higher-density homes built on smaller 50-foot-wide lots. Diverting the runoff from the drainage ditch into the bay rather than the canals could lower enterococci levels at these sites.

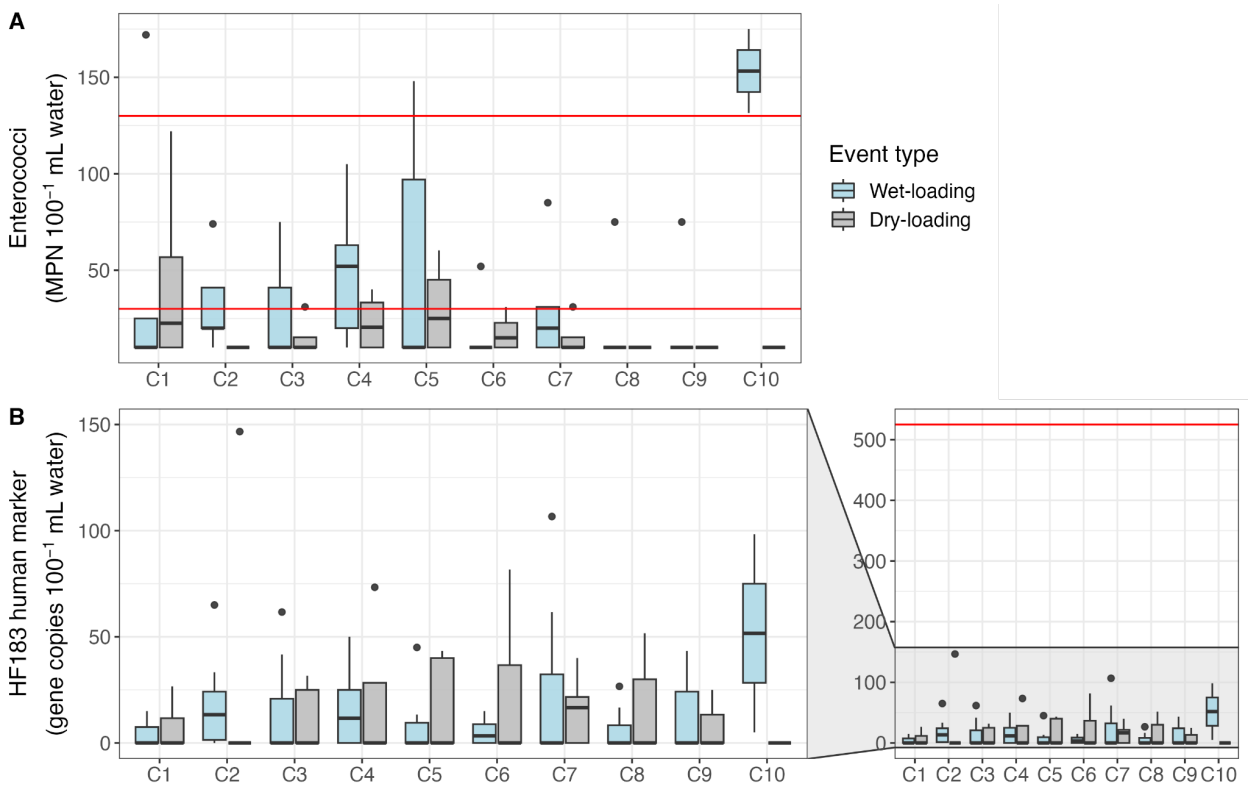

Figure 2. A) Concentrations of enterococci at the ten sampling sites; wet-loading samples are shown in light blue on the left side boxplots; dry-loading samples are shown in gray on the right side boxplots. Enterococci were significantly higher after wet-loading (censored two-way ANOVA (cen2way); $p<0.05$). The two red lines represent the USEPA's recommended limits for enterococci concentrations: geometric mean below 35 MPN and a statistical threshold value of 130 MPN that should not be exceeded in more than 10% of samples. **B)** HF183 human marker concentrations at the ten sampling sites; wet-loading samples are shown in light blue on the left side boxplots; dry-loading samples are shown in gray on the right side of the boxplots. HF183 was significantly higher after dry-loading (two-way ANOVA; $p<0.05$). The red line on the right-hand panel represents the risk-based threshold (equivalent to the USEPA's recommended risk threshold of fewer than 32 illnesses per 1,000 primary contact recreators) of 525 gene copies.

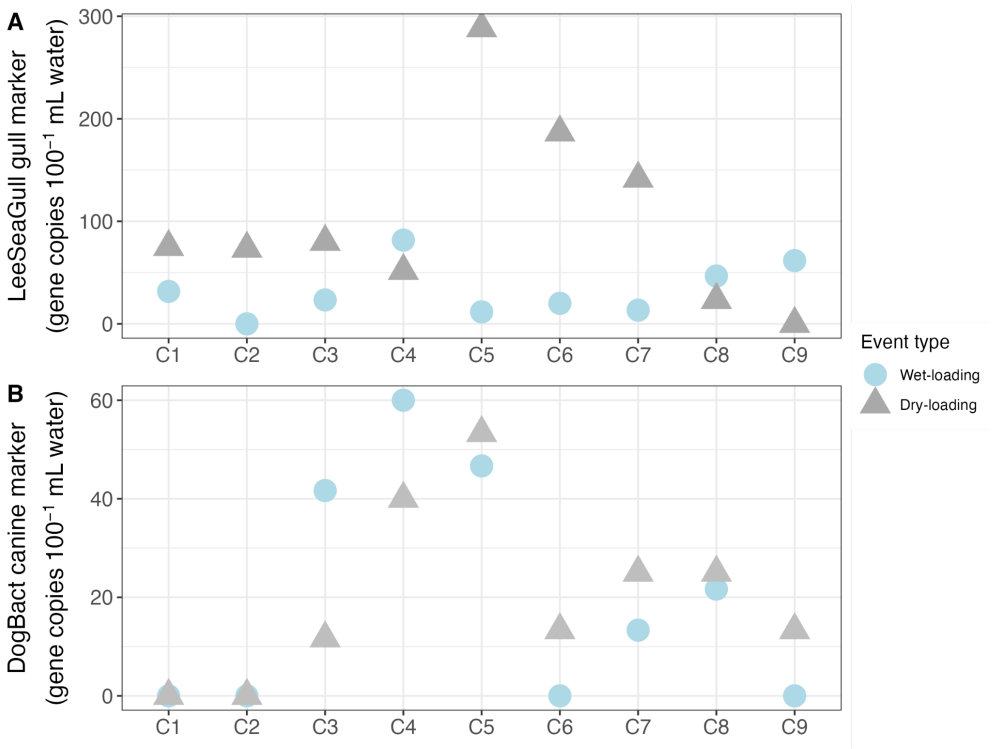

Figure 3. A) Gull (n=18) and **B)** canine (n=18) marker concentrations from sites C1-C9. Wet-loading samples are shown as blue circles; dry-loading samples are shown as gray triangles. Gull markers were marginally higher during dry-loading, although the results were not significant. Canine markers were not affected by weather conditions, although sites C4 and C5 had significantly higher concentrations than sites C1 and C2 (ANOVA; $p<0.05$). Note the overlap of wet-loading and dry-loading data points for sites C1 and C2 for the canine marker.

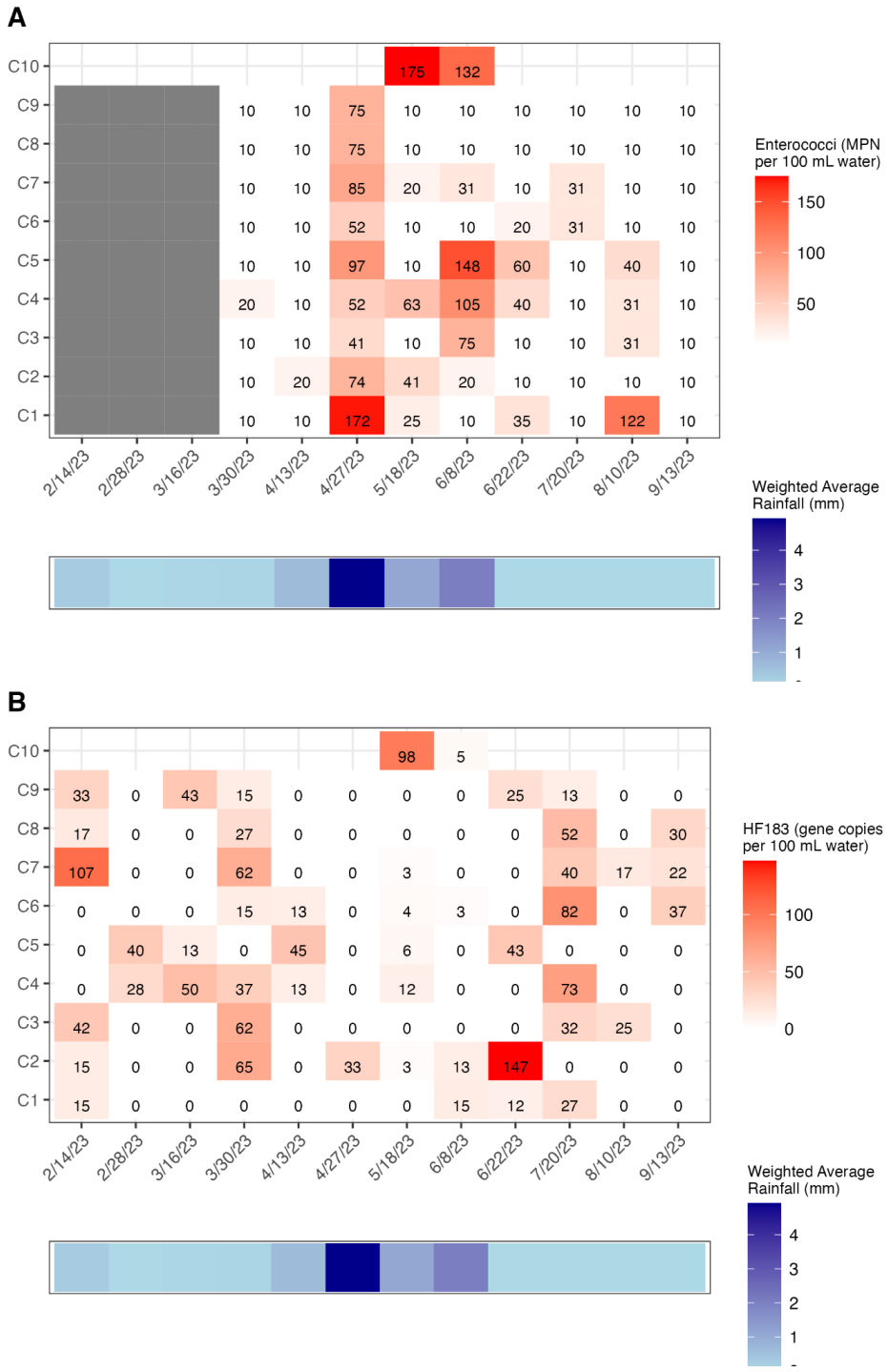

Figure 4. **A)** Enterococci (MPN) reported for each site by sampling date. The volume of rainfall (weighted average; mm) from the preceding week is shown in blue. The shaded gray area represents samples removed from the dataset due to improper dilutions during Enterolert testing. **B)** HF183 concentrations (gene copies 100 mL^{-1} water) reported for each site by sampling date. The volume of rainfall (weighted average; mm) from the preceding week is shown in blue.

The enterococci linear model was significant (chi-squared test; $p<0.01$), and the Q-Q plot residuals and Shapiro-Francia test ($p>0.1$) indicated the model was a good fit for the data. The Likelihood R^2 value was 0.41, meaning the model explained 41% of variability between samples. The volume of preceding rainfall (weighted average) and pH had positive coefficients in the model (11.49 and 0.19, respectively) and were positively correlated with enterococci (the strong relationship between enterococci and volume of preceding rainfall is shown in Figure 4). Conversely, nitrate had a negative coefficient (-0.18), indicating an inverse correlation with enterococci. The small coefficients of DO, salinity, and water temperature (i.e., <0.1) suggest weak or biologically irrelevant correlations with enterococci.

In contrast to the enterococci model, the HF183 model was not significant, and it had a low adjusted R^2 (0.01), skewed Q-Q plot, and significant Shapiro-Wilk test ($p<0.01$), suggesting the model was not a good fit for explaining variability between HF183 samples. These results could be due to non-linear relationships between variables, although no trend was observed between HF183 and the majority of variables in scatterplots of the data. One exception was the volume of preceding rainfall; a scatterplot showed an inverse association between HF183 and rainfall (Pearson correlation: -0.21; $p<0.05$; Figure 4). The results and output of the linear model suggest HF183 was not significantly influenced by and could therefore not be predicted by the environmental parameters measured in this study. Although the Q-Q plots and Shapiro-Wilk test results were acceptable in the gull and canine models, neither of these models was statistically significant. Subsequent visual inspection of scatterplots suggested positive trends between the gull marker and the volume of preceding rainfall, nitrate, salinity, and water temperature.

Figure 5 shows a PCA of the environmental data recorded during this study, which was able to explain 48.75% of variability between samples. PC1 explained 32.5% of the variability between samples and was associated with variables related to wet-loading events: rainfall volume, water temperature, specific conductance, days preceding rainfall, and salinity. PC2 explained 16.25% of variability and was associated with pH, depth, and transparency. The PCA biplot also shows distinct clustering between dry-loading samples with higher HF183 and wet-loading samples with higher enterococci. A negative association between enterococci and salinity, days preceding rainfall, and specific conductance can also be seen in the biplot; this inverse relationship is not surprising, given that enterococci were strongly correlated with rainfall. Higher HF183 levels were associated with dry-loading samples, although HF183 was not strongly influenced by the other environmental variables included in the PCA, further supporting the results of the linear model that HF183 was not strongly influenced by these environmental parameters.

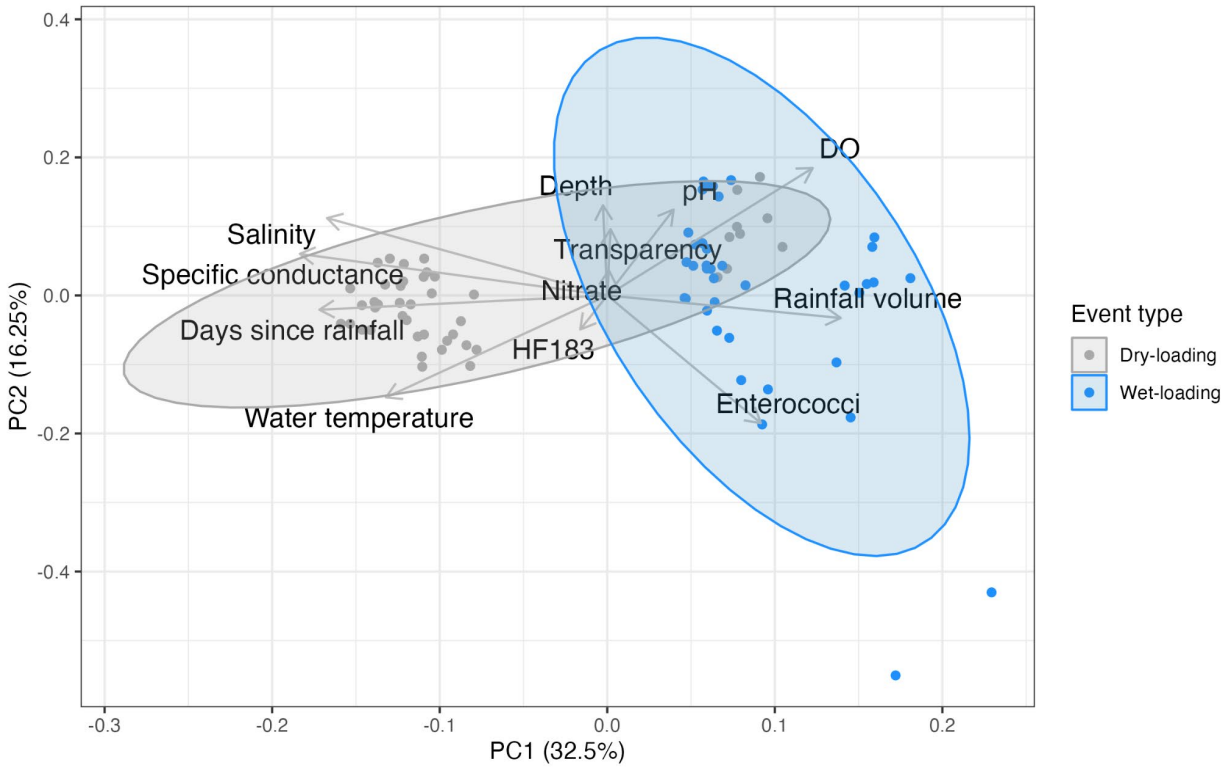

Figure 5. Principal component analysis (PCA) showing relationships between relevant environmental variables. Samples collected after dry-loading are shown in gray and samples collected after wet-loading are shown in blue.

Conclusions

- Enterococci were detected in 56.4% of samples, ranging from <10 to 175 MPN. Enterococci levels throughout the study were below the USEPA's risk-based threshold of 32 illnesses per 1,000 primary recreation events (geometric mean < 35 MPN; less than 10% of samples exceeded the statistical threshold value of 130 MPN).

- The human-associated fecal marker was detected in 43.6% of samples, ranging from 0 to 146.67 gene copies 100 mL^{-1} water. This marker was below the USEPA's risk-based threshold of 32 illnesses per 1,000 primary recreation events (525 gene copies 100 mL^{-1} water), even in combination with the gull marker.

- Enterococci were not correlated with the host-associated markers, suggesting enterococci originated from different sources. Enterococci may be an inaccurate indicator of health risks from human fecal pollution in this system.

- Although the levels of enterococci and the human marker were not significantly different between sites, elevated levels of enterococci were detected at sites C1 and C5 following rainfall. These bacteria could have been transported in drainage from the culverts connecting a drainage ditch along Highway 35 to the canal system.

- Enterococci were significantly higher after rainfall, whereas the human-associated fecal marker was significantly higher during dry weather events. These results and the lack of correlation between both indicators suggest the human marker did not originate from the same inland source as enterococci. Rather, the human marker may have originated from a more local source, such as CBTS septic system malfunction or leaks in the sewage collection infrastructure outside CBTS.

References

Bernhard AE, Field KG. 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Applied Environmental Microbiology*. 66(10):4571−4574.

Boehm AB, Soller JA. 2020. Refined ambient water quality thresholds for human-associated fecal indicator HF183 for recreational waters with and without co-occurring gull fecal contamination. *Microbial Risk Analysis*. 16:100139.

Boehm AB, Van De Werfhorst LC, Griffith JF, Holden PA, Jay JA, Shanks OC, Wang D, Weisberg SB. 2013. Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research*. 47:6812–6828.

Cann K, Thomas DR, Salmon RL, Wyn-Jones AP, Kay D. 2013. Extreme water-related weather events and waterborne disease. *Epidemiology & Infection*. 141:671–686.

DeFlorio-Barker S, Wing C, Jones RM, Dorevitch S. 2018. Estimate of incidence and cost of recreational waterborne illness on United States surface waters. *Environmental Health*. 17(3).

Dick LK, Bernhard AE, Brodeur TJ, Santo Domingo JW, Simpson JM, Walters SP, *et al.* 2005. Host distributions of uncultivated fecal Bacteroidales bacteria reveal genetic markers for fecal source identification. *Applied and Environmental Microbiology*. 71(6):3184 –3191.

Engqvist L. 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. *Animal Behaviour*. *70*(4).

Horikoshi M, Tang Y. 2018. *ggfortify: Data Visualization Tools for Statistical Analysis Results*. https://CRAN.R-project.org/package=ggfortify.

Johnston EL, Roberts DA. 2009. Contaminants reduce the richness and evenness of marine communities: a review and meta-analysis. *Environmental Pollution*. *157*(6):1745 –1752.

Julian P, Helsel D. 2023. NADA2: Data Analysis for Censored Environmental Data. R package version 1.1.5. https://github.com/SwampThingPaul/NADA2.

Lawson PA, Collins MD, Falsen E, Foster G. 2006. *Catellicoccus marimammalium* gen. nov., sp. nov., a novel Gram-positive, catalase-negative, coccus-shaped bacterium from porpoise and grey seal. *International Journal of Systematic Evolutionary Microbiology*. 56:429–432.

Layton BA, Cao Y, Ebentier DL, Hanley K, Ballesté E, Brandão J, *et al*. 2013. Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Research*. 47:6897–6908.

Li D, Van De Werfhorst LC, Holden PA. 2022. Genetic sequence data evidence that human faecal-associated HF183 sequences are on human skin and in urine. *Journal of Applied Microbiology*. 133(2):232–40.

Lee C, Agidi S, Marion JW, Lee J. 2012. Arcobacter in Lake Erie beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Applied and Environmental Microbiology*. 78:5511–5519.

Lee C, Marion JW, Lee J. 2013. Development and application of a quantitative PCR assay targeting *Catellicoccus marimammalium* for assessing gull-associated fecal contamination at Lake Erie beaches. *Science of the Total Environment*. 1(8):454–455.

Lee L. 2020. NADA: Nondetects and data analysis for environmental data. R package version 1.6 − 1. https://CRAN.R- project.org/package=NADA.

Levin SA, Lubchenco J. 2008. Resilience, robustness, and marine ecosystem-based management. *Bioscience*. *58*(1):27 –32.

Lewis M, Berman S. 2022. Safe for Swimming 2022 Texas Edition: Pollution at our beaches and how to prevent it. Environmental Texas Research & Policy Center.

Lu J, Santo Domingo JW, Lamendella R, Edge T, Hill S. 2008. Phylogenetic diversity and molecular detection of bacteria in gull feces. *Applied and Environmental Microbiology*. 74:3969 –3976.

Malham SK, Rajko-Nenow P, Howlett E, Tuson KE, Perkins TL, Pallett D, *et al.* 2014. The interaction of human microbial pathogens, particulate material and nutrients in estuarine environments and their impacts on recreational and shellfish waters. *Environmental Science: Processes & Impacts*. 16(9):2145 –2155.

Millard SP. 2013. EnvStats: an R package for environmental statistics. Springer Science & Business Media.

Peperzak, L., & van Bleijswijk, J. (2021). False-positive enterococci counts in seawater with the IDEXX Enterolert-E most probable number technique caused by *Bacillus licheniformis*. *Environmental Science and Pollution Research*. 28(9):10654 –10660.

Powers NC, Wallgren HR, Marbach S, Turner JW. 2020. Relationship between rainfall, fecal pollution, antimicrobial resistance, and microbial diversity in an urbanized subtropical bay. *Applied Environmental Microbiology*. 86(19).

Powers NC, Pinnell LJ, Wallgren HR, Marbach S, Turner JW. 2021. Water quality dynamics in response to rainfall along an estuarine ecocline. *ACS Environmental Science & Technology Water*. 1(6), 1503-1514.

Schriewer A, Goodwin KD, Sinigalliano CD, Cox AM, Wanless D, Bart-Kowiak J, *et al*. 2013. Performance evaluation of canine-associated Bacteroidales assays in a multi-laboratory comparison study. *Water Research*. 47:6909–692.

Senhorst H, Zwolsman J. 2005. Climate change and effects on water quality: a first impression. *Water Science & Technology*. 51:53–59.

Seurinck S, Defoirdt T, Verstraete W, Siciliano SD. 2005. Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology*. 7(2):249−259.

Shahin SA, Keevy H, Dada AC, Gyawali P, Sherchan SP. 2022. Incidence of human associated HF183 Bacteroides marker and *E. coli* levels in New Orleans Canals. *Science of The Total Environment*. 806, 150356.

Sinigalliano CD, Fleisher JM, Gidley ML, Solo-Gabriele HM, Shibata T, Plano LR, *et al*. 2010. Traditional and molecular analyses for fecal indicator bacteria in non-point source subtropical recreational marine waters. *Water Research*. 44(13):3763 –3772.

Sinigalliano CD, Ervin JS, Van De Werfhorst LC, Badgley BD, Ballesté E, Bartkowiak J, *et al*. 2013. Multi-laboratory evaluations of the performance of *Catellicoccus marimammalium* PCR assays developed to target gull fecal sources. *Water Research*. 47:6883–6896.

Tang Y, Horikoshi M, Li W. 2016. "ggfortify: Unified Interface to Visualize Statistical Result of Popular R Packages." *The R Journal*. 8(2):474–485. doi:10.32614/RJ-2016-060, https://doi.org/10.32614/RJ-2016-060.

U.S. Environmental Protection Agency. Recreational water quality criteria. 2012. https://www.epa.gov/sites/production/files/ 2015-10/documents/rwqc2012.pdf.