Distribution and persistence of fecal indicators in a Texas waterway impacted by Hurricane Harvey

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Abstract

Hurricane Harvey has caused unprecedented damages to huge parts of southeastern Texas, particularly damaging the wastewater infrastructure resulting in release of sewage contamination into environmental waters. The purpose of this study was to conduct a baseline assessment of fecal indicator bacteria (*E. coli* and enterococci) including human-associated fecal genetic markers (Human-specific *Bacteroidales*) across a Texas river impacted by Hurricane Harvey. Water samples were collected along the Guadalupe River during September – December 2017. The most heavily flooded sites showed highest abundance of fecal indicator bacteria and human-specific *Bacteroidales* indicating that the large number of sewage overflows and stormwater runoff occurred during Harvey flooding introduced high levels of fecal bacteria into waterways draining into the Gulf of Mexico and impaired surface water quality. The human-specific *Bacteroidales* markers exhibited low to moderate correlation with conventional fecal indicators using qPCR results, suggesting the variable persistence of different markers and uncertainty of enterococci and *E. coli* for detection of human fecal pollution. In general, results of this initial microbiological contaminant assessment will serve as baseline information for ongoing follow-on studies to monitor existing and emerging public health risks to residents of Texas and potential long-term environmental impacts upon the water resources in the impacted regions.

Keywords. Fecal source tracking, human-specific *Bacteroidales*, quantitative PCR, genetic markers, water quality, Hurricane Harvey

Introduction

Hurricane Harvey originated as a tropical storm on August 17, 2017 over the Atlantic Ocean, becoming a Category 4 hurricane on August 25 as it approached the coast of Texas.[1,2] The hurricane made landfall at peak intensity on the southeastern coast of Texas (Rockport) with winds of over 215 km/h. After initial landfall, the storm moved over the Copano Bay and made a second landfall in Texas just north of Holiday Beach on August 26 as a Category 3 hurricane. After that, for about two days the storm stalled just inland, dropping very heavy rainfall and causing widespread flash flooding. Though most of the damage was in the city of Houston, TX due to flooding of a significant portion of the metropolitan area, several other cities including Rockport, Corpus Christo and Victoria were also severely affected by the storm.[3,4] The widespread flooding had resulted in sewage overflows containing high levels of fecal bacteria and potentially pathogenic organisms posing a serious risk to human and environmental health via waterborne disease outbreaks, deterioration of recreational and drinking water quality, and degradation of aquatic ecology. Of particular apprehension is the potential impact of exposure to

microbial pathogens originating from human and animal waste. Human exposure to pathogenic microorganisms can result in an increased risk for infectious diseases. Understanding and identifying the sources of fecal contamination is thus paramount to protecting water quality and mitigating pollution and risk to human health. Surveys in the aftermath of Katrina identified several cases of Vibrio infections and gastrointestinal illness. High levels of fecal pollution are the primary cause of river and stream impairment in the United States according to the National Water Quality Inventory, with numerous often uncharacterized contamination sources including municipal waste from household sewage treatment systems (HSTS), combined sewer overflows (CSO), sanitary sewer overflows (SSO) and other factors like agriculture and urban runoff. Hence, accurate and reliable fecal source identification methods are essential for mitigating bacterial contributions to waterways and maintaining water quality.

The main objectives of the current research are to evaluate the presence, abundance and fate of fecal indicator bacteria and selected human pathogens in flood-impacted environmental waters. We applied a suite of quantitative polymerase chain reaction (qPCR) assays targeting *E. coli*, enterococci, and human-specific *Bacteroidale*s markers (HF183 and BacHum) to identify and quantify the fecal contamination in water.

Methodology

Sampling: The sampling was performed biweekly in September-November 2017, immediately in the weeks following Hurricane Harvey. Water samples were collected from 9 locations along Guadalupe River ranging from sites in Victoria, TX to its outfall and delta region in the Texas coast, as shown in Figure 1. The San Antonio River was used as a control site since it is in close proximity to Guadalupe River and was not severely impacted due to flooding. Triplicate samples were collected from each site using sterilized Nalgene bottles and transported on ice to the PI's laboratory at UTSA for microbial analysis. 300ml of samples were filtrated using sterile polycarbonate membrane filters with 0.45 µm pore size (Section 9222 B, Standard Methods for the Examination of Water and Wastewater). After filtration, the filter membranes were stored at -80ºC for further analysis.

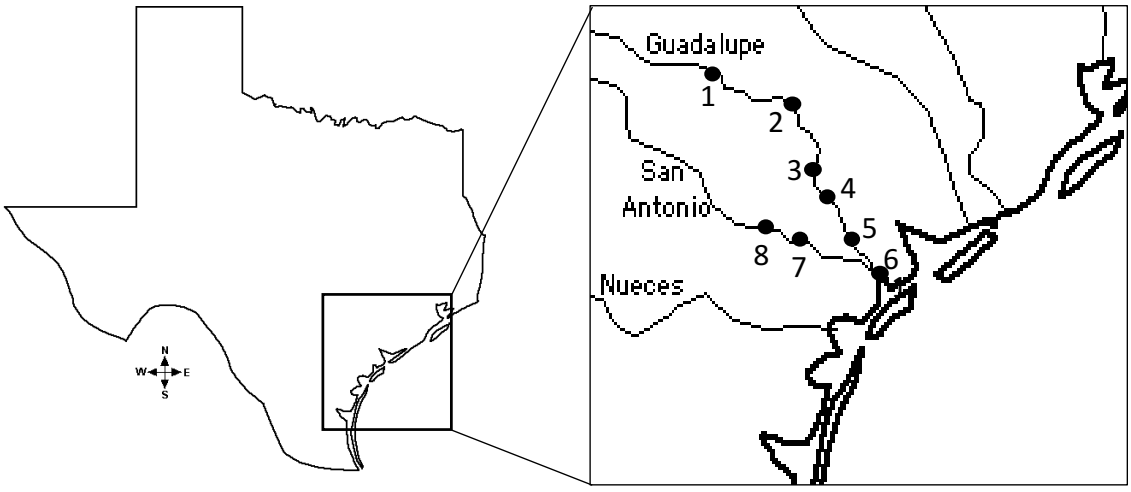

Figure 1. . Locations of sampling sites.

qPCR analyses. The occurrence and relative abundance of four different fecal bacterial markers in environmental water samples was measured using TaqMan qPCR assays and DNA extracts as the templates. DNA was extracted from filter samples using the DNeasy PowerLyzer PowerSoil Kit (Qiagen, Germantown, MD) according to the manufacturer's protocol. The targeted fecal bacterial groups were *E. coli* (EC23S857 assay),[18] *Enterococcu*s spp. (Entero1 assay),[19] and human-specific *Bacteroidales* (HF183 and BacHum assays).[20,21] The qPCR assays were performed as previously described.[22]

Data analyses. The marker copy number per 100 mL of water was calculated for all samples subjected to qPCR with a Ct value above background, and all data were log10 transformed before statistical analysis. Differences in marker concentrations were analyzed using non-parametric Kruskal-Wallis one-way analysis of variance. The correlation between human-specific Bacteroidales and conventional fecal indicators was analyzed using the logistic regression analysis. All analyses were performed using Microsoft Excel (2016) and correlation strength was interpreted according to an accepted scale for biological statistics.23 All statistical test outcomes were regarded as significant at $p < 0.001$.

Results and Discussion

Initial grab water samples (n = 4) were collected on September 8, 2017 near sites 4 and 5. The average concentration of fecal bacterial indicators in these samples were 1.31×10^2 copies and 5.05×10^5 copies per 100 mL water for *E. coli* and enterococci, respectively. The average concentration of human-specific *Bacteroidales* in these samples were 6.74×10^1 copies and 5.67×10^1 copies per 100 mL water for BacHum and HF183, respectively. This was followed by a more intensive sampling campaign from September to December 2017.

The targeted fecal bacterial groups were frequently detected in the surface water samples (n = 32) (40–100%). *E. coli* and enterococci were present in all the samples using the EC23S857 and Entero1 assays, respectively while the human-specific *Bacteroidales* were detected less frequently (Table 1). Enterococci exhibited the highest levels across all the sampling sites with mean marker abundance greater than 10^3 copies per 100 mL water. The spatial distribution of the levels of markers across the study sites is represented in Figure 2. The two human-specific *Bacteroidales* markers, HF183 and BacHum, exhibited a similar spatial distribution pattern across the sampling sites, although the level of BacHum marker was slightly higher for all the samples. Both of the markers tested positive at most of the sites, with the exception of sites 1 and 8 where HF183 was not detected in any of the samples. The levels of the human-specific *Bacteroidales* markers were statistically different ($p < 0.001$, Kruskal-Wallis one-way analysis of variance) from each other among the study sites for both the markers. Site 4 had the highest mean copy number for the human-specific *Bacteroidales* markers which is consistent with the excessive flooding at this site, and also because it was located right after a WWTP and the discharge from the WWTP was fed into this region. Site 5 also had a relatively high concentration of human-specific *Bacteroidales* compared to other sites. However, for site 6 which is located right after the confluence of Guadalupe River and San Antonio River, the concentrations of both human-specific *Bacteroidales* markers decreased, indicating that San Antonio River was not severely impacted by human fecal contamination. This was also true for sites 7 and 8 (control sites on San Antonio River) which had considerably lower levels of human-specific *Bacteroidales.*

Table 1. Distribution of molecular markers (log_{10} copies per 100 mL) used in this study detected via qPCR assays (n = 32).

Assay	Mean	Range	+(%)
E. coli (EC23S857)	1.91	0.55 – 4.13	100
General *Enterococcus* (Entero1)	3.54	2.09 – 4.68	100
Human-specific *Bacteroidales* (BacHum)	0.70	0.00 – 3.46	43
Human-specific *Bacteroidales* (HF183)	0.61	0.00 – 3.44	40

+(%) = percentage of samples detected positive for the marker

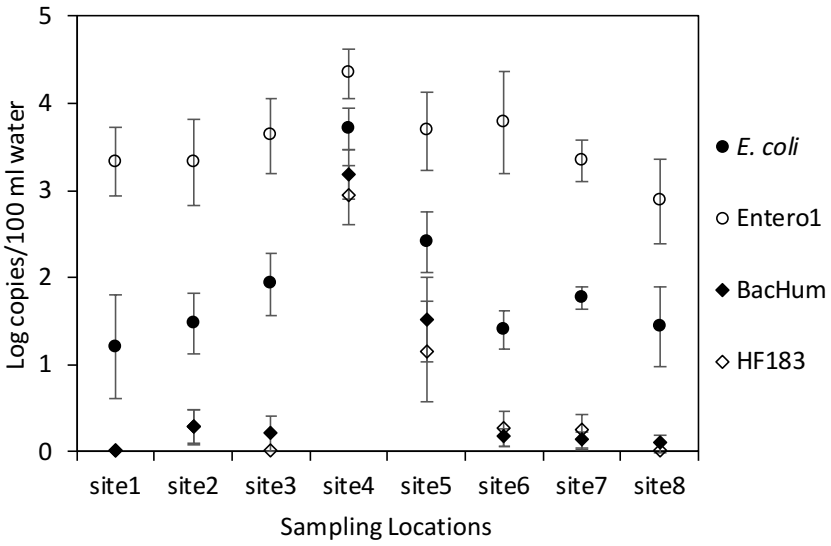

Figure 2. Spatial variation in levels of markers across study sites as determined using qPCR assays.

The temporal distribution of the average levels of fecal indicator bacteria and human-specific markers is presented in Figure 3. Sites 4, 5 and 6 were classified as flooded regions of the Guadalupe River, while sites 1, 2 and 3 were located in the non-flooded areas. Sites 7 and 8 were located in the non-flooded segments of the San Antonio River. On average, all the markers had the highest concentrations for the flooded regions of the Guadalupe River. Samples collected on September 22, 2017, ~3 weeks after flooding, demonstrated highest levels for *E. coli*, BacHum and HF183, both in the flooded and non-flooded segments. The concentrations of

human-specific *Bacteroidales* was significantly higher for the flooded sites than the non-flooded sites.

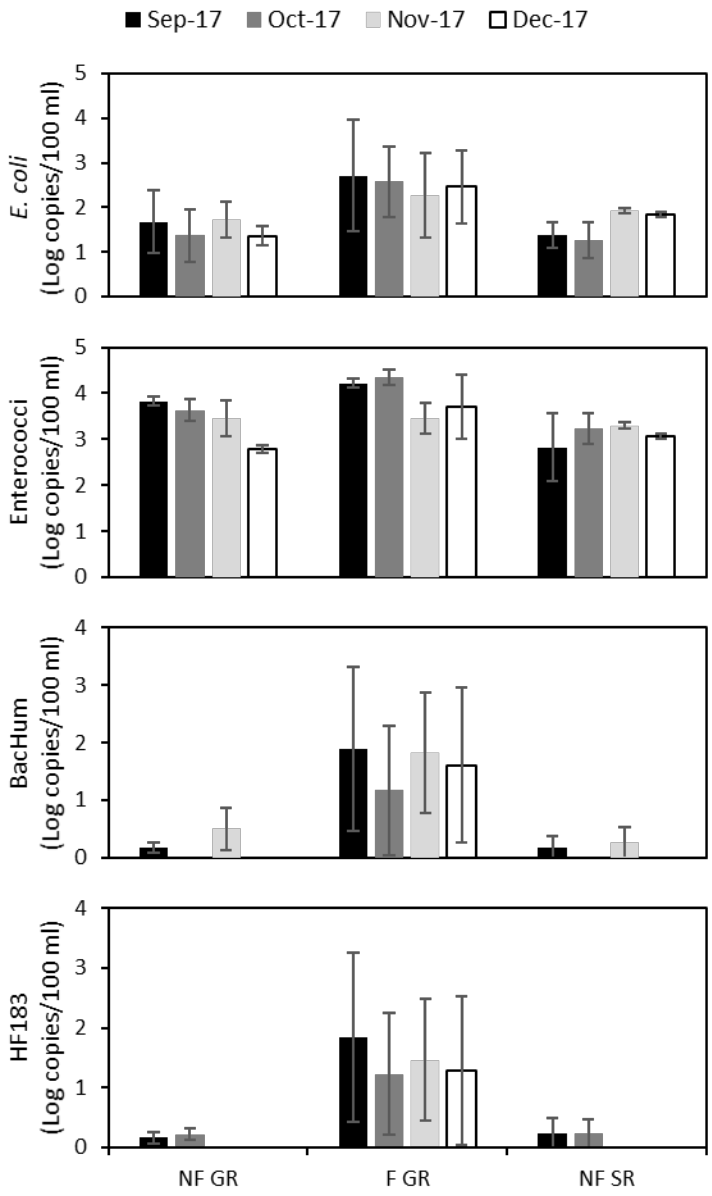

Figure 3. Temporal variation in levels of *E. coli*, enterococci and human-specific *Bacteroidales* in water samples collected in this study. Sites 1, 2 and 3 were classified as non-flooded regions of the Guadalupe River (NF GR), while sites 4, 5 and 6 were located in the flooded catchment of the Guadalupe River (F GR). Sites 7 and 8 were located in the non-flooded segments of the San Antonio River (NF SR).

The presence of high concentrations of bacterial indicators in surface water samples collected from flooded regions of the river were indicative of the large volumes of human waste that were present in the sewer system of the impacted areas. The most heavily flooded sites showed the highest abundance of fecal indicator bacteria and human-specific *Bacteroidales*

indicating that the large number of sewage overflows and stormwater runoff which occurred during Harvey flooding introduced high levels of fecal bacteria into waterways draining into the Gulf of Mexico and thereby impairing surface and coastal water quality. All initial water samples collected from the flooded regions of the Guadalupe River had *E. coli* and enterococci concentrations above the regulatory level for contact recreational waters,[9] although it should be noted that our results were measured by qPCR methods while the Recreational Water Quality Criteria is based on culturable *E. coli* or enterococci.

Conclusion

Successful accomplishment of project objectives is expected to provide a wealth of information regarding the fate and transport of fecal indicators along the sampling sites. The qPCR analysis will provide a means to identify the location and abundance of contamination. The data will provide the required information to produce a dynamic fecal source model for surface waters impacted by sewage overflows caused due to extreme flooding. Longer term, this research will serve as a precursor for more advanced studies for assessing public health risk and the development of more representative recreation management programs.

Acknowledgements

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