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Evaluation of potential *E. coli* transport from on-site sewage facilities in a Texas watershed

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Abstract: Potential *E. coli* contamination in surface waters from on-site sewage facilities was investigated in the Dickinson Bayou watershed, Texas. This watershed is listed as impaired due to bacteria by the Texas Commission on Environmental Quality. Two water quality monitoring stations, with flow meters and automatic water samplers, were installed in the watershed to assess *E. coli* concentrations in surface runoff. One monitoring station was installed in a neighborhood that solely used an on-site sewage facility (OSSF) and the second monitoring station, the control site, was installed in a neighborhood connected to a municipal sewage plant. For 16 runoff events at the OSSF site, the combined geometric mean *E. coli* concentration was 639 colony forming units (CFU)/100milliters while the geometric mean *E. coli* concentration for 13 runoff events at the control site was 371 CFU/100milliliters. The *E. coli* concentrations from the 2 sites were not statistically different, suggesting that OSSFs may not be the major cause of bacterial contamination in the Dickinson Bayou watershed. In addition, a bacterial source tracking method was employed, which concluded that a portion of the *E. coli* from both sites were of human origin.
Keywords: *E. coli*, on-site sewage facilities, surface water, Texas coastal watershed, bacterial source tracking

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Terms used in paper

Acronym	**Descriptive term**
BST	bacteria source tracking
CFU	colony forming unit
EPA	Environmental Protection Agency
E. coli	*Escherichia coli*
ERIC-PCR	repetitive intergenic consensus sequence-polymerase chain reaction
RP	riboprinting
HGAC	Houston-Galveston Area Council
OSSF	on-site sewage facility
NELAP	National Environmental Laboratory Accreditation Program
TCEQ	Texas Commission on Environmental Quality
USGS	U.S. Geological Survey

INTRODUCTION

The Dickinson Bayou watershed is located in Fort Bend and Galveston counties in southeast Texas and contains portions of nearby cities including Alvin, Dickinson, Friendswood, League City, Manvel, Santa Fe, and Texas City (Figure 1). Dickinson Bayou flows through Dickinson Bay to arrive ultimately in Galveston Bay. Even though all surrounding point sources, which include many wastewater treatment plants, are constantly monitored and assessed, Dickinson Bayou, Dickinson Bay, and Galveston Bay all have high levels of bacteria. All 3 water bodies are on the Texas Commission on Environmental Quality's (TCEQ) 303(d) list, a summary of waters in and around Texas that fail to meet their intended use regulatory standard, and have been since 1996 due to impairment by elevated bacterial concentrations (TCEQ 2012).

Dickinson Bayou and Dickinson Bay are used by many residents of the area for fishing, boating, and other recreational activities. However, nearly half of all residents in the Dickinson Bayou watershed are not aware of the bacterial problem in the watershed even though excess bacteria in the area has been widely reported (Quigg et al. 2009; TAMUPPRI 2012). Specifically, *E. coli*, which is found in excess in both Dickinson Bay and Dickinson Bayou, causes intestinal problems in humans (Smith Jr. et al. 2004; Teague 2007; Riebschleager et al. 2012) and has been documented as an economic issue (Overstreet 1988; Soller et al. 2010). High levels of bacteria in fish and shellfish limit the amount of seafood that can be sold and cause significant economic problems in areas that rely on fishing as a livelihood.

Previous research has suggested that failing OSSFs may be a factor in elevated bacterial levels in nearby Buffalo Bayou (Platt 2006). Both anaerobic and aerobic on-site sewage facilities (OSSFs) are found in the Dickinson Bayou watershed. Anaerobic systems use a holding tank (septic tank) for primary treatment and utilize soil microbes for secondary treatment when the effluent is discharged through a series of subsurface drainage pipes (TAMAE 2008). When the soil surrounding the drainage field has low permeability, infiltration of the wastewater through the soil profile is greatly reduced and has been shown to be a factor in septic system failure (Carr et. al. 2009; Conn et al. 2011; Withers et. al. 2011). When infiltration rates are low, the wastewater may rise to the surface and untreated wastewater can runoff directly into nearby surface waters. In addition, previous research has also shown that when high water tables are present near the drainage pipes, anaerobic systems have the ability to directly contaminate groundwater (Scandura and Sobsey 1997; Humphrey et. al. 2011; Lapworth et. al. 2012).

Aerobic systems employ a holding tank, an aerobic treatment unit with a disinfectant system (typically chlorine), and a spray system to dispose of the effluent (TAMAE 2008). If the aerobic system is not well maintained, the efficiency of aerobic OSSFs is greatly diminished and the surface soil becomes the primary treatment medium (Levett et. al. 2010). If the soil has low infiltration rates, the irrigated wastewater may pond on the surface and run off to nearby ditches and streams. Furthermore, studies have shown that *E. coli* is capable of attaching to suspended solids during runoff (Parker et. al. 2010; Soupir et. al. 2010). Bacteria sprayed onto the soil surface from improperly maintained aerobic OSSFs may be transported by sediment in runoff to nearby ditches and streams.

There are approximately 5,000 OSSFs in the Dickinson Bayou watershed (DBWP 2007). The vast majority of OSSFs built before 1997 were anaerobic systems. However, in 1997 Texas began requiring a soil inspection before an OSSF could be installed (TCEQ 2014). Heavy clay soils with shallow groundwater present in most of Galveston County prevented homeowners from building new anaerobic OSSFs. Therefore, aerobic OSSFs started becoming the most commonly installed OSSF type after 1997.

A project was developed to explore the potential for local OSSFs to cause bacterial loads in stormwater runoff in the Dickinson Bayou watershed by sampling runoff from 2 sites within the watershed. One monitoring site was in a neighborhood that used only OSSFs (the OSSF site) to treat wastewater. The second monitoring site was in a neighborhood that used a municipal sewage plant to treat wastewater (the Control site). Various indicator bacteria can be used to gauge bacterial contamination in coastal water bodies. Groundwater, potentially affected from anaerobic systems, was not taken into consideration for this study. To directly compare with results from previous studies in the Dickinson Bayou watershed, *E. coli* was chosen as the indicator bacteria for this project. The project's main objective was to determine if OSSFs in residential areas were contributing to the elevated *E. coli* concentrations in Dickinson Bayou.

METHODS

Two water quality monitoring stations were installed in the Dickinson Bayou watershed, as indicated by the star symbols in Figure 1. The first, known as the OSSF site, was located in Santa Fe, Texas (29° 25' 00.82"N, 95° 06' 18.69"W), in a neighborhood that uses only OSSFs for wastewater treatment. Of the 28 houses in the watershed, 19 use the anaerobic OSSF and the remaining 9 use the aerobic OSSF (HGAC 2013a). Approximately 10% of the OSSF watershed consisted of impervious surfaces.

The second water quality monitoring station, known as the Control site, was located in Dickinson, Texas (29° 27' 02.54"N, 95° 03' 40.43"W) in a neighborhood that used a municipal

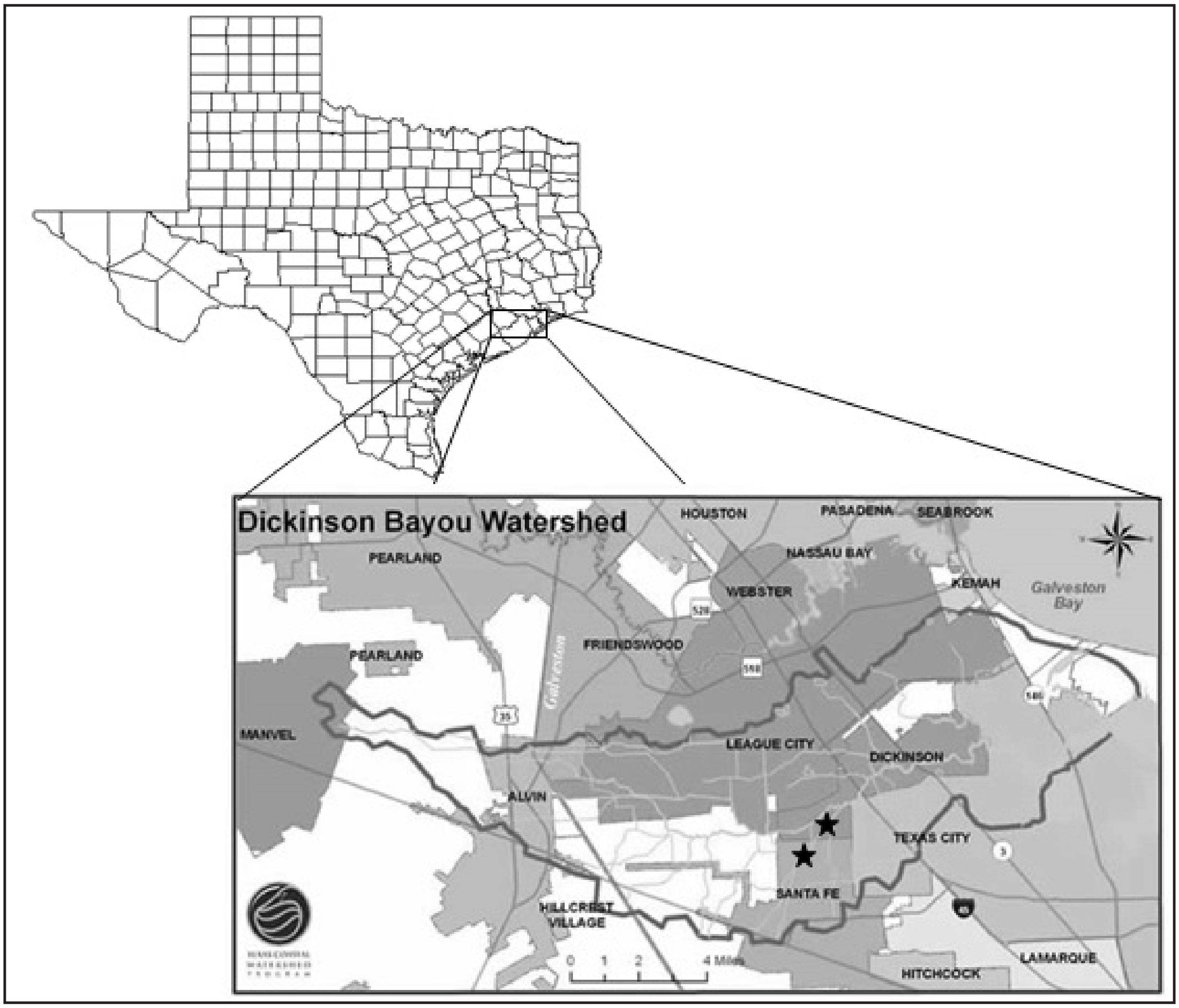

Figure 1. Map of the Dickinson Bayou watershed and the locations of the OSSF and Control sites indicated by the southern and northern star symbols, respectively..

treatment plant for wastewater treatment. All of the houses are connected to the municipal wastewater treatment plant via a series of clay sewer pipes. Impervious surfaces account for approximately 38% of the watershed.

The 2 monitoring stations were approximately 8 kilometers from each other and in both watersheds, a system of drainage ditches direct surface runoff to a single location before it flowed into Dickinson Bayou. The monitoring stations were installed at these runoff collection points. Meteorological data were collected from a nearby weather station that is located approximately 4 kilometers from the OSSF site (WU 2013).

Both monitoring sites were instrumented with bubbler flow meters (4230, Teledyne ISCO, Lincoln, NE) and automatic water samplers (3700, Teledyne ISCO, Lincoln, NE). The bubbler flow meter was interfaced with the automatic water sampler and the sampler was triggered to collect samples when the runoff levels were approximately 32 millimeters deep. In order to ensure that no cross-contamination of bacteria occurred in the field, 1 bottle, out of 24 total, remained empty and was used as a field control.

Preliminary hydrographs from the bubbler flow meter were used to create a sample programming schedule for the automatic water samplers. Water samples were obtained during pre-peak (rising limb), peak, and post-peak (recession limb) runoff time periods to assess how *E. coli* concentrations were changing during runoff events.

Within 8 hours of the first samples being taken during a runoff event, the sample bottles were put on ice, transported immediately to the laboratory, and tested within 24 hours of the first sample, using Environmental Protection Agency (EPA) Method 1603 (Stumpf et al. 2010; Hathaway and Hunt 2011). No samples were composited in the laboratory. Seven samples bottles were selected from each runoff event to be used as representative samples. For most runoff events, 3 samples were chosen to characterize the pre-peak runoff, 1 was chosen for the peak runoff, and 3 were chosen to represent the post-peak runoff. Due to the natural variability in the duration of each runoff event, these guidelines could not be used for every event but were used whenever possible. In total, 17 samples were analyzed for each runoff event: 1 lab control, 1 field control from the OSSF monitoring site, 7 samples from the OSSF monitoring site, 1 field control from the Control site, and 7 samples from the Control site.

Antecedent moisture conditions were assessed for each rainfall-runoff event to help determine if periods without rainfall were causing a buildup of treated wastewater from the surface application from the aerobic OSSFs. Antecedent moisture conditions were based on the amount of rain received during the 7 days prior to the sampling event (James and Roulet 2009). Antecedent moisture was considered dry if the previous 7 days received less than 6.35 millimeters of rainfall. Average antecedent moisture conditions were assigned if the previous 7 days received between 6.35 and 25.4 millimeters of rainfall. Wet antecedent moisture conditions were assigned if the previous 7 days received greater than 25.4 millimeters of rainfall.

EPA Method 1603 was used to enumerate *E. coli* in the runoff (EPA 2009). This process uses membrane filtration and a specific agar to allow the growth of *E. coli* for enumeration. *E. coli* counts lower than the lower detection limit, 10 CFU/100milliliters, were reported as non-detect. The lower detection limit was estimated by dividing the lowest possible colony count (1 colony) in the maximum undiluted sample volume (10 milliliters), then multiplying by 100 to convert to CFU/100milliliters. All non-detects were included in figures and statistical calculations as 5 CFU/100milliliters (1/2 of lower detection limit). To rule-out cross-contamination, both lab and field blanks were analyzed for every sampling event. Samples were periodically split with a third-party laboratory that was National Environmental Laboratory Accreditation Program (NELAP)-approved to validate the *E. coli* concentrations.

To determine the source of *E. coli* in runoff, a bacterial source tracking (BST) assessment was performed on *E. coli* isolates from a set of runoff samples taken on 3/4/14. A previous BST analysis performed on *E. coli* isolates from Oyster Creek watershed (northwest of the Dickinson Bayou watershed) indicated that 43% of *E. coli* was coming from wildlife, 19% was from livestock, 14% was from humans, and 9% was from domestic pets (Martin 2013). One isolate was taken from each of the 7 *E. coli* samples from the OSSF site (n=7 from the OSSF site) and 1 isolate was taken from each of the 7 samples from the Control site (n=7 from the Control site) using EPA Method 1603. Isolates were then DNA-fingerprinted using enterobacterial repetitive intergenic consensus sequence-polymerase chain reaction (ERIC-PCR) and riboprinting (RP) as described by Casarez et al. (2007). A DNA fingerprint was performed on 1 individual *E. coli* colony, or isolate, from each sample. Fingerprints for each of the isolates were compared against the Texas *E. coli* BST Library (ver. 6-13). This library contains DNA fingerprints for 1,524 *E. coli* isolates from 1,358 different fecal samples representing over 50 animal subclasses (Di Giovanni et al. 2013). Source-identified *E. coli* water isolates were divided into 3 source categories: human, wildlife, and livestock/domestic animals. A water isolate's category was chosen based on the highest percentage match to a known-source isolate in the library, with 80% being the lowest acceptable percentage match (Di Giovanni et al. 2013). If a water isolate's DNA fingerprint was not at least 80% similar to any known-source isolate in the library, then the water isolate was classified as unidentified with respect to its source.

RESULTS

E. coli concentrations

E. coli were found in 13 of 16 sampling events at the OSSF site and in 12 of 13 sampling events at the Control site. The *E. coli* concentrations detected at the OSSF site and the Control site are summarized in the box plots in Figure 2. The dashed horizontal line in Figure 2 represents the EPA and Texas state contact standard (126 CFU/100milliliters) of *E. coli* in recreational freshwaters. For the 16 runoff events at the OSSF site, the combined geometric mean *E. coli* concentration was 639 CFU/100millilters while the geometric mean *E. coli* concentration for 13 runoff events at the control site was 371 CFU/100milliliters.

E. coli concentrations at both the OSSF site and the Control site were typically well above the Texas state standard: 126 CFU/100milliliters. The geometric mean *E. coli* concentration for 12 of the runoff events (16 total events) at the OSSF site exceeded the regulatory use standard and at the Control site the geometric mean for 9 of the runoff events (13 total events) exceeded the regulatory use standard. It should be noted that there were 3 runoff events at the OSSF site and 1 runoff event at the Control site that yielded no culturable *E. coli* in all samples. With these exceptions, all runoff events had at least 1 sample that exceeded the regulatory standard. These samples showed that not only was there *E. coli* present at both sites, it was present in concentrations that routinely exceeded the EPA and Texas state recreational freshwater contact standard.

Statistical analysis

A paired t-test was used to compare the untransformed (normal) *E. coli* concentrations at the 2 sites to determine if

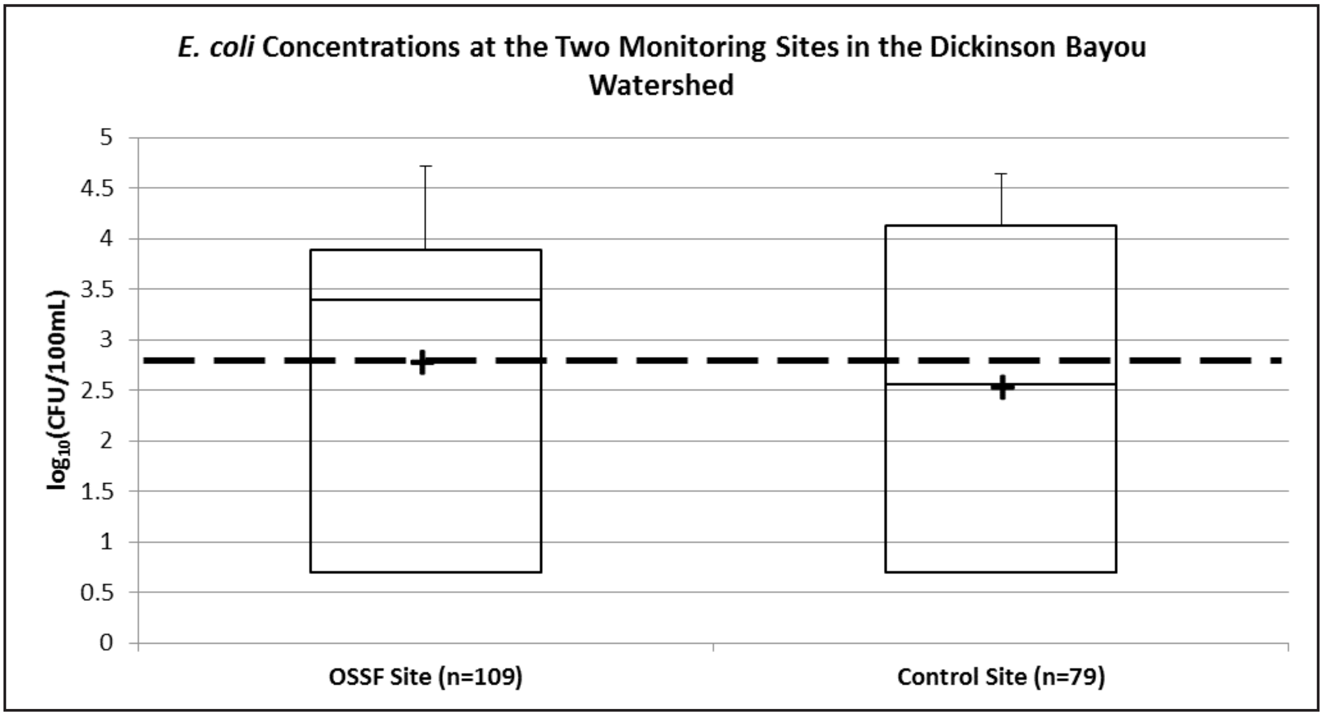

Figure 2. Cumulative distribution plots of *E. coli* concentrations found in runoff at the 2 monitoring sites in the Dickinson Bayou watershed. The dashed line represents the Texas state recreational contact standard for *E. coli*.

there was a significant difference between the 2 sample sets ($\alpha = 0.05$). Results from this analysis showed that there was no statistical difference between the concentrations found at the OSSF site and those found at the Control site ($p = 0.9335$). Previous research performed in the Dickinson Bayou watershed by the Galveston County Health District between 1992 and 1996 also concluded that "There was no clear difference in coliform concentrations between sewered and unsewered areas" (GCHD 1998).

A paired t-test was also used on a case-by-case basis to determine if there were any individual rainfall events that had *E. coli* concentrations that were statistically different between the 2 monitoring sites. Three events were found that had statistically different concentrations. The first 2 runoff events with statistically different *E. coli* concentrations occurred on 9/20/13 and 5/26/14, and the concentrations were higher at the OSSF site for both events ($p = 0.0451$ and $p = 0.0039$, respectively). These dates produced the 2 largest runoff volumes at the Control site during dry antecedent moisture conditions (21.17 millimeters and 11.52 millimeters, respectively). It is possible that the larger-than-typical runoff amounts at the Control site led to higher dilution and therefore lower concentrations at the Control site. The third event with statistically different *E. coli* concentrations occurred on 5/30/14 and had concentrations that were higher at the Control site ($p = 0.0002$). This particular event had the second highest runoff volume during wet antecedent moisture conditions at the Control site (50.64 millimeters) while the runoff volume at the OSSF site was typical for wet antecedent moisture conditions (4.31 millimeters). It should be noted that the largest runoff volume for wet antecedent moisture conditions at the Control site was due to an intense storm that also caused flooding at the OSSF monitoring site, leading to the largest runoff volume at the OSSF site.

Potential correlations considered for each individual sample were flow rate, temperature, antecedent moisture conditions, and the amount of time since the last sampling event. The linear regression analysis (R^2) values for each of the correlation variables at the OSSF site were less than 0.0301, while the R^2 values for each of the correlation variables at the Control site were less than 0.1963. The U.S. Geological Survey (USGS) had found elevated *E. coli* concentrations for high flow rates and seasonal differences, but these results were not correlated in this study (USGS 2003). In a fecal coliform study in nearby Buffalo and White Oak bayous by Petersen et al. (2006), there were almost no statistical differences between cooler and warmer months at multiple stations in the 2 bayous.

While no correlations were made for the individual sampling events between *E. coli* concentration and flow rate, temperature, antecedent moisture conditions, and the time between sampling events, statistical differences were found when the combined concentrations from all events at each of the sites were based on antecedent moisture conditions. Figure 3 shows the combined *E. coli* concentrations from the 2 sites divided by

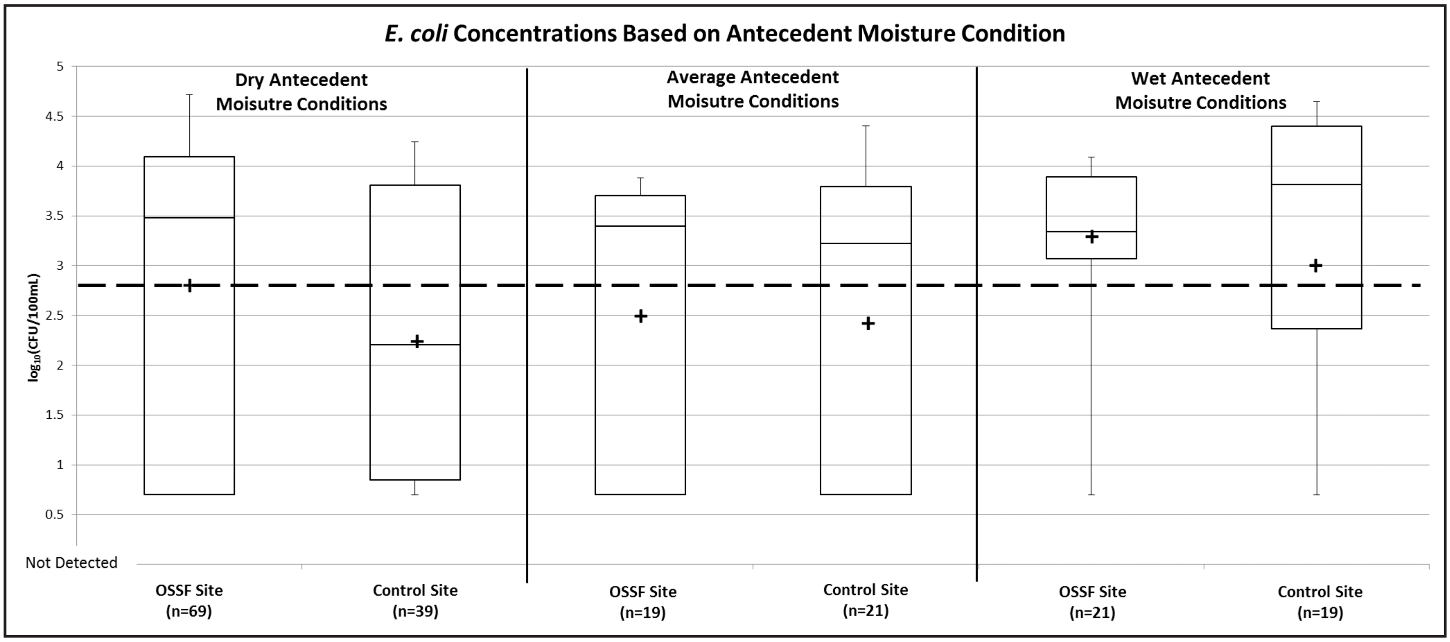

Figure 3. Quartile plots of *E. coli* concentrations found in runoff at the 2 monitoring sites in the Dickinson Bayou watershed separated by antecedent moisture conditions. The plus symbol represents the mean value of each of the 2 datasets. The dashed line represents the Texas state recreational contact standard for *E. coli*.

antecedent moisture conditions. As shown in Figure 3, runoff from the OSSF site had the highest *E. coli* concentrations during dry antecedent moisture conditions (p = 0.0170). On the other hand, runoff from the Control site had the highest *E. coli* concentrations during wet antecedent moisture conditions (p = 0.0226).

Evidence of elevated *E. coli* concentrations before the peak runoff rate, first flush, should be present if contaminated wastewater from aerobic systems had pooled on the surface of the low permeability soils. First flush was not found to occur at either the OSSF site or the Control site (p = 0.7711 and p = 0.3965, respectively; see Figure 4). In addition, no first flush

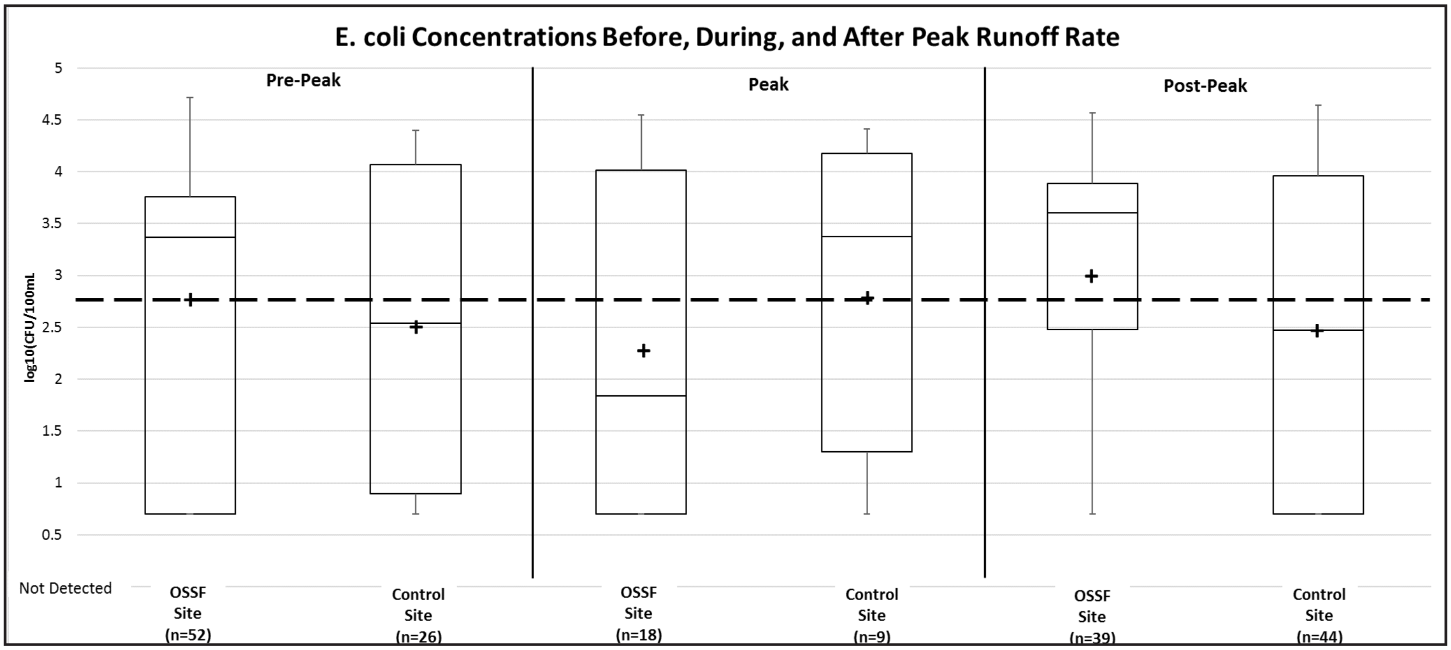

Figure 4. Quartile plots of *E. coli* concentrations found in runoff at the 2 monitoring sites in the Dickinson Bayou watershed separated by occurrence before, during, or after the peak flow rates. The plus symbol represents the mean value of each of the 2 datasets. The dashed line represents the Texas state recreational contact standard for *E. coli*.

Table 1. The statistical analysis of *E. coli* concentrations in the first flush runoff for the OSSF and Control sites based on antecedent moisture conditions.

Site	Antecedent Moisture Condition	p Value
OSSF site	Entire Dataset	0.7711
	Dry	0.1540
	Average	0.6139
	Wet	0.4298
Control site	Entire Dataset	0.3965
	Dry	0.5215
	Average	0.1936
	Wet	0.4299

effects for *E. coli* concentrations were observed at either site when the sampling events were divided based on antecedent moisture conditions. The respective *p* values for each site and antecedent moisture condition is shown in Table 1.

Bacterial source tracking analysis

Similar *E. coli* concentrations at both sites during each rainfall event can lead to a number of possible conclusions with varying combinations of *E. coli* sources. When looking specifically for the possibility of failing OSSFs as the primary contamination source, 2 main possibilities exist. First, the OSSFs may be the primary contributors to the contamination at the OSSF site, and at the Control site a combination of wildlife, domestic animals, and the possibility of the municipal sewage pipes in the neighborhood failing could equal the OSSF site. However, it may also be possible that the OSSFs at the OSSF site and the sewer pipes at the Control site are operating properly and all *E. coli* in the runoff from the 2 sites is coming from either wildlife or domestic animals. Therefore, additional site investigations were undertaken.

While BST analyses have been performed in neighboring watersheds, this study was the first to employ the analysis in the Dickinson Bayou watershed (Martin 2013). As discussed in the Methods section, 7 *E. coli* isolates were taken from each site from the runoff event on 3/4/14 to perform the BST. Results of the BST indicated that human fecal material contributed to *E. coli* levels at both the OSSF and Control sites. Human fecal contamination was a larger source of *E. coli* in runoff at the Control site, 43% of isolates, than the OSSF site, 14% of isolates. Breaking down the remainder of the isolates from the OSSF and Control sites, wildlife accounted for 86% and 28%, respectively and domesticated animals accounted for 0% and 28%, respectively.

DISCUSSION

Continuous monitoring efforts performed by both the USGS, in cooperation with the TCEQ, and the Houston-Galveston Area Council (HGAC), with the help of the Texas Stream Team, have found similarly high, and variable, *E. coli* concentrations in Dickinson Bayou and Dickinson Bay. The USGS performed a major study of the Dickinson Bayou watershed from 2000 to 2002 and found *E. coli* concentrations ranging from 0–16,000 CFU/100milliliters (USGS 2003). Likewise, data from HGAC shows *E. coli* concentrations ranging from 5–20,000 CFU/100milliliters (HGAC 2013b). Both of these ranges are consistent with what was found at both the OSSF site (0–52,000 CFU/100milliliters) and the Control site (0 – 44,000 CFU/100milliliters). The USGS also noted that "Densities of both bacteria varied over wide ranges, particularly in Dickinson Bayou," both bacteria being *E. coli* and fecal coliforms (USGS 2003).

The maintenance and complaint records for 2013 and 2014 for the sewer pipes in the Control site watershed were obtained from the Galveston County Water Control and Improvement District #1. These documents showed that there had been cracks and leaks found in the sewage pipes caused by invasive roots and shifting soils. Also, a maintenance engineer with the Galveston County Water Control and Improvement District #1 said that occasionally during exceptionally large rainfall events or periods of rain for many days the sewage lines sometimes overflow through manhole covers found in dead-end streets (District #1, personal communication, April 18, 2014). Therefore, failing sewage pipes could potentially be a reason for the high *E. coli* concentrations at the Control site. In addition, this study found that wet antecedent moisture conditions led to higher *E. coli* concentrations at the Control site. The BST evidence agrees with the possibility of leaking clay pipes at the Control site being a cause of *E. coli* concentrations in the runoff.

Detection of *E. coli* from human sources at the OSSF site would point towards OSSFs since they are the only identifiable human source in this watershed. This study's finding that dry antecedent moisture conditions have higher *E. coli* concentrations than wet conditions may also agree with the OSSF finding. If OSSFs are the cause of the human-borne *E. coli* the bacteria on the surface would collect on the surface during the dry events and be carried away during the first runoff event. The wet conditions would contribute less *E. coli* because there had been less time for *E. coli* to collect since the last runoff event. While first flush was not found for each event, it is likely that at the OSSF site the first runoff event during the dry conditions were effectively acting as a long-term first flush event for the *E. coli*.

In order to effectively remedy the excess bacteria levels, the major source(s) of contamination should be identified and verified. As in most cases, finding the source of nonpoint source pollution has proven difficult. With BST analyses, the primary sources of bacterial contamination can be identified. Similar projects near coastal areas should consider the use of a BST analysis or another analysis to measure human-specific bacterial markers, during every sampled rainfall event to determine the extent of the human fecal source presence. Using BST analyses in future studies should also provide more information as to the specific cause of the contamination. In addition, future projects in coastal areas should look into monitoring not only residential areas but also natural areas in an attempt to create a baseline *E. coli* concentration from natural sources.

CONCLUSIONS

Dickinson Bayou is contaminated with *E. coli* concentrations higher than the EPA and Texas state recreational freshwater contact standard. Stormwater runoff collected from the site containing OSSFs and the site connected directly to the municipal sewage facility consistently exhibited *E. coli* concentrations higher than the EPA and Texas recreational freshwater contact standard, yet no statistical difference between the overall *E. coli* concentrations at the 2 sites was found. Further differentiation between the various potential *E. coli* sources was made by using a BST analysis; this was the first time such analysis has been performed on runoff samples in the Dickinson Bayou watershed. Results from the BST analysis confirmed a human fecal presence at both sites.

While it was not confirmed that OSSFs were failing at the OSSF site, OSSFs are the only ostensible source of human fecal contamination and are most likely in part to blame for the increased bacterial contamination in the Dickinson Bayou watershed. Homeowners of OSSFs should follow a regular maintenance and check-up schedule with a qualified professional to minimize the possibility of failure. Conversely, there are no apparent human sources of fecal material at the Control site, yet *E. coli* from human sources was still confirmed. Broken or leaky municipal sewage lines may be the cause of the human fecal material present in runoff and should be investigated further since no definitive source of pollution was identified in this study.

Assuming the single BST analysis performed in this study is indicative of all runoff events, less than half of the *E. coli* at both sites are from human sources. From this it can be assumed that animals, both domestic and wildlife, are the primary contributors of bacteria to Dickinson Bayou. Besides picking up after pets, little can be done to prevent contamination due to animals. The primary way of preventing bacterial contamination in the Dickinson Bayou watershed would be to focus on reducing the human sources by means discussed above.

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