ASSESSMENT OF E. COLI POLLUTION FROM FAILING ON-SITE SEWAGE FACILITIES TO DICKINSON BAYOU

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ABSTRACT

Since 1996, the *E. coli* levels in Dickinson Bayou have been considerably higher than the Texas state maximum of 126 colony forming units (CFU) per 100 mL for recreational waters. One hypothesis is that failing onsite sewage facilities (OSSFs) in the nearby residential areas are causing an increase of *Escherichia coli (E. coli)* concentrations in Dickinson Bayou. There are two types of OSSFs in the watershed; anaerobic and aerobic systems. The anaerobic systems discharge partially treated effluent below the soil surface from gravel drainage trenches while the aerobic systems disperse treated effluent on the soil surface using spray nozzles. This project was designed to determine if either of the two systems was contributing to the elevated *E. coli* concentrations in Dickinson Bayou.

Two water quality monitoring stations were installed in the Dickinson Bayou watershed to estimate *E. coli* concentrations in surface runoff. One of the monitoring stations was placed in a neighborhood that uses OSSFs and the second station was placed in a neighborhood connected to a municipal sewage plant. Each monitoring station was equipped with a flow meter and an automatic water sampler.

Runoff/rainfall relationships were established for each monitoring station. Water quality samples were obtained for sixteen rainfall events at the site with OSSFs and twelve events at the site with no OSSFs. Nearly all sampling events had at least one sample with an *E. coli* concentration greater than the state boundary. However, the concentrations from both sites were very similar to one another. A bacterial source tracking method was employed to conclude that a portion of the *E. coli* from both sites were of human origin. Further studies should focus on bacterial source tracking to determine the exact extent of human-based bacterial contamination in the Dickinson Bayou watershed.

ACKNOWLEDGEMENTS

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NOMENCLATURE

TCEQ	Texas Commission on Environmental Quality
OSSF	Onsite Sewage Facilities
E. coli	*Escherichia coli*
km^2	Square Kilometers
ac	Acres
mm	Millimeters
in	Inches
km	Kilometer
mi	Mile
m	Meters
ft	Feet
m^2	Square Meters
LiDAR	Light Detection and Ranging
TNRIS	Texas Natural Resources Information System
GIS	Geographical Information System
SWAT	Soil Water Assessment Tool
EPA	Environmental Protection Agency
^oC	Degrees Centigrade
CFU	Colony Forming Unit
NELAP	National Environmental Laboratory Accreditation Program

Hr	Hours
m^3/s	Cubic Meters per Second
mL	Milliliter
USGS	United States Geological Survey
H-GAC	Houston-Galveston Area Council
BST	Bacterial Source Tracking

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Dickinson Bayou is located in southeast Texas, near Galveston. The bayou is connected to Dickinson Bay, which then flows into Galveston Bay, as shown below in Figure 1. 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 three water bodies have been listed on the Texas Commission on Environmental Quality's (TCEQ) 303(d) list since 1996. These water bodies are impaired by elevated bacteria concentrations and do not meet the intended use regulatory standard (TCEQ, 2012).

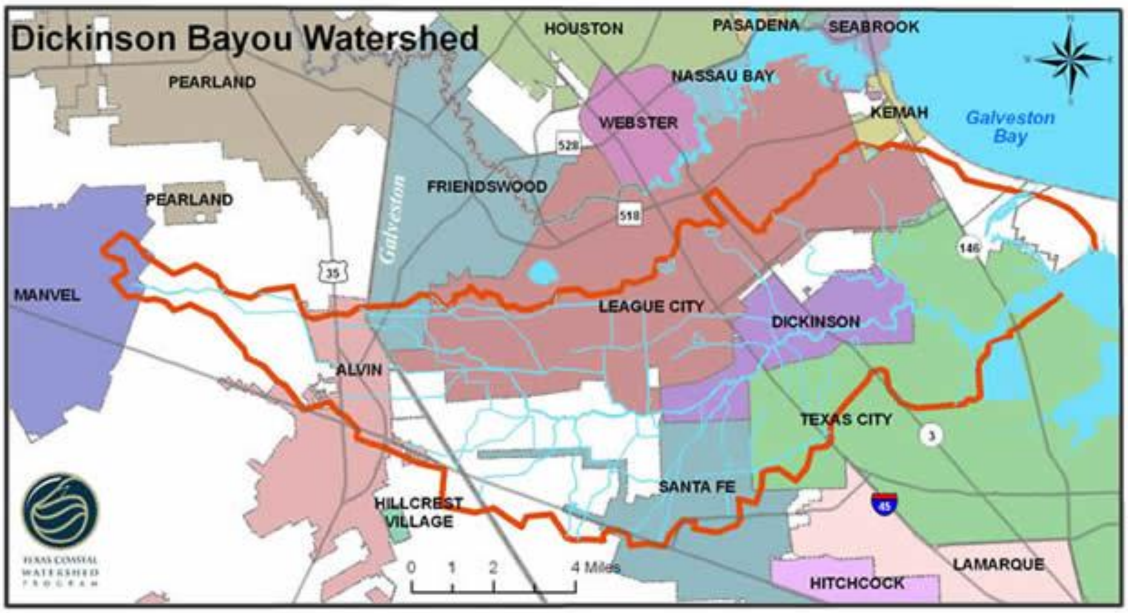

Figure 1. Map of the Dickinson Bayou watershed boundary and the stream network (DBWP, 2007).

Failing anaerobic and aerobic onsite sewage facilities (OSSFs) may be contributing to the increased bacteria levels in Dickinson Bayou. Both systems have shown increased bacteria concentrations in nearby coastal areas when they are malfunctioning (Conn et. al., 2011). Failing anaerobic systems may cause high concentrations of *Escherichia coli* (*E. coli)* in the groundwater because of the high water table found in the majority of the Dickinson Bayou watershed. Aerobic systems improperly operated or maintained may also cause an increase in *E. coli* concentration in runoff because the dense clay soils inhibit the effluent from these types of systems to percolate into the ground.

"Water Quality in the Dickinson Bayou Watershed and Health Issues", an article in *Marine Pollution Bulletin*, discusses the negative effects of excess bacteria in the project area, such as illnesses in humans and degrading ecosystem services in the Dickinson Bayou, and the necessity to prevent further pollution (Quigg et. al., 2009). Other research has also pointed toward excess bacteria as both an environmental and economic issue that should be addressed (Overstreet, 1988; Soller et. al., 2010). Some of the issues discussed include gastrointestinal illnesses in humans and infections in fish and shellfish, which limits the amount of seafood that can be sold therefore, causing a major economic issue in areas that rely on fishing as a means of financial substance.

In addition to the environmental and economic matters, Dickinson Bayou and Dickinson Bay are used by many residents of the area for fishing, boating, or other recreational activities. However, nearly half of all residents in the Dickinson Bayou watershed are not aware of the bacterial problem in the watershed (TAMUPPRI, 2012). These bacteria, in high enough concentrations, are capable of causing illnesses in the citizens using the bayou. Specifically, *E. coli*, which is found in excess in both Dickinson Bay and Dickinson Bayou, may cause intestinal problems in humans (Smith and Perdek, 2004; Teague, 2007; Riebschleager, 2012). In order to decrease the bacteria concentration in Dickinson Bayou and prevent further pollution the source must be known. Specifically, where does the excess bacteria come from? There are four main sources of fecal contamination in water bodies as designated by the indicator organism *E. coli*: failing onsite sewage facilities (OSSFs), wastewater treatment plants, domestic animals, livestock, and wildlife (Smith and Perdek, 2004). Previous research has suggested that failing OSSFs may be a major factor in elevated bacterial levels in nearby Buffalo Bayou (Platt, 2006). This thesis discusses the possibilities of local OSSFs causing excess bacterial loads and presents the results of a monitoring program to determine *E. coli* concentrations from OSSFs. *E. coli* concentrations and flow rates were monitored during runoff events at two monitoring locations since December 2012. 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 clays present in most of Galveston County prevented homeowners from building new anaerobic systems. Aerobic systems started becoming the most installed OSSF type after 1997, especially in areas that could not accept anaerobic systems.

Anaerobic Systems

A conventional septic tank with a drain field is an example of an anaerobic system. In this type of system the wastewater from the residence first enters a holding tank (septic tank) that is used to settle out the solids in the waste stream. The effluent is then considered partially treated because the holding tank has removed the solids that are capable of settling but has not treated any other contaminants that may still be suspended in the effluent. The partially treated wastewater then flows into a distribution system. Perforated distribution pipes allow the wastewater to flow out and percolate into the surrounding soil. Distribution pipes are at least 6 inches below the surface of the soil but may be deeper depending on the surrounding soil type (CUCES, 2010). Gravel or chipped tires typically surround the distribution pipes, while a geotextile membrane is set on top of this layer to separate it from a layer of loamy soil placed near the surface (TAMAE, 2008). Native soil is added on top of the layer of loamy soil, a typical set-up of a drainage field can be seen in Figure 2. The soil acts as the final treatment for the anaerobic system by allowing microbes in the soil to feed on the excess waste and nutrients in the effluent (TAMAE, 2008). Anaerobic systems in the Dickinson Bayou watershed use a septic tank to settle out the solids and then the effluent flows by gravity to an underground gravel drainage field.

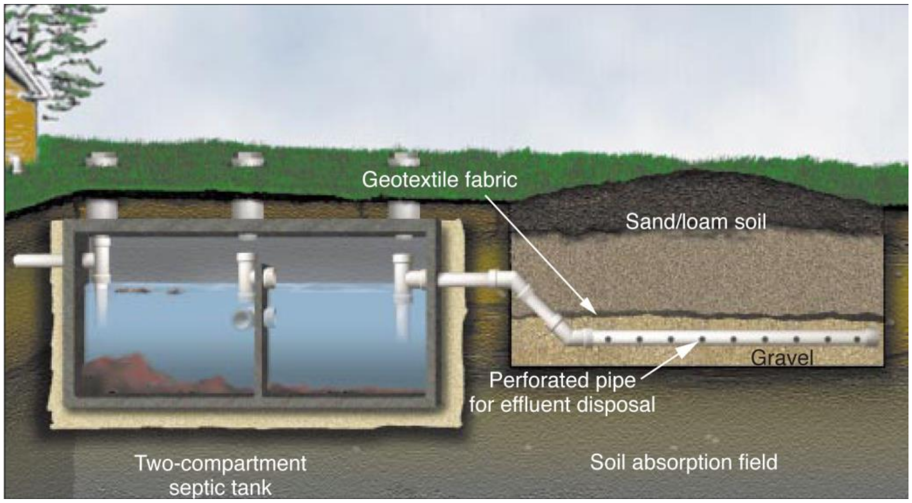

Figure 2. Cross-section of a typical anaerobic OSSF showing the septic tank and the disposal system (TAMAE, 2008).

When the soil surrounding these drainage fields is dense clay the ability of the wastewater to infiltrate into the ground is greatly reduced and has been shown to be a significant factor in septic system failures (Carr et. al., 2009; Withers et. al., 2011). Previous research has also shown that when high water tables are present, anaerobic systems have the ability to contaminate groundwater (Scandura and Sobsey, 1997; Humphrey et. al., 2011; Lapworth et. al., 2012).

Aerobic Systems

An aerobic system is fundamentally different from an anaerobic system. In an aerobic treatment system the wastewater first enters a trash tank that removes non-biodegradable solids. The wastewater then enters the aerobic treatment unit where aerobic microorganisms decompose the biodegradable waste in the effluent. A clarifier (settling chamber) is then used to remove the microbes. In addition, most aerobic systems also treat the wastewater with a disinfectant, typically through chlorination, ultraviolet light, or ozone. Spray heads are then used to distribute the treated wastewater onto the land surface (TAMAE, 2008). Figure 3 shows a cross-sectional diagram of a typical aerobic OSSF system in the Dickinson Bayou watershed.

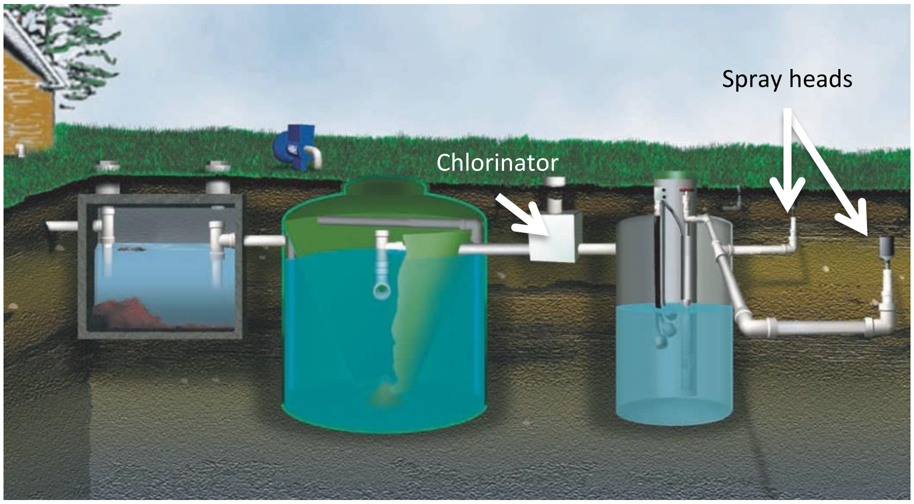

Figure 3. Cross-section of a typical aerobic OSSF showing the septic tank, disinfection tank, and disposal system (TAMAE, 2008).

The aerobic systems used in the project area generally disinfect the wastewater with chlorine before it is sprayed onto the surface of the soil. The disinfection process does not completely remove all pathogens, rather it greatly reduces the concentration. Chlorination should also result in a residual amount of chlorine in the effluent. Effluent from OSSFs with chlorination should "contain at least 0.1 milligram of chlorine per liter of wastewater or have no more than 200 fecal coliforms (bacteria from human wastes) per 100 milliliters of wastewater" (TAMAE, 2008). However, if the aerobic system is not well maintained the efficiency of this type of OSSF is greatly diminished (Levett et. al., 2010). In the case of an improperly maintained aerobic OSSF the surface soil then becomes the primary treatment medium. If the soil consists largely of clays then the infiltration capacity is generally very low and the 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). Therefore bacteria sprayed onto the soil surface from improperly maintained aerobic OSSFs may be transported by sediment in runoff to nearby ditches and streams and eventually to Dickinson Bayou as well.

Failing OSSF Contamination Potential

Human fecal material contains approximately $1*10^6$ – $4.2*10^6$ colony forming units (CFUs) per 100 mL (Riebschleager et. al., 2012; TCEQ, 2014). In theory, if the OSSF were failing then the *E. coli* concentration being emitted to the soil would be approximately $1*10^6$ CFUs per 100 mL. An implementation plan developed by the Dickinson Bayou Watershed Partnership estimated that 35% of OSSFs installed before the year 2000 were failing while only 25% of OSSFs installed after the year 2000 are failing (TCEQ, 2014). According to the United States Census Bureau there are approximately 3 persons per household in Galveston County, the county in which the majority of the watershed lies (USCB, 2014). Each person will typically use between 60 and 70 gallons of water per day (Riebschleager et. al., 2012; TCEQ, 2014). This means that the average household with a failing OSSF will discharge between 680 and 795 liters of water per day with a theoretical *E. coli* concentration of $1*10^6$ CFUs per 100 mL. A rainfall event that captured a discharge from a home with a failing OSSF could contain elevated *E. coli* concentrations.

Objectives and Hypothesis

The main objective of this project was to determine if failing OSSFs in residential areas were contributing to the elevated *E. coli* concentrations in Dickinson Bayou. This was accomplished by monitoring the quality of stormwater runoff from two neighborhoods in the Dickinson Bayou watershed. One neighborhood utilizes OSSFs while the houses at the second site are directly connected to a municipal wastewater treatment plant. This research project recorded total rainfall, calculated the total runoff, and estimated the *E. coli* concentrations in runoff from two sites in the Dickinson Bayou watershed.

It is hypothesized that runoff from neighborhoods with OSSFs will have higher *E. coli* concentrations than runoff from neighborhoods connected to a municipal sewage line. If it is found that failing OSSFs are a major source of bacteria in the Dickinson

Bayou watershed, alternative OSSF practices will need to be developed to improve water quality in Dickinson Bayou.

CHAPTER II

MATERIALS AND METHODS

The Dickinson Bayou watershed is located in south-east Texas, near Galveston (Figure 4). The water draining from this watershed flows into Dickinson Bayou and then into Galveston Bay. The watershed is approximately 258.3 km^2 (63,827 ac) and contains portions of major nearby cities including Alvin, Dickinson, Friendswood, League City, Manvel, Santa Fe, and Texas City (Figure 4). Dickinson Bayou receives discharge from 11 wastewater treatment plants, 8 of which treat domestic wastewater (DBWP, 2007). Soils in the watershed are “somewhat poorly drained, very slowly permeable, clays and clay loams, loams and silt loams, and fine sandy loams” and receives an average annual rainfall of 1,219 mm (48 in) (GCPD, 2005). The topography of the watershed is very flat with low slopes of 0-3%. Main land uses in the watershed are grassland (43%), woodland (27%), agriculture (9%), low intensity development (9%), and high intensity development (6%) (DBWP, 2007).

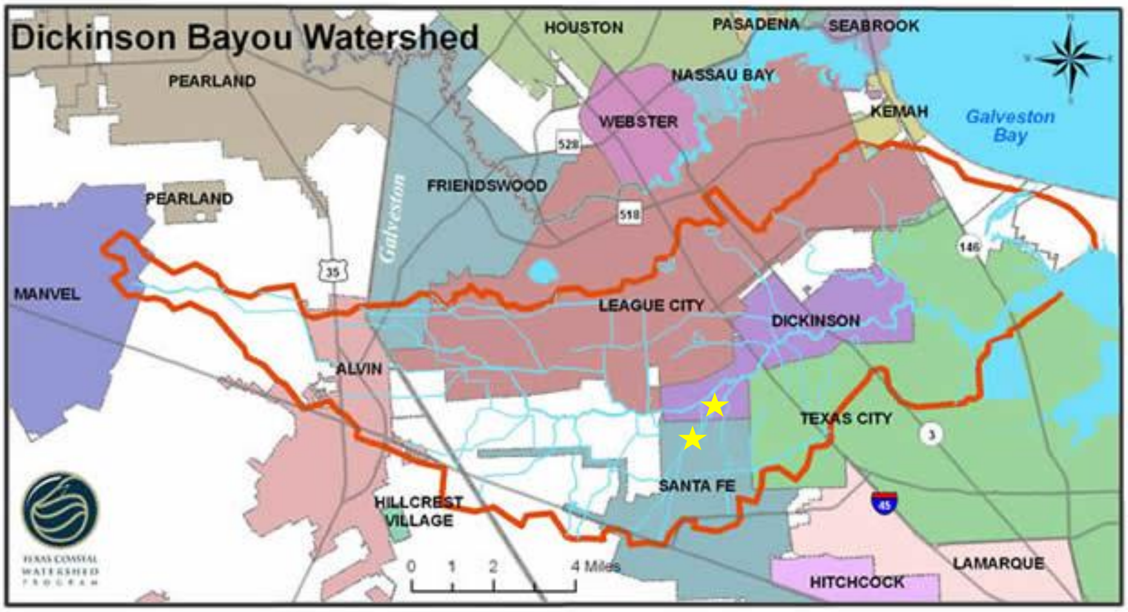

Figure 4. Locations of the two monitoring sites in the Dickinson Bayou watershed are indicated by the star symbols (DBWP, 2007).

There are a total of about 5,000 OSSFs in the Dickinson Bayou watershed (DBWP, 2007). The total number of OSSFs in the watershed includes both permitted and unpermitted OSSFs. Texas Coastal Watershed Program found the total number of OSSFs by collecting the permitted OSSF location information and the map of the sewage main using the following procedure. These two pieces of information were overlaid with the locations of all the houses in the area. Houses that were not near the sewage main but did not have a permitted OSSF were assumed to be using an unpermitted OSSF. The remaining households were assumed to be directly connected to a municipal sewer system.

Two water quality monitoring stations were installed, as indicated by the star symbols in Figure 4. The first is located in Santa Fe, Texas, in a neighborhood that uses only OSSFs. Approximately 8 km (5 mi) away the second water quality monitoring station, the control site, is located in the town of Dickinson in a neighborhood connected to a sewer main. In both neighborhoods a system of drainage ditches converge at a single location before entering Dickinson Bayou. The points where the drainage ditches connected were used to collect runoff samples. The two neighborhoods are similar in size, age, and percent imperviousness. Meteorological data was collected from a nearby weather station that is located approximately 4 km (2.5 mi) from the Santa Fe neighborhood (WU, 2013). Rainfall intensity and total rainfall are updated every 15 min to the Weather Underground website (wunderground.com). Rain gages were installed at both sites to confirm the accuracy of the Weather Underground data.

OSSF Monitoring Site

The watershed for the OSSF monitoring site is shown in Figure 5. There are no houses in the watershed that are connected to a municipal sewer system. Of the 28 houses in the watershed, 19 utilize the anaerobic type OSSF and the remaining 9 use the aerobic type OSSF.

The soil found in this area consists of Mocarey loam, Mocarey-Algoa complex, and Mocarey-Cieno complex and all soils are in hydrologic group D (WSS, 2013). The slope of the land is very gentle, ranging from 0-3 percent. The houses in the neighborhoods range from 1,011 to 4,047 m^2 (0.25 to 1 ac) lots. Homes in the neighborhood north of the ditch were built in the early 2000’s and are typically connected to aerobic systems while the homes south of the ditch were built in the 1980’s and are mainly connected to anaerobic systems.

Light Detection and Ranging (LiDAR) data was obtained from the Texas Natural Resources Information System (TNRIS) and input into a Geographic Information System (GIS) program. The LiDAR data was used in the Soil Water Assessment Tool's (SWAT) automatic watershed delineation program to determine the shape and size of the watershed associated with the sampling point. Results from this analysis showed that the watershed was approximately 0.146 km^2 (36 ac). Using satellite photos from TNRIS the total impervious area was found using the area calculation tool in a GIS program. Impervious areas accounted for approximately 10% of the total area of the watershed. The red arrow in Figure 5 indicates the location of the manhole that is being used as the sampling point.

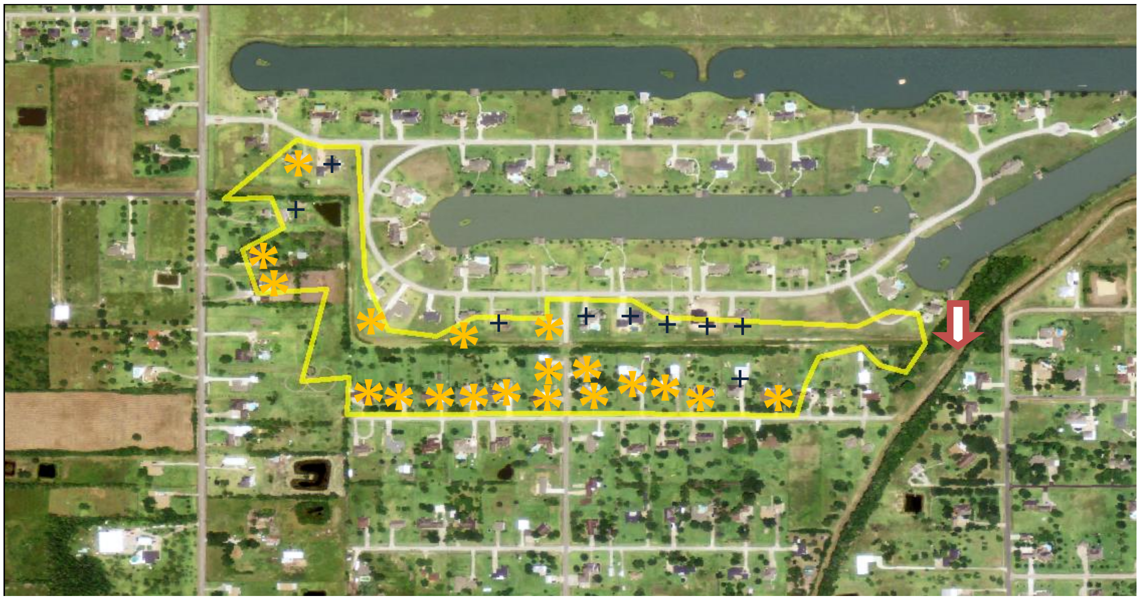

Figure 5. The red arrow indicates the location of the OSSF monitoring station used to collect runoff water sample for *E. coli* analysis in the Dickinson Bayou watershed. The watershed boundary associated with the OSSF monitoring station is shown. Houses marked by the asterisk symbol have anaerobic systems and houses marked with the plus symbol use aerobic systems.

The monitoring point at this location is in a manhole approximately 335 m (1,100 ft) east of the neighborhood, as shown below in Figure 6. Stormwater runoff is collected in a ditch that is then diverted into an underground 1.2 m (4 ft) corrugated metal pipe that flows into the manhole. Water levels in the larger ditch, shown in the background of Figure 6, were taken with a Rugged Troll (100, In-Situ, Ft. Collins, CO) and were normalized with a BaroTroll (In-Situ, Ft. Collins, CO). A hydrograph of the data collected from the large ditch is shown at the end of Appendix A.

Figure 6. Manhole used for equipment storage and runoff collection at the OSSF site.

Control Site

The control site is located in the town of Dickinson in a neighborhood where all of the houses are directly connected to a sewer main. There are no houses in the neighborhood that are connected to an OSSF system. The monitoring point is the outlet of a detention basin in the middle of the neighborhood. Stormwater runoff from the surrounding neighborhood is collected by a ditch network and routed to the basin. Three drainage pipes take the runoff to a larger retention pond immediately behind the fence seen in Figure 7. The two outer pipes were partially blocked off so that the runoff would flow toward the center pipe, which was used for runoff collection.

Figure 7. Detention basin used to collect runoff from the control site. All instrumentation is kept inside of a storage box on the apex of the basin.

Soil found in this area consists of Lake Charles clay and Vamont clay and both soils are in hydrologic group D (WSS, 2013). Topographically, the land is very flat with slopes ranging from 0-3 percent. The houses in the neighborhoods range from 1,011 to 2,023 m^2 (0.25 to 0.5 ac) lots. Homes on the eastern side of the watershed were built in the 1960's while the homes on the western side of the watershed were built in the later 2000's. Impervious surfaces account for approximately 38% of the watershed. The same method used to determine the watershed size for the OSSF monitoring site was also used to determine the watershed associated with the control site. A total of 29 houses are included in the watershed that was found to be approximately 0.03 km^2 (7.3 ac). Figure 8 shows the location of the control site. The red arrow shows the location of the monitoring point.

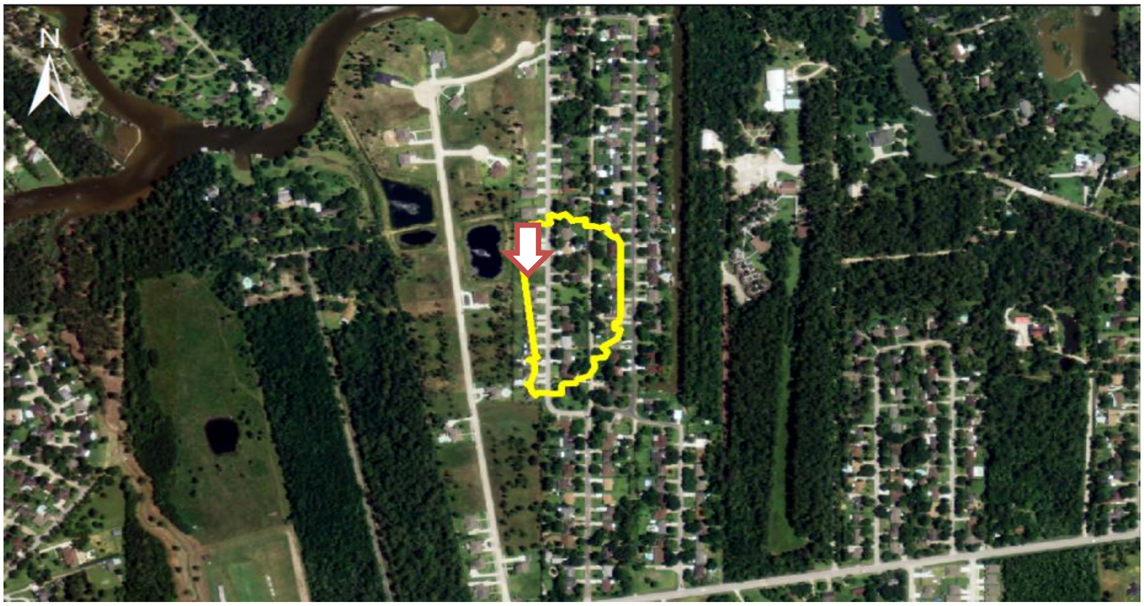

Figure 8. The red arrow indicates the location of the control site monitoring station used to collect runoff water samples for *E. coli* analysis in the Dickinson Bayou watershed. The watershed boundary associated with the control station is shown.

Runoff Collection Methods

Both monitoring sites were instrumented with a bubbler flow meter (4230, Teledyne ISCO, Lincoln, NE), an automatic water sampler (3700, Teledyne ISCO, Lincoln, NE), and the necessary support equipment to run the two devices. A picture of the bubbler flow meter (left) and automatic water sampler (right) used at the control site is shown in Figure 9. The OSSF site also contained the same instrumentation inside the corrugated metal manhole.

Figure 9. Bubbler flow meter (left) and automatic water sampler (right) used to collect runoff at the control site.

The bubbler flow meter was interfaced with the automatic water sampler which allowed the sampler to be initiated by the flow meter once runoff levels were deep enough to fully submerge the sampler tip, approximately 31.75 mm (1.25 in). The automatic water samplers held 24, one liter polypropylene bottles. In order to ensure that no bacteria were present in the bottles, one bottle always remained empty so that it could be used as a field control. The remaining 23 bottles were used to collect the runoff samples. The flow meter was installed in early December 2012 and collected preliminary data before the addition of the sampler in April 2013. Both the flow meter and the automatic water sampler were installed at the control site in April 2013.

Preliminary data from the bubbler flow meter was used to create hydrographs and rainfall-runoff ratios at the OSSF monitoring site. This information was used to program the automatic samplers. Since the goal of the project was to determine if OSSFs are contributing to *E. coli* concentrations during runoff events an assessment of how *E. coli* concentrations may be changing during runoff events was important. Therefore, water samples were needed during pre-peak (rising limb), peak, and post-peak (recession limb) runoff time periods. The preliminary runoff data was used to determine the average time it took the runoff event to reach the peak level and to determine how long the total runoff lasted for each rainfall event. In addition, the water level must be at least 31.75 mm (1.25 in) to fully submerge the sampler tip. Thus, the flow meter was set to trigger the sampler when the water level in the pipe reached this level. If the rainfall event did not provide enough rainfall to reach the trigger amount then the samplers would not be enabled. Initially the sampler collected eight samples every three minutes and the remaining fifteen bottles every ten minutes. After more rainfall-runoff information was collected, the timing was changed to better reflect the current information. The sampling times were changed once so that the sampler collected five samples every five minutes, eight samples every ten minutes, and the remaining ten bottles every twenty minutes. This timing system was used for both the OSSF site and the control site.

Sampling Methods

After the water samples were deposited into the bottles by the sampler, the following steps were used to preserve the samples. The sample bottles were put on ice, transported immediately to the laboratory, and tested within 24 hours using EPA Method 1603 (Stumpf et. al., 2010; Hathaway and Hunt, 2011). No sample was composited in the laboratory. However, to save time and money only seven of the 23 samples were actually chosen to be analyzed. These seven samples were selected by referring to the hydrographs. For most runoff events three samples were chosen to characterize the pre-peak runoff, one was chosen for the peak runoff, and three 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. Therefore, a total of 17 samples in total were analyzed for each runoff event. The 17 samples analyzed included one lab control, one field control from the OSSF monitoring site, seven samples from the OSSF monitoring site, one field control from the control site, and seven samples from the control site.

Water Quality Analysis

EPA Method 1603 was used to estimate the *E. coli* concentrations in the runoff (EPA, 2009). This process uses membrane filtration and a nutrient medium to allow the growth of *E. coli* for enumeration. Millipore 0.45 µm membrane filters (Millipore, Billerica, MA) were used to capture bacteria from the runoff and modified membrane-thermotolerant *E. coli* (modified mTEC) agar powder (BD, Franklin Lakes, NJ) was used for *E. coli* growth. These same materials have been used in previous *E. coli* research (Padia et. al., 2010; Gallagher et. al., 2012). Each of the selected samples was serially diluted, passed through the membrane filter, and each of the dilutions' membranes was put onto an agar plate. Two dilutions were performed on each sample, therefore three dilutions from each sample were filtered. The first dilution contained 10 mL of the sample, the second dilution contained 1 mL of the sample in 9 mL of autoclaved deionized water, and the third dilution contained 0.1 mL of the sample in 9.9 mL of autoclaved deionized water. After 2 hours in an incubator at 35 ± 0.5^{o}C the samples were placed in Whirl-Pak bags and left in a water bath for 22 hours at 44.5 ± 0.2^{o}C. The plates were then removed from the bath and the number of colonies for each sample was counted. In accordance with Method 1603 practices the dilutions with the closest to between 30 and 300 CFUs were reported. No duplication was performed. Subtleties and uncertainties in both the sampling procedure and the sampling method can lead to inconsistent data. To minimize these uncertainties both lab and field controls were used during every sampling event. Also, *E. coli* concentrations were verified by a third-party laboratory that is approved by the National Environmental Laboratory Accreditation Program (NELAP).

CHAPTER III

RESULTS AND DISCUSSION

Runoff Characteristics

Since April 2013, sixteen sampling events have been aggregated at the OSSF monitoring site and twelve events have been aggregated at the control site. The times to reach the peak runoff and the total length of the runoff event for the OSSF site can be seen below in Table 1 and for the control site in Table 2. Times were based on when the rainfall began and antecedent moisture conditions were based on the amount of rain that had fallen within the seven days prior to the runoff event. The total runoff time was calculated by subtracting the time at which the runoff reached a predetermined depth on the trailing end of the runoff and the time when the runoff began.

Table 1. Summary of rainfall-runoff conditions at the OSSF monitoring site since April 2013.

Date	Antecedent Moisture Conditions[a]	Time Between Rainfall Initiation and Peak Runoff (hr)	Total Runoff Time (hr)
5/10/13	Dry	2.65	110.42[c]
8/11/13	Dry	4.67	18.12
8/26/13	Dry	1.87	82.50
9/20/13	Dry	4.33	13.96[b]
9/21/13	Wet	3.52	75.00

Table 1. Continued

Date	Antecedent Moisture Conditions[a]	Time Between Rainfall Initiation and Peak Runoff (hr)	Total Runoff Time (hr)
10/27/13	Dry	2.60	61.67
10/31/13	Wet	6.02	200.62
11/22/13	Dry	2.57	77.50
11/25/13	Average	14.93	138.25[c]
1/13/14	Dry	2.58	69.79
2/2/14	Dry	7.42	87.92[b]
2/4/14	Average	5.30	113.12
3/4/14	Average	5.98	n/a
5/13/14	Dry	3.23	27.42
5/26/14	Dry	2.83	90.58[c]
5/30/14	Wet	3.58	70.42

Table 2. Summary of rainfall-runoff conditions at the control site since April 2013.

Date	Antecedent Moisture Conditions[a]	Time Between Rainfall Initiation and Peak Runoff (hr)	Total Runoff Time (hr)
8/26/13	Dry	1.45	18.54
9/20/13	Dry	2.92	22.08[b]
9/21/13	Wet	2.02	32.08

Table 2. Continued

Date	Antecedent Moisture Conditions[a]	Time Between Rainfall Initiation and Peak Runoff (hr)	Total Runoff Time (hr)
10/27/13	Dry	0.68	29.58
10/31/13	Wet	4.10	79.17
11/22/13	Dry	3.06	7.29
11/25/13	Average	12.76	15.62[c]
2/4/14	Average	4.05	29.58
3/4/14	Average	4.15	n/a
5/13/14	Dry	3.90	3.75
5/26/14	Dry	0.67	5.17
5/30/14	Wet	0.25	18.83

[a] Dry conditions were less than 0.25 inches of rain in the previous seven days, average conditions were between 0.25 and 1 inches of rain in the previous seven days, and wet conditions were greater than 1 inch of rain in the previous seven days.
[b] These runoff events were interrupted by the initiation of the next rainfall event
[c] Rainfall occurred during the runoff period

Using the information provided in Tables 1 and 2 the average time between the initial rainfall and the peak runoff at the OSSF monitoring site was found to be approximately 4.63 hr, while the average time at the control site was found to be 3.33 hr. The average runoff duration at the OSSF site was found to be 82.49 hr and the control site’s average total runoff time was 23.79 hr. Information from the first few rainfall events were used to program the automatic samplers. Similarities of the time between the initial rainfall and the peak runoff at both sites allowed the automatic sampler for the control site to be programmed in the same manner as the sampler at the OSSF monitoring site.

Quantifying Runoff

The percentage of runoff generated during a rainfall event, the runoff/rainfall ratio, was calculated using the rainfall information and the runoff monitoring data from each site. The runoff/rainfall ratios provided general guidelines as to how much runoff would be generated by a rainfall event. This information was used to determine the minimum rainfall event that would trigger the samplers. Total rainfall amounts were obtained from the Weather Underground (wunderground.com) website. Total runoff was calculated by first determining the flow rate through the pipe. The flow rate for a given time was found by multiplying the cross-sectional area of water in the pipe, found by using the water depth from the bubbler flow meter, and the result of Manning's equation, which was also found using the same water depth information. The equations used are shown below in equations 1, 2, and 3.

$$P = d * 2\cos^{-1}(\tfrac{d-h}{d}) \qquad \text{Eqn. 1}$$

$$A = \frac{d^2 * 2\cos^{-1}\left(\frac{d-h}{d}\right) - \sin(2\cos^{-1}\left(\frac{d-h}{d}\right))}{2} \qquad \text{Eqn. 2}$$

$$Q = \left(\frac{1}{n} * A^{\frac{2}{3}} * S^{\frac{1}{2}}\right) * A \qquad \text{Eqn. 3}$$

Where P is the wetted perimeter (m), d is the diameter of the pipe (m), h is the depth of the water in the pipe (m), A is the cross-sectional area of water in the pipe (m^2), Q is the flow rate of water through the pipe (m^3/s), n is the unitless Manning's roughness coefficient, and S is the slope of the pipe (%). The slopes of the pipes at both sites were calculated by using surveying equipment in the field. In order to obtain accurate flow rate information, the actual flow rate was found in-situ using a timer and a container with a known volume. Flow rates calculated using the timer and container were used with the equations discussed above to solve for the n value for both the pipe at the OSSF site and the pipe at the control site. Final n values for the pipes were found by taking the average of all the calculated n values for each site. Manning's n value used for the OSSF site was 0.033 and the control site was 0.015.

The equations above provided a flow rate in m^3/s which was converted to L/min. Using the flow rate data and the timing of the runoff events inside of the trapz function of MATLAB the total runoff in liters was found for each sampling event. To determine the total runoff in millimeters the total volume of runoff was divided by the area of the sub-watershed. Runoff/rainfall ratios were used to determine the minimum rainfall amount necessary to trigger the automatic water samplers at either site. Tables 3 and 4 show the total rainfall amount, the total runoff amount, and the runoff/rainfall ratio for the OSSF monitoring site and the control site, respectively.

Table 3. Total rainfall, runoff, and runoff/rainfall ratios from sampled rainfall events at the OSSF monitoring site.

Date	Total Rainfall, mm (in)	Total Runoff, mm (in)	Runoff/Rainfall Ratio (%)
5/10/13	27.69 (1.09)	6.59 (0.26)	23.82
8/11/13	27.94 (1.10)	0.49 (0.02)	1.75
8/26/13	42.67 (1.68)	12.58 (0.49)	29.48
9/20/13	42.92 (1.69)	0.77 (0.03)	1.81
9/21/13	17.53 (0.69)	3.73 (0.15)	21.31
10/27/13	28.19 (1.11)	1.94 (0.08)	6.87
10/31/13	88.646 (3.49)	239.20 (9.42)	269.84
11/22/13	9.91 (0.39)	2.39 (0.09)	24.15
11/25/13	18.03 (0.71)	11.79 (0.46)	65.38
1/13/14	14.22 (0.56)	1.63 (0.06)	11.44
2/2/14	10.67 (0.42)	1.84 (0.07)	17.20
2/4/14	6.60 (0.26)	2.51 (0.09)	38.06
3/4/14	22.09 (0.87)	n/a[a]	n/a[a]
5/13/14	26.67 (1.05)	1.67 (0.06)	6.26
5/26/14	82.81 (3.26)	48.76 (1.92)	58.89
5/30/14	12.19 (0.48)	4.31 (0.17)	35.36

Table 4. Total rainfall, runoff, and runoff/rainfall ratios from sampled rainfall events at the control site.

Date	Total Rainfall, mm (in)	Total Runoff, mm (in)	Runoff/Rainfall Ratio (%)
8/26/13	42.67 (1.68)	3.43 (0.13)	8.04
9/20/13	42.93 (1.69)	21.17 (0.83)	49.33
9/21/13	17.53 (0.69)	11.57 (0.45)	66.02
10/27/13	28.19 (1.11)	9.2 (0.36)	32.66
10/31/13	88.646 (3.49)	113.31 (4.46)	127.82
11/22/13	9.91 (0.39)	2.59 (0.10)	26.24
11/25/13	18.03 (0.71)	6.37 (0.25)	35.31
2/4/14	10.67 (0.26)	3.25 (0.13)	49.24
3/4/14	22.09 (0.87)	n/a[a]	n/a[a]
5/13/14	26.67 (1.05)	1.13 (0.04)	4.24
5/26/14	29.97 (1.18)	11.52 (0.45)	38.43
5/30/14	12.19 (0.48)	50.64 (1.99)	415.42

[a] Runoff data is not available for the entire runoff event

October 31, 2013 had an exceptionally long, heavy rainfall. In fact, the amount of rain was so significant that the larger drainage ditch next to the manhole at the OSSF site began rising to a very high level and caused the runoff from the neighborhood to back up into the manhole. At the control site a similar issue occurred with the detention pond that the runoff flows into. These back-ups are most likely the reason for the runoff/rainfall ratios greater than 100%.

Antecedent Moisture Conditions

Comparing the three antecedent moisture condition groups to the runoff amounts and the runoff/rainfall ratios for both monitoring sites yields slightly different results than what would be expected. For the OSSF site, drier antecedent moisture conditions and average antecedent moisture conditions had similar runoff amounts, while the wet antecedent moisture conditions led to noticeably higher runoff amounts. However, at the control site the average antecedent moisture condition had a lower runoff amount than the dry condition, although the runoff/rainfall ratios were still as expected. This higher average runoff value for the dry antecedent moisture condition is most likely the result of the 9/20/13 and 5/26/14 events. During those events there was an exceptionally high runoff amount which significantly increased the average runoff amount for the dry antecedent moisture conditions. Average runoff/rainfall ratios for both sites were as expected; dry antecedent moisture conditions had the lowest percentage of rainfall that was converted to runoff and the wet antecedent moisture condition had the highest percentage of rainfall that was converted to runoff. The results for both sites are shown below in Table 5.

Table 5. Average runoff amounts and runoff/rainfall ratios for three antecedent moisture conditions at both sites.

Site	Antecedent Moisture Condition	Runoff, mm (in)	Runoff/Rainfall Ratio (%)
Control Site	Dry	8.17 (0.32)	26.49
	Average	4.81 (0.19)	42.28
	Wet	58.51 (2.30)	203.09
OSSF Site	Dry	7.87 (0.31)	18.17
	Average	7.15 (0.28)	51.72
	Wet	82.41 (3.24)	108.84

E. coli Concentrations

E. coli concentrations were found for all sixteen sampling events at the OSSF site and for the twelve sampling events at the control site. Results from these analyses for the OSSF site and the control site are shown below in Tables 6 and 7, respectively. Total CFUs were calculated by multiplying the concentration at one point in time by the volume of runoff that had occurred in a certain time interval. The runoff event was divided into a number of intervals that equaled the number of sampling points. Each interval began at the time that was half-way in between the sampling point and the previous sampling point and the interval ended at the time half-way between the sampling point and the proceeding sampling point. An example of the interval spacing used to determine the total flow and total *E. coli* is shown below in Figure 10. Total flow, in liters, was calculated by finding the area in each of the seven sections and adding them together.

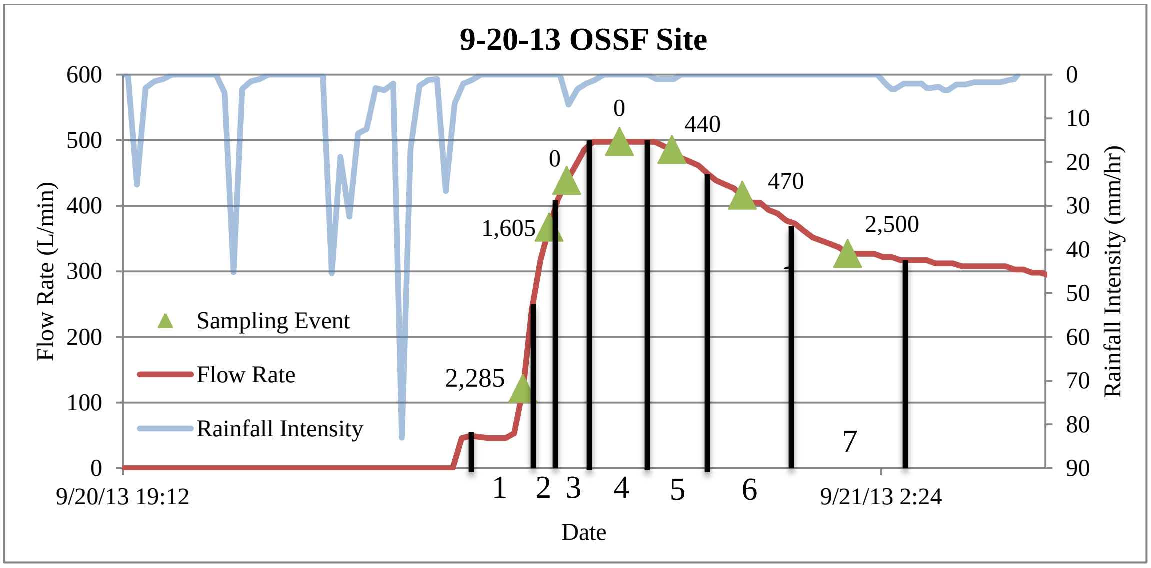

Figure 10. Example hydrograph showing how total runoff was calculated.

For the first sampling region, area 1 in Figure 10, the beginning time was selected so that the sampling point would be the center of the region. The same concept was also used for the last sample. The end time of the last sampling region, area 7 of Figure 10, was selected so that the sampling point would be the center of the region because there was no proceeding point. Runoff that occurred after the last sampling region was not used to determine the total CFUs. This method raised some reservations because the entire runoff was not taken into account.

Table 6. ***E. coli*** **concentration data for all sampling events at the OSSF site.**

Date	Minimum Concentration (CFU/100mL)	Geometric Mean (CFU/100mL)	Maximum Concentration (CFU/100mL)	Total CFUs (x 10^9)
5/10/13	1,500	4,607	17,200	3.97
8/11/13	210	3,417	9,200	1.66
8/26/13	900	5,437	37,000	123.26
9/20/13	ND[a]	152	2,285	0.74
9/21/13	ND[a]	147	1,710	0.52
10/27/13	ND[a]	7	3,640	0.979
10/31/13	1,830	3,011	11,600	1,401.08
11/22/13	ND[a]	ND[a]	ND[a]	ND[a]
11/25/13	ND[a]	ND[a]	ND[a]	ND[a]
1/13/14	ND[a]	ND[a]	ND[a]	ND[a]
2/2/14	ND[a]	1,023	15,650	4.13
2/4/14	1,664	2,344	2,755	1.66
3/4/14	3,830	5,792	7,530	n/a[b]
5/13/14	12,400	23,545	52,000	5.42
5/26/14	13,500	21,311	35,000	15.45
5/30/14	4,800	8,396	12,300	10.33

Table 7. *E. coli* concentration data for all sampling events at the control site.

Date	Minimum Concentration (CFU/100mL)	Geometric Mean (CFU/100mL)	Maximum Concentration (CFU/100mL)	Total CFUs (x 10^6)
5/10/13	4,000	5,467	9,500	n/a[b]
9/20/13	ND[a]	2	160	269. 22
9/21/13	ND[a]	15	260	83.32
10/27/13	10	126	740	819.75
10/31/13	50	2,127	44,000	291,483.34
11/22/13	ND[a]	10	250	24.68
11/25/13	ND[a]	ND[a]	ND[a]	ND[a]
2/4/14	1,515	1,857	2,359	1,399.21
3/4/14	1,550	8,994	25,450	n/a[b]
5/13/14	13,400	14,840	17,500	877.90
5/26/14	4,200	7,156	16,100	27,237.69
5/30/14	14,600	21,527	30,600	360,461.93

[a] *E. coli* was not detected

[b] No water depth information was available for this event.

E. coli concentrations at both the OSSF site and the control site are typically well above the Texas state standard, 126 CFU/100 mL. The geometric mean *E. coli* concentration for each of the rainfall events at the OSSF site exceeded the state requirement twelve of the sixteen events and at the control site the geometric mean *E. coli* concentration for each of the rainfall events exceeded the state limit eight of the twelve events. With the exception of the three rainfall events at the OSSF site and the one event at the control site that yielded no culturable *E. coli,* all events had at least one sample that exceeded the regulatory standard. These samples showed that not only was there *E. coli* present at both sites, it was present in high concentrations.

During periods without much rainfall manual samples would be taken from the larger ditch at the OSSF site, seen in the background of Figure 6, and at the retention pond at the control site, behind the fence in Figure 7. This was done to determine a baseline *E. coli* concentration for the two areas. Results from these analyses showed highly variable concentrations in the retention pond at the control site but relatively consistent, low concentrations in the larger ditch at the OSSF site. *E. coli* concentrations for the larger ditch at the OSSF site and the retention pond at the control site are shown below in Table 8.

Table 8. *E. coli* concentrations of the large ditch and retention pond.

Date	Large Ditch (CFU/100mL)	Retention Pond (CFU/100mL)
6/18/13	30	17,600
8/1/13	0	41,000
8/22/13	10	1,450
9/8/13	0	0
9/28/13	10	0
10/6/13	60	120

Table 8. Continued

Date	Large Ditch (CFU/100mL)	Retention Pond (CFU/100mL)
10/20/13	0	0
11/10/13	30	0
1/11/14	0	0

Previous *E. coli* Studies

Continuous monitoring efforts performed by both the United States Geological Survey (USGS), in cooperation with the TCEQ, and the Houston-Galveston Area Council (H-GAC), with the help of the Texas Stream Team, have found similarly high, and variable, *E. coli* concentrations in Dickinson Bayou and Dickinson Bay. 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/100 mL (USGS, 2003). Likewise, data from H-GAC shows *E. coli* concentrations ranging from 5 – 20,000 CFU/100 mL (HGAC, 2013). Both of these ranges are consistent with what was found at both the OSSF site (0 – 52,000 CFU/100 mL) and the control site (0 – 44,000 CFU/100 mL). 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). No reason for the high variability was given by either study but USGS did suggest two possible correlations for the higher concentrations: high flow rates and winter/fall seasons (USGS, 2003).

Statistical Analysis

Potential correlations considered for each individual sample were flow rate, temperature, antecedent moisture conditions, and the amount of time since the last sampling event. Peak rainfall intensity, peak flow rate, maximum temperature, and the time since the last sampling event were all used as potential correlations to the estimated total amount of *E. coli* that flowed through the pipe during each rainfall event. All respective linear regression analysis R^2 values for each of the correlation variables, found using Microsoft Excel, are shown below in Table 9. Details of each of the correlation variables can be found in Appendix C.

Table 9. Weather and temporal variables used to determine correlations between individual *E. coli* samples and total *E. coli* samples

Site	***E. coli* sample set**	**Variable**	**R^2**
OSSF Site	Individual *E. coli* samples	Flow Rate	0.0008
		Temperature	0.0284
		AMC	0.0228
		Last Sampling Event	0.0301
	Total *E. coli*	Peak Rainfall Intensity	0.1927
		Peak Flow Rate	0.7160
		Maximum Temperature	0.0475
		Last Sampling Event	0.0757

Table 9. Continued

Site	***E. coli* sample set**	**Variable**	**R^2**
Control Site	Individual *E. coli* samples	Flow Rate	0.0844
		Temperature	0.0164
		AMC	0.1963
		Last Sampling Event	<0.0001
	Total *E. coli*	Peak Rainfall Intensity	0.0281
		Peak Flow Rate	0.7535
		Maximum Temperature	0.1849
		Last Sampling Event	0.1809

The only correlation variable that had any significance was the peak flow rate relating to the total *E. coli*. This variable had an R^2 value of 0.7535 for the control site and an R^2 value of 0.7160 for the OSSF site. This finding agrees with what was found by the USGS, however the second observation made by USGS, seasonal differences, was not seen in this data. What is more, the opposite was found in a study performed in Buffalo and Whiteoak Bayou's. There was mainly no statistical difference between cooler and warmer months when looking at each station in the two bayous. However, in the few instances when there was a difference the warmer months had higher fecal coliform concentrations (Petersen et. al., 2006).

Student's *t*-test was used to compare the *E. coli* concentrations at the two sites to see if there was a significant difference between the two sample sets. If a statistical difference was found then the difference between the two sites would be considered a result of failing OSSFs at the OSSF site. However, 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*= 0.9862). 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).

Student's *t*-test was also used on each of the individual rainfall events to determine if there were any singular rainfall events that had *E. coli* concentrations that were statistically different. Three events were found that had statistically different concentrations. The first two events with statistically different *E. coli* concentrations, 9/20/13 and 5/26/14, had concentrations that were higher at the OSSF site (*p* = 0.0449 and *p* = 0.0039, respectively). However, the third event with statistically different *E. coli* concentrations, 5/30/14, had concentrations that were higher at the control site (*p* = 0.0002). Both events where the OSSF site had significantly higher *E. coli* concentrations had considerably high runoff amounts for a dry antecedent moisture condition. The larger than average runoff amounts most likely played a major role in the statistical differences between the two sites. Higher runoff values most likely led to a higher dilution and therefore a lower concentration at the control site.

Other statistical differences were found when each of the two sites were separated based on antecedent moisture condition. At the OSSF site dry antecedent moisture conditions had higher *E. coli* concentrations (*p* = 0.0466) while the control site had higher *E. coli* concentrations during wet antecedent moisture conditions (*p* = 0.0307). When the two sites were compared against one another based on antecedent moisture conditions it was found that during average antecedent moisture conditions there was no statistical difference between the two sites (*p* = 0.2499). However, during wet antecedent moisture conditions the control site had higher *E. coli* concentrations (*p* = 0.0313) and during dry antecedent moisture conditions the OSSF site had higher *E. coli* concentrations (*p* = 0.0418).

Evidence of first flush was not found at either the OSSF site or the control site (*p* = 0.7709 and *p* = 0.4803, respectively). No first flush effect was 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 below in Table 10.

Table 10. First flush effect analysis for the OSSF site and the control site based on antecedent moisture conditions.

Site	**Antecedent Moisture Condition**	***p* Value**
OSSF Site	Dry	0.3427
	Average	0.3568
	Wet	0.4300
Control Site	Dry	0.4828
	Average	0.1936
	Wet	0.7350

Possible Explanations

Similar *E. coli* concentrations at both sites during each rainfall event can lead to one of two potential conclusions. First, the OSSFs may be failing and there may be an issue with the municipal sewage lines in the neighborhood at the control site, which would lead to high *E. coli* concentrations at both sites. Secondly, the OSSFs may be operating properly and all *E. coli* was coming from either wildlife or domestic animals. In order to conclude that there was minimal human attributable *E. coli* in the storm water runoff the first potential conclusion had to be proven false.

Galveston County Water Control and Improvement District #1 in Dickinson, TX, provided maintenance and complaint records for the neighborhoods' sewage lines for the past two years. These documents did show that there had been cracks and leaks found in the sewage lines caused by invasive roots. Also, a maintenance engineer with the city said that occasionally during an exceptionally large rainfall event or during a period of many days with rain that the sewage lines may overflow through manhole covers found in dead-end streets. Failing sewage pipes may be a reason for the high *E. coli* concentrations at the control site. It was concluded that wet antecedent moisture conditions led to higher *E. coli* concentrations at the control site which would agree with the hypothesis that failing sewage pipes overflow during rainfall events that last many days.

The results from the analysis of the larger ditch at the OSSF site and the retention pond at the control site would seem to point toward wildlife being an issue at the retention pond during the warmer months. However, after an inspection of the site no nests or dens could be found. Also after a discussion with a homeowner in the neighborhood there seems to not be any major wildlife habitats surrounding the pond.

Bacterial Source Tracking

A bacterial source tracking (BST) method was performed on a total of fourteen samples, seven from the OSSF site and seven from the control site, taken on 3/4/14. *E. coli* colonies were grown in the lab using EPA Method 1603 and were taken to Dr. Terry Gentry's lab in Texas A&M University's Department of Soil and Crop Science for the DNA fingerprinting and analysis. A DNA fingerprint is performed on one individual *E. coli* colony, called an isolate. One isolate was selected from each sample that contained *E. coli* grown using EPA Method 1603. Fingerprints for each of the isolates were compared against the Texas *E. coli* BST Library (ver. 6-13). Isolates were divided into 3–way and 7-way sources splits. Human, Wildlife, and Livestock/Domestic Animals were the three possible categories that each isolate could fit into for the 3-way split. Human, Cattle, Other Livestock (non-avian), Other Livestock (avian), Pets, Avian Wildlife, and Non-avian Wildlife were the seven possible categories for the 7-way split. An isolate's category was chosen based on the highest percentage match, with an 80% being the lowest acceptable percentage match. If an isolate's DNA fingerprint did not match at least 80% of any source in the library then it would be left as unclassified. Due to time and budget constraints, only one *E. coli* isolate from each of the fourteen samples tested in the lab (seven from the OSSF site and seven from the control site) was fingerprinted. Results from the BST analyses at both the OSSF site and the control site are shown below in Figures 11 and 12, respectively.

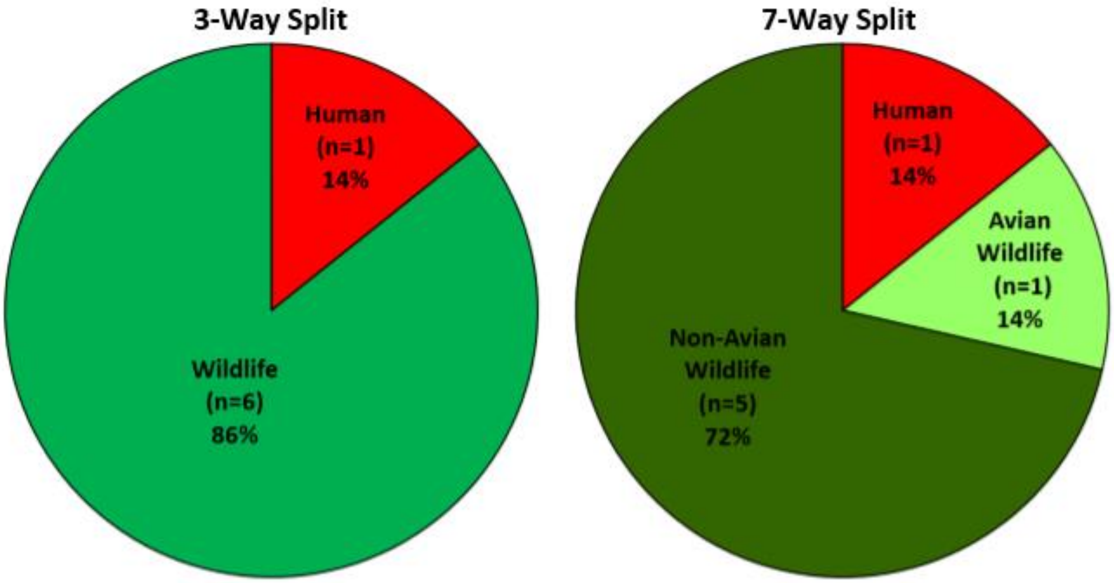

Figure 11. Source classification of *E. coli* isolates from the OSSF sites using a 3-way split (L) and a 7-way split (R).

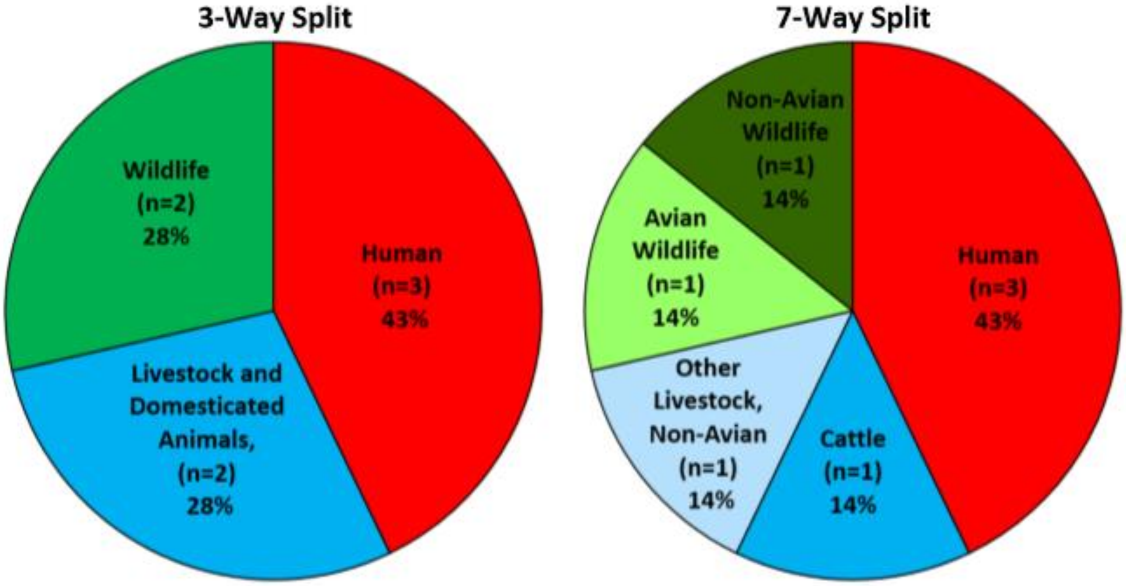

Figure 12. Source classification of *E. coli* isolates from the control site using a 3-way split (L) and a 7-way a split (R).

One immediately recognizable element from the BST analysis is that there seems to be human feces present at both the OSSF site and the control site. Not only is there a human presence at the OSSF site, which was hypothesized, but there was an even stronger presence at the control site. Typically, human-based *E. coli* can be emitted into nature through failing OSSFs, however this can't possibly be true for the control site because there are no homes using OSSFs anywhere near the area. There are no indications that there should be any human feces present at the control site. The single human marker at the OSSF site was expected but the amount of human isolates was hypothesized to be much more substantial. A BST analysis performed on *E. coli* isolates from Oyster Creek watershed (south 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). This same study also found that in the Oyster Creek watershed (north of the Dickinson Bayou watershed) nearly 80% of *E. coli* was from wildlife, 12% was from domestic animals, and 8% was coming from human sources. The percentages found in this study are similar to the OSSF site percentages but are quite different from the control site.

Estimated Failure Rates

Malfunction rates for the OSSFs at the OSSF site were found by utilizing the total *E. coli* load during the runoff events, the runoff duration used to calculate the total *E. coli* load, and equation 4 (Riebschleager, et. al., 2012).

$$EC = \#OSSFs * Malfunction\ Rate * \frac{1 * 10^{6}\ CFU}{100\ mL} * \frac{60\ gal}{\frac{person}{day}} * \frac{Ave\ \#}{household} * \frac{3758.2\ mL}{gal} * 0.5 \qquad \text{Eqn. 4}$$

Where EC is the *E. coli* load per day, # OSSFs is the number of OSSFs in the watershed, $1*10^{6}$ CFU/100 mL is the estimated *E. coli* concentration from sewage, 60 gal/person/day is the approximate number of gallons one person will use every day, and the Ave # / household is the average number of people per household. At the OSSF site there are 28 OSSFs (19 anaerobic, 9 aerobic) and the average number of people per household in Galveston County is 3. Total *E. coli* loads for each runoff event were used for the *E. coli* load in equation 4. However, because the times used to calculate the total *E. coli* loads were less than 1 day equation 4 was multiplied by the duration of the runoff and then divided by 24 hours per day. It was assumed that the *E. coli* load estimated using equation 4 is uniform throughout the entire day. This allowed the amount of *E. coli* to be calculated for only the time of the runoff, not per day. It was also assumed that both the aerobic and anaerobic systems had the same failure rate. The malfunction rate is the maximum possible because it was assumed that all *E. coli* came from failing OSSFs. Table 11 shows the total *E. coli* amount, the runoff time used for the total *E. coli* calculations, and the OSSF failure rate found using equation 4 for the OSSF site.

Table 11. Maximum OSSF failure rates based on observed *E. coli* loads at the OSSF site

Date	Total *E. coli* (CFUs)	Runoff Time (hr)	Maximum Failure Rate (%)
5/10/2013	3.97	3.33	30
8/11/2013	1.66	3.08	13
8/26/2013	123.26	2.83	1,103
9/20/2013	0.74	4.00	4
9/21/2013	0.52	3.75	3
10/27/2013	0.98	5.50	4
10/31/2013	1,401.08	7.33	4,843
2/2/2014	4.13	3.58	29
2/4/2014	1.66	4.08	10
5/13/2014	5.42	7.00	19
5/26/2014	15.45	2.42	161
5/30/2014	10.33	5.92	44

The majority of the runoff events had malfunction rates that were reasonable nonetheless three events had malfunction rates greater than 100%. This is most likely because of the assumption that 100% of the *E. coli* came from human sources. Bacterial source tracking concluded that not all of the *E. coli* was from human sources so the failure rates shown in Table 11 should only be viewed as potential maximum failure rates and not conclusive evidence that a certain percentage of the OSSFs are failing.

This same process was used at the control site to estimate the maximum failure rate in the clay pipes. Equation 4 was modified so that instead of the number of OSSFs in the watershed it was the number of homes in the watershed. There are 29 homes in the watershed at the control site. Again, the failure rates presented in Table 12 should only be viewed as a potential maximum failure rate of the clay pipes and not evidence that a certain percentage of the pipes are failing. Table 12 shows the total *E. coli* amount, the runoff time used for the total *E. coli* calculations, and the sewage pipe failure rate found using equation 4 for the control site.

Table 12. Maximum municipal pipe failure rates based on observed *E. coli* loads at the control site

Date	Total *E. coli* (CFUs)	Runoff Time (hr)	Maximum Failure Rate (%)
9/20/2013	269.22	3.75	1
9/21/2013	83.32	3.58	0
10/27/2013	819.75	3.50	5
10/31/2013	291,483.34	5.33	1,338
11/22/2013	24.68	4.92	0
2/4/2014	1399.21	4.67	7
5/13/2014	877.90	1.42	15
5/26/2014	27,237.69	3.08	216
5/30/2014	360,461.93	5.42	1,627

As at the OSSF site three events had failure rates above 100% however the three events are not the same at both sites. At both sites the failure rates less than 100% were compared against antecedent moisture conditions, rainfall amounts, runoff amounts, runoff/rainfall ratios, and total runoff duration. None of these variables were significantly correlated to the failure rates at either site.

CHAPTER IV

CONCLUSIONS

Two monitoring sites were installed inside of the Dickinson Bayou watershed and used to collect runoff for sixteen events at the OSSF site and twelve events at the control site. Analysis of the events using EPA Method 1603 indicated that high concentrations of *E. coli* were present in the runoff. Correlation analyses showed that the only significant relationship existed between the total *E. coli* load during an event and the peak runoff. This correlation was almost a perfect linear association at both sites. Student's *t*-test was performed to determine if a statistically significant difference was present between the *E. coli* concentrations at the two sites. Results from this analysis showed that a significant difference between *E. coli* concentrations at the two sites did not exist.

A definitive conclusion on whether or not OSSFs were contributing to the elevated bacteria levels in Dickinson Bayou could not be made at this time. Nearly all sampling events had at least one *E. coli* concentration that was above the Texas state recreational contact standard. In fact, it was quite common to have samples well over 10 times this requirement. After an initial BST analysis a human fecal presence was confirmed at both sites.

Human fecal material is most likely coming from failing OSSFs at the OSSF site. Conversely, there are no apparent human sources of fecal material at the control site yet *E. coli* from human sources was still found at this site. Broken or leaky municipal sewage lines may be the cause of the human fecal material present in runoff and should be investigated further.

The future of this project should involve the use of a BST analysis during every sampled rainfall event to determine the exact extent of the human presence. Using BST analyses on future samples should also provide more information as to the specific cause of the contamination. The cause of these human-based *E. coli* needs to be conclusively found to prevent further contamination to Dickinson Bayou.

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APPENDIX A

OSSF SITE HYDROGRAPHS

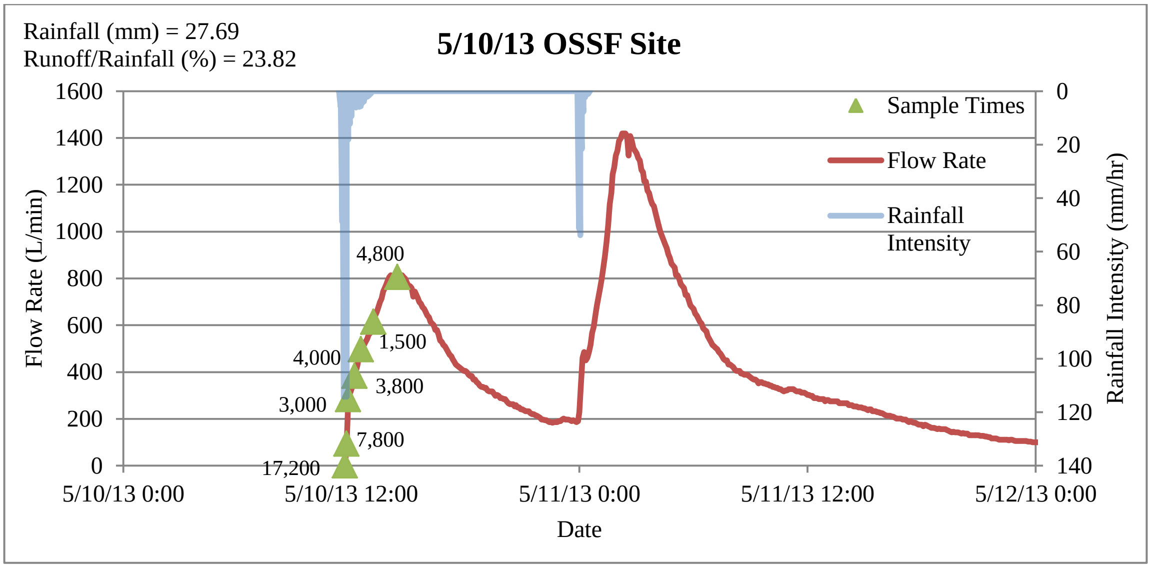

Figure 13. Hydrograph for sampling event on 5/10/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

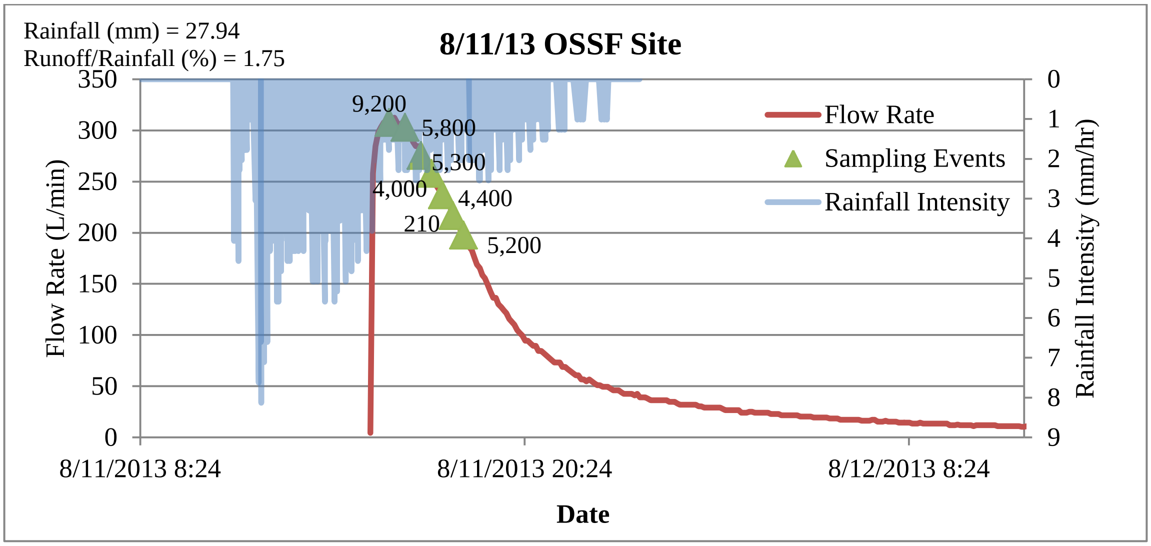

Figure 14. Hydrograph for sampling event on 8/11/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

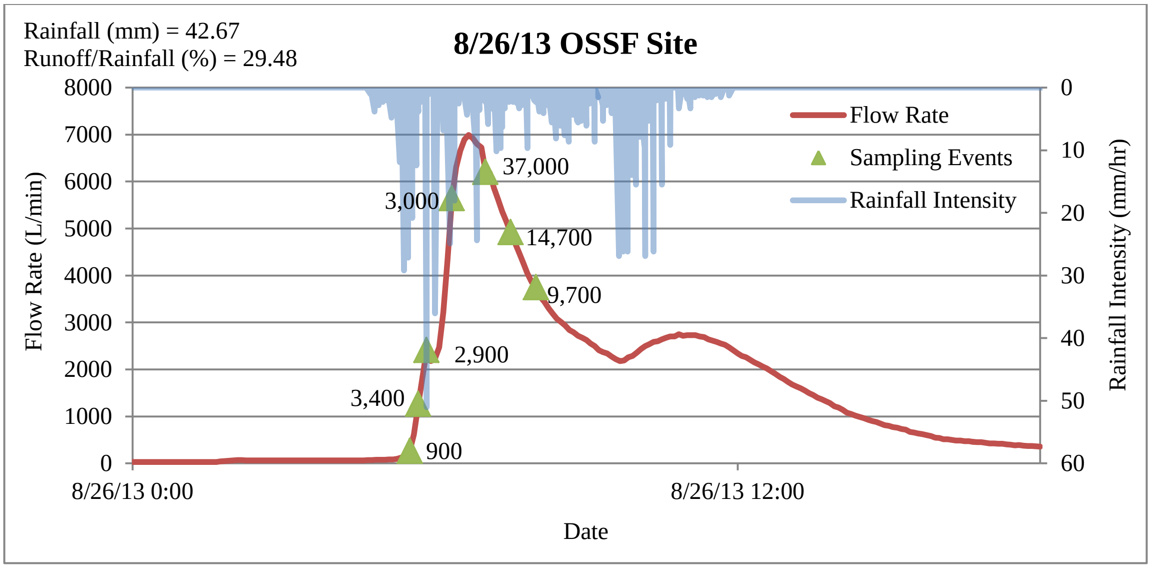

Figure 15. Hydrograph for sampling event on 8/26/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

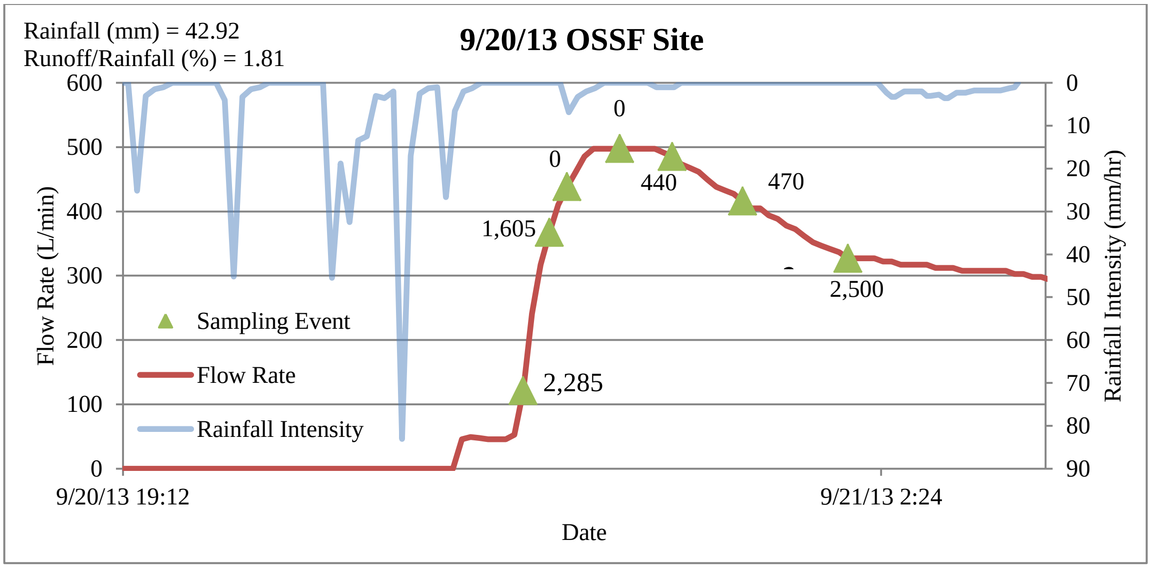

Figure 16. Hydrograph for sampling event on 9/20/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

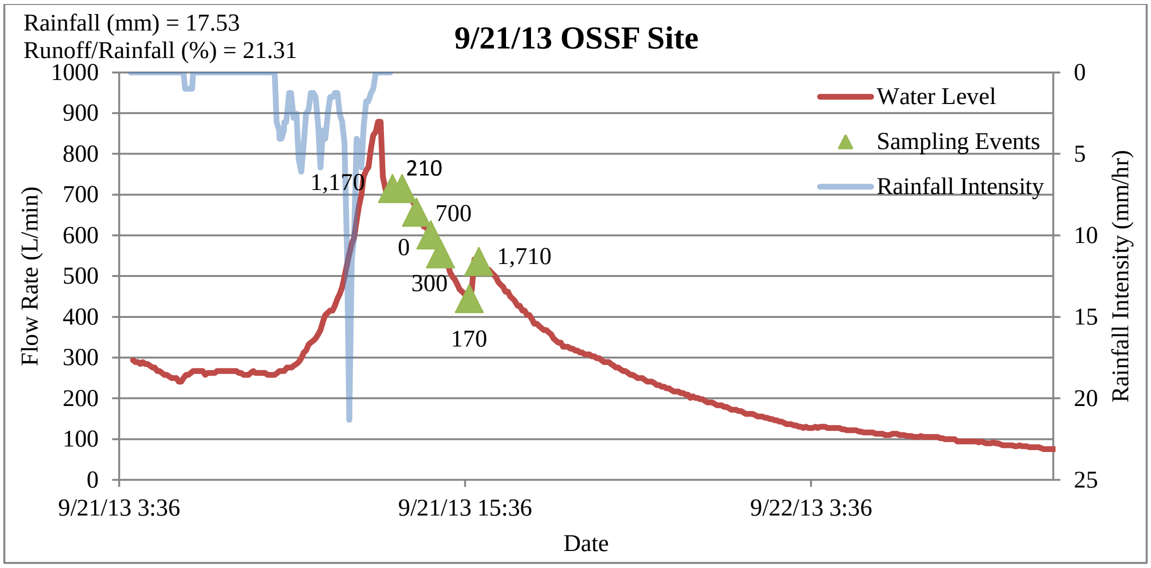

Figure 17. Hydrograph for sampling event on 9/21/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

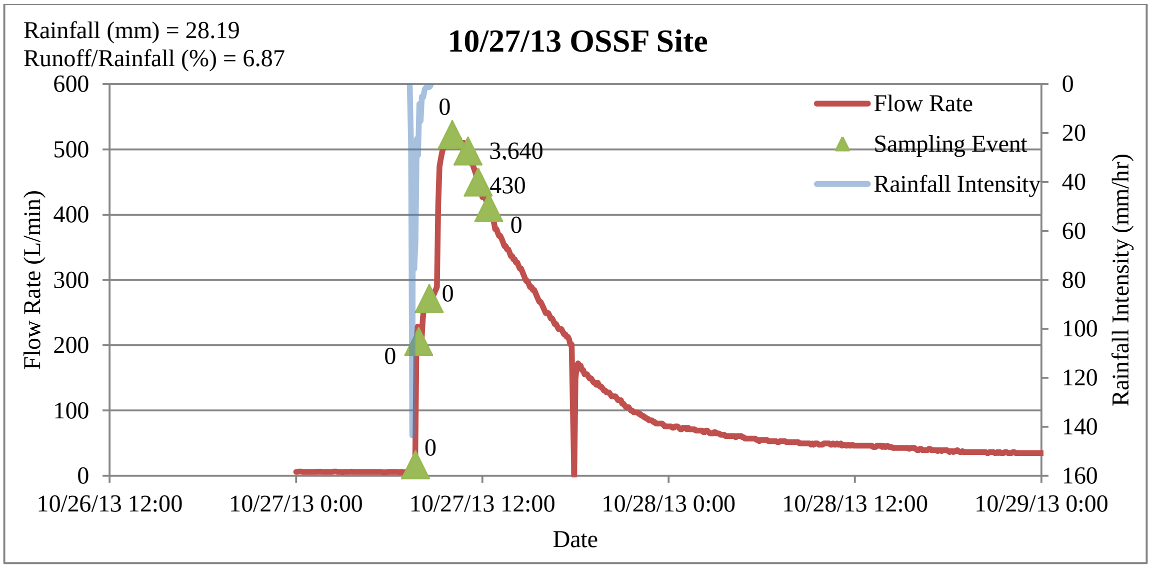

Figure 18. Hydrograph for sampling event on 10/27/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

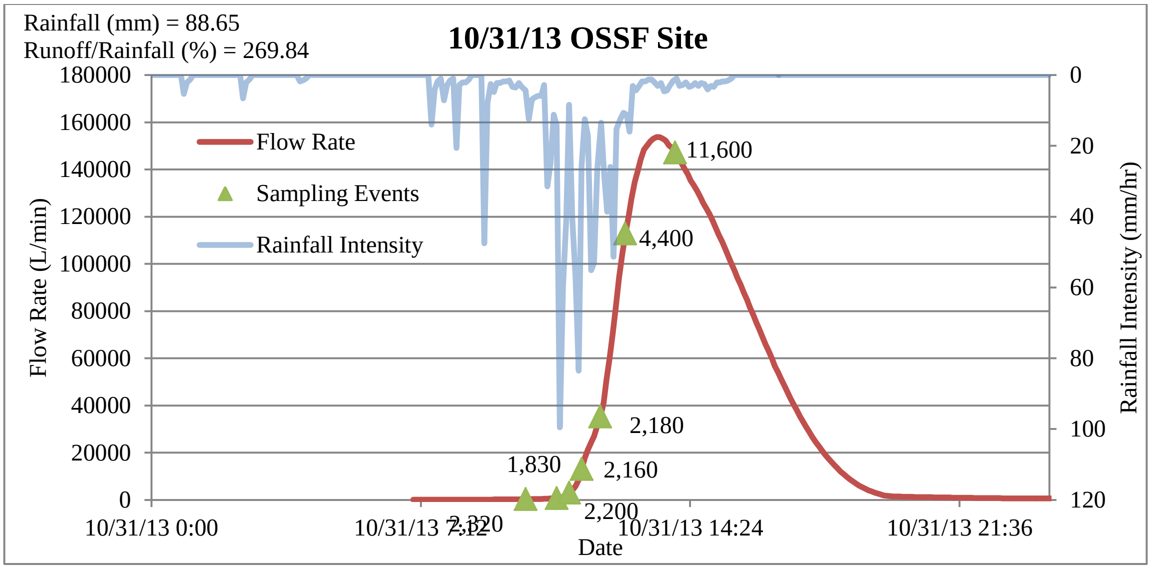

Figure 19. Hydrograph for sampling event on 10/31/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

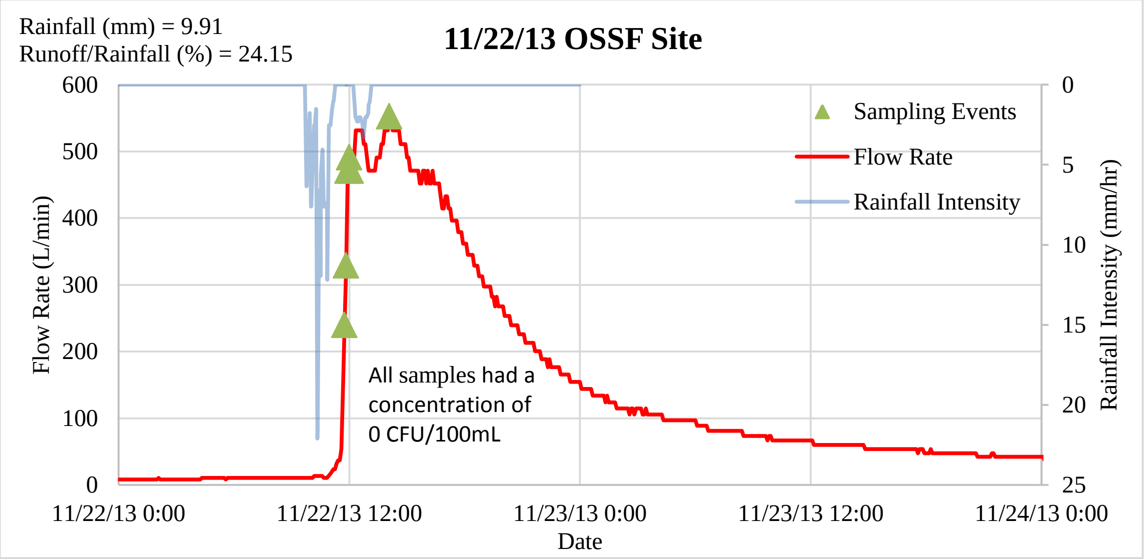

Figure 20. Hydrograph for sampling event on 11/22/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

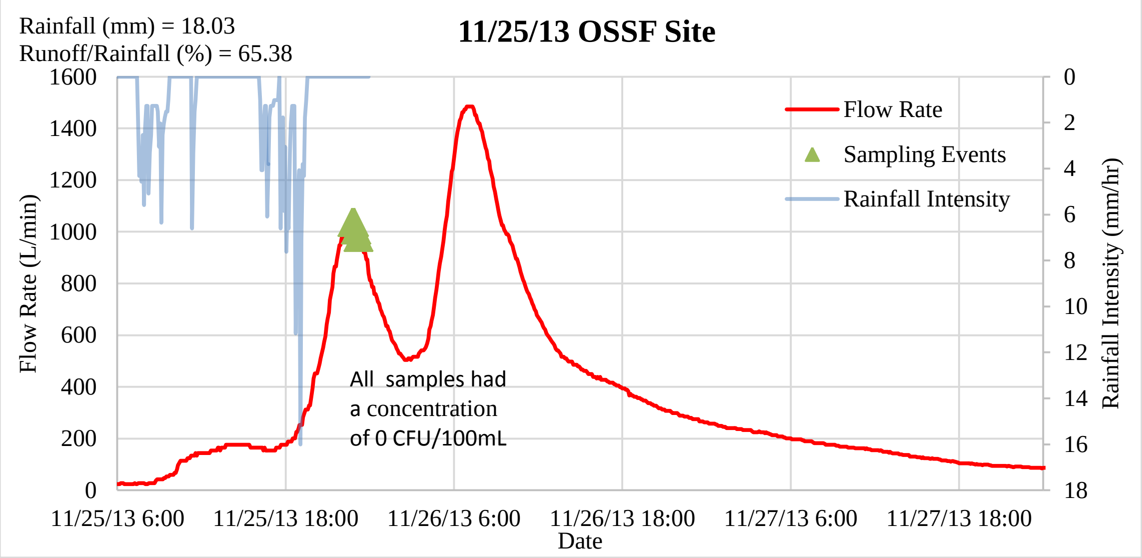

Figure 21. Hydrograph for sampling event on 11/25/13 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

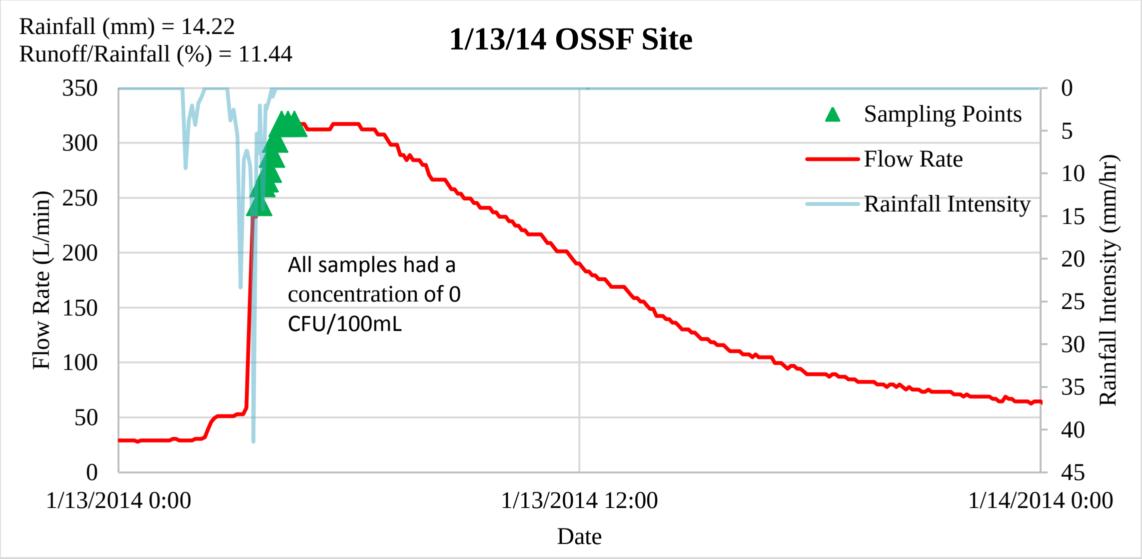

Figure 22. Hydrograph for sampling event on 1/13/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

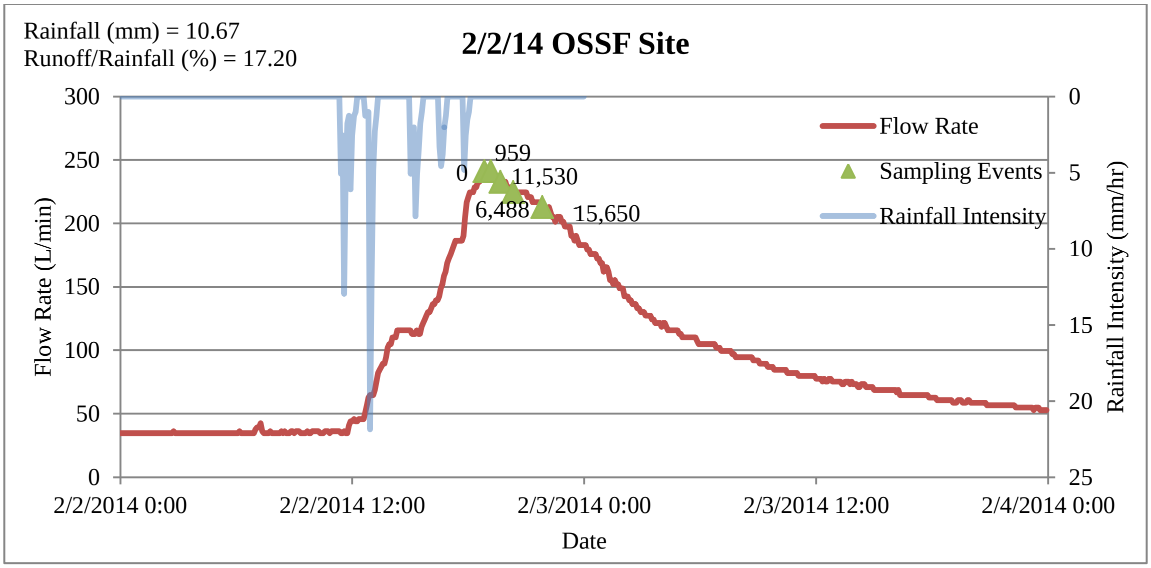

Figure 23. Hydrograph for sampling event on 2/2/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

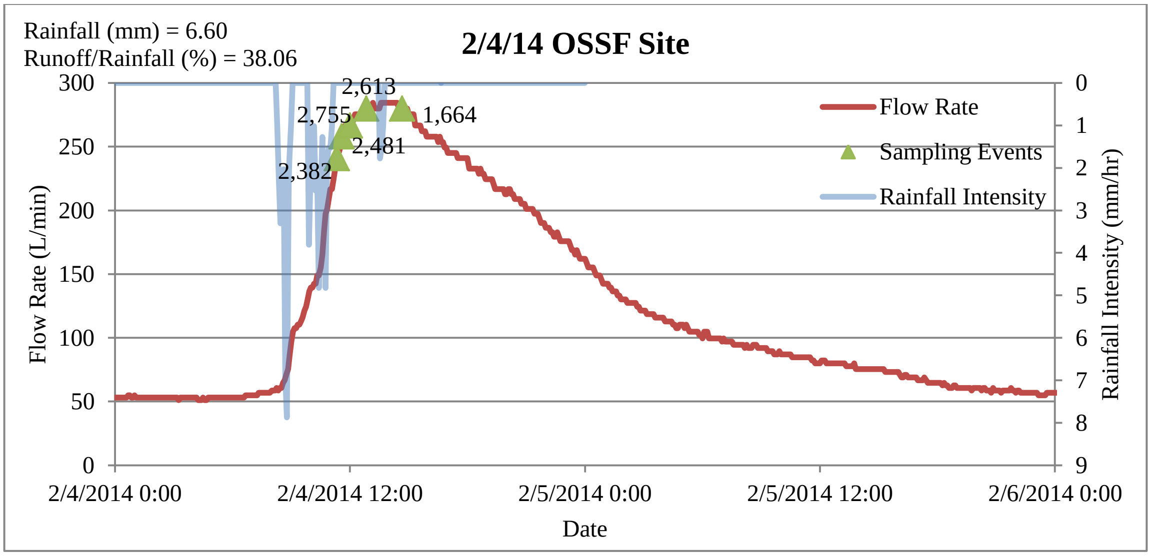

Figure 24. Hydrograph for sampling event on 2/4/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

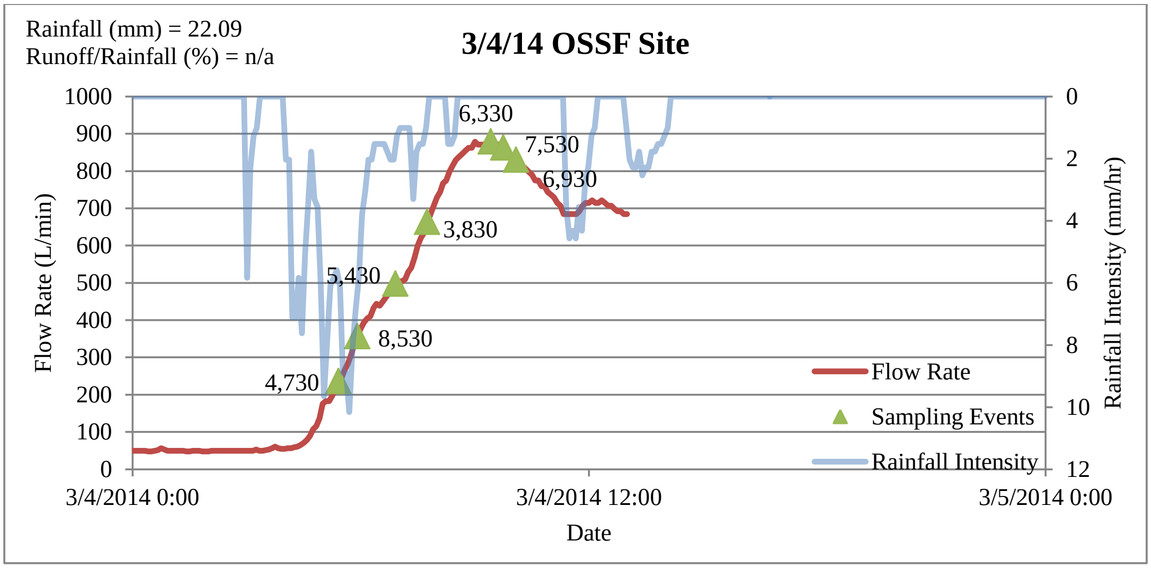

Figure 25. Hydrograph for sampling event on 3/4/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

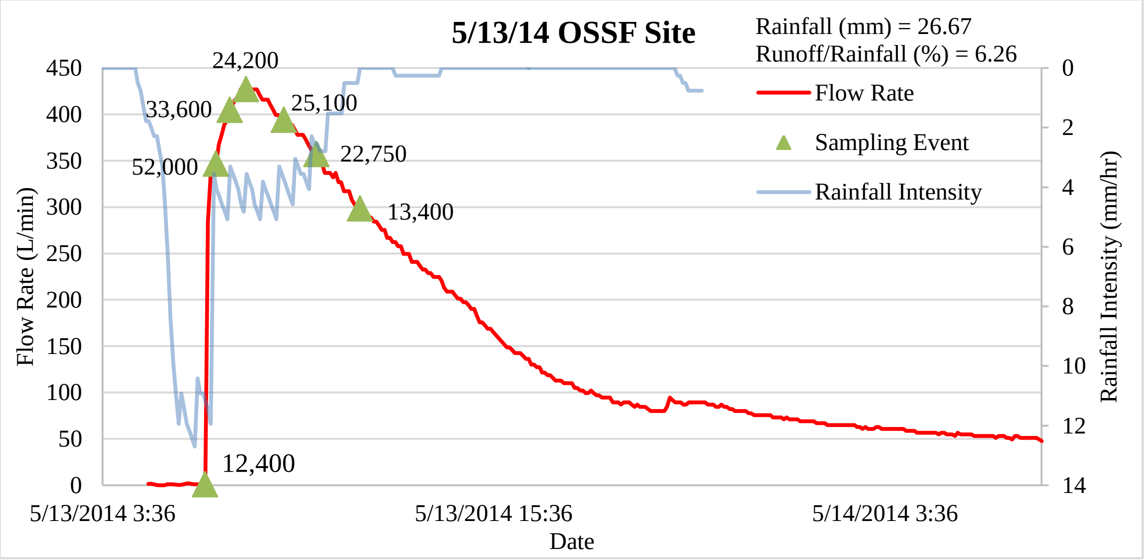

Figure 26. Hydrograph for sampling event on 5/13/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

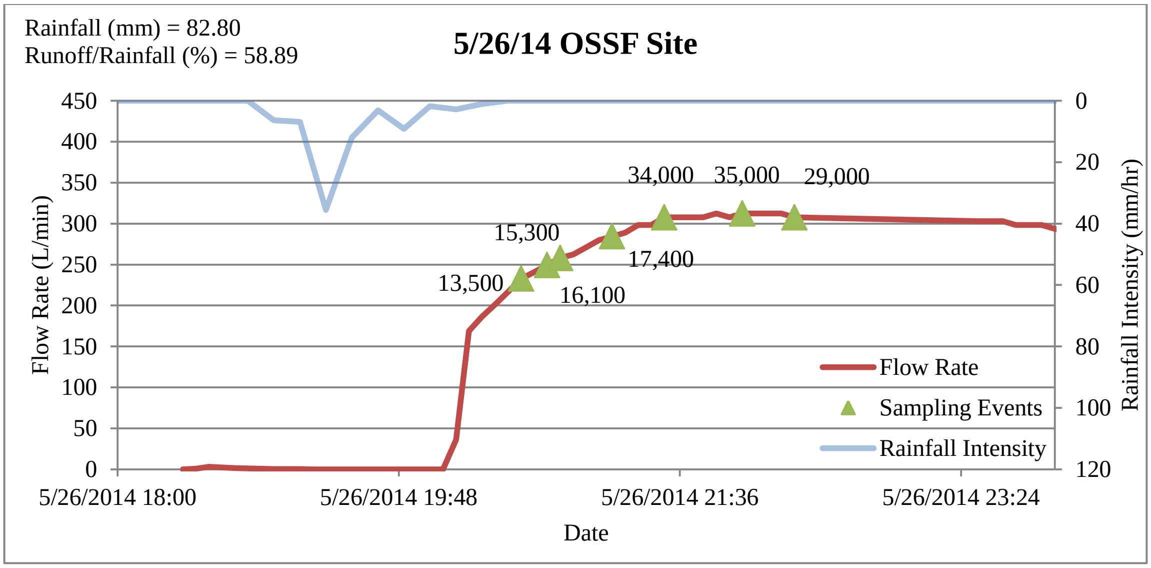

Figure 27. Hydrograph for sampling event on 5/26/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

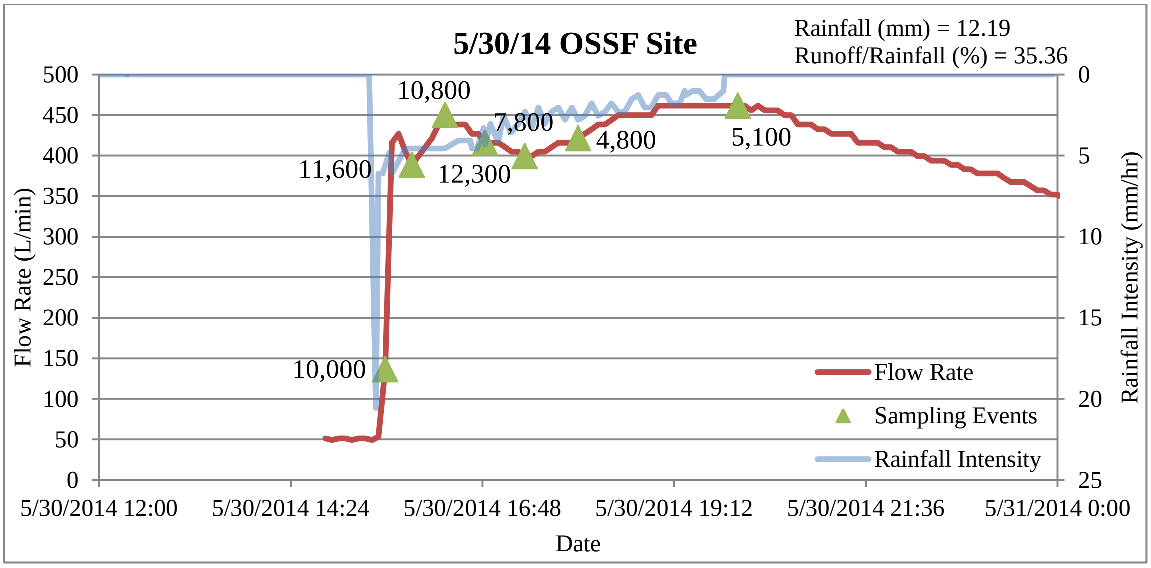

Figure 28. Hydrograph for sampling event on 5/30/14 at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

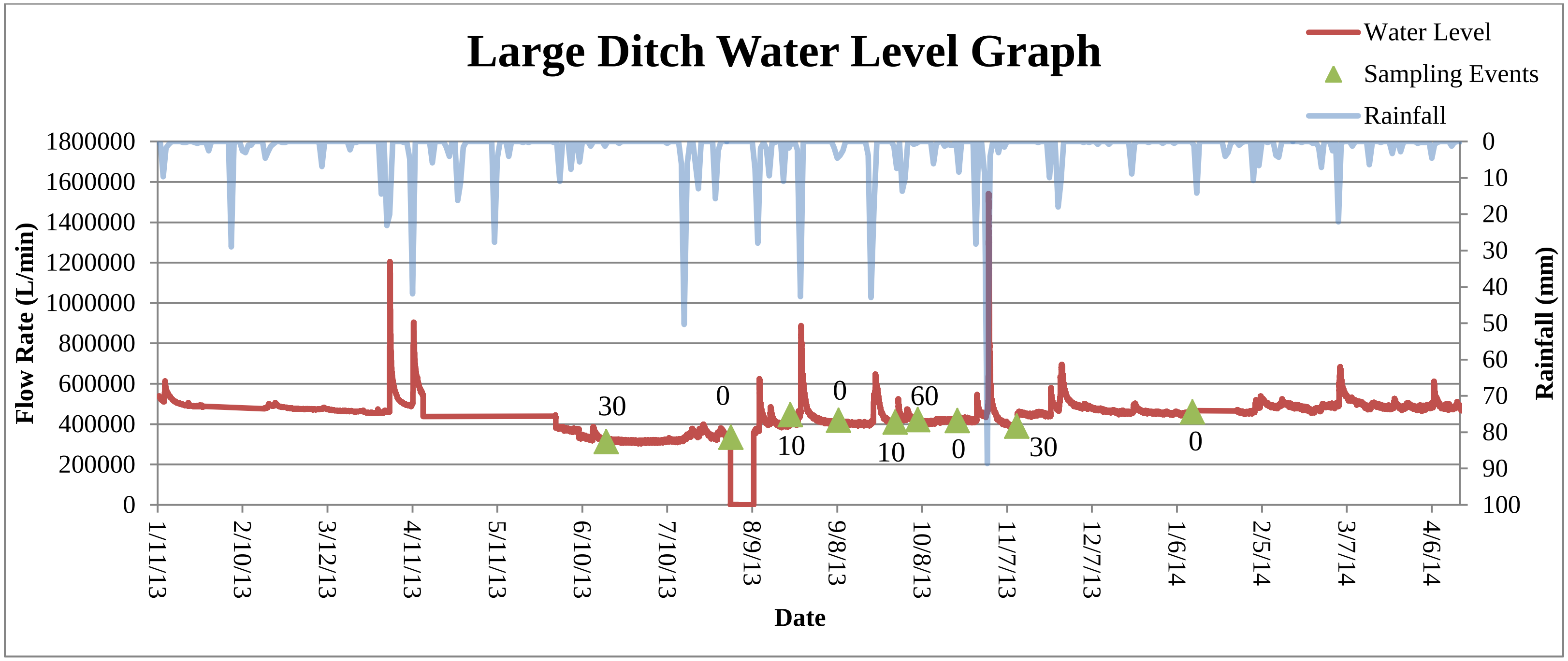

Figure 29. Hydrograph for the large ditch at the OSSF site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100mL.

APPENDIX B

CONTROL SITE HYDROGRAPHS

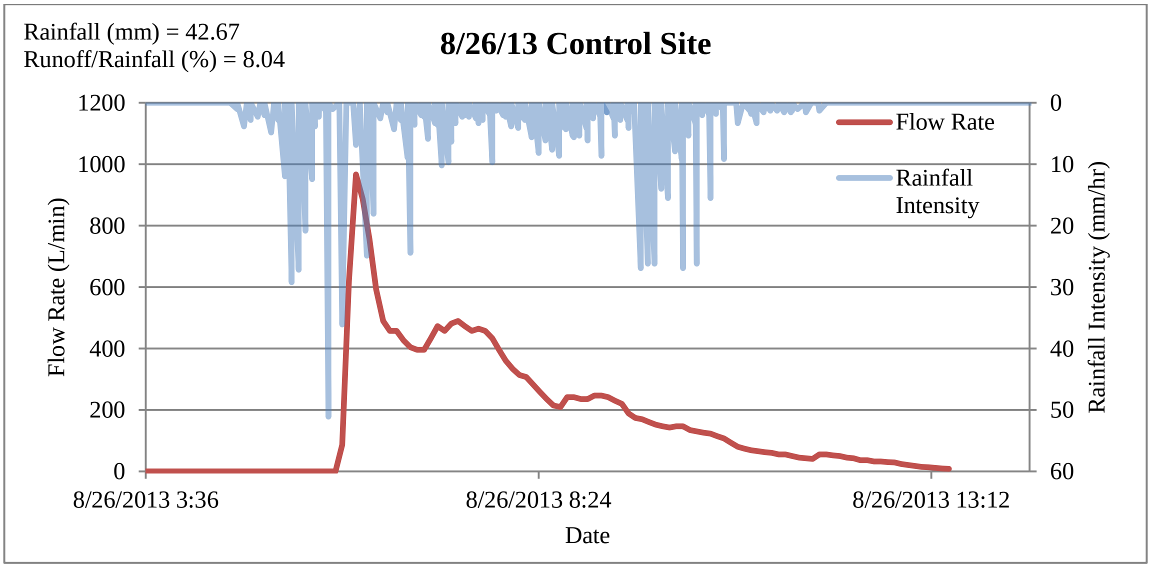

Figure 30. Hydrograph for sampling event on 8/26/13 at the control site. No samples were taken during this rainfall event.

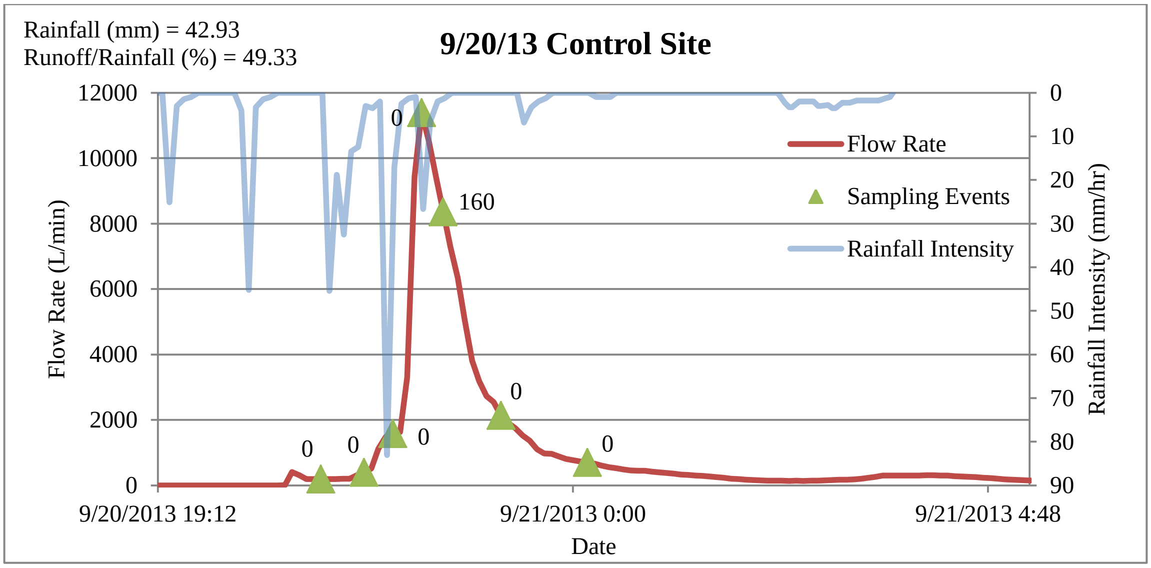

Figure 31. Hydrograph for sampling event on 9/20/13 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

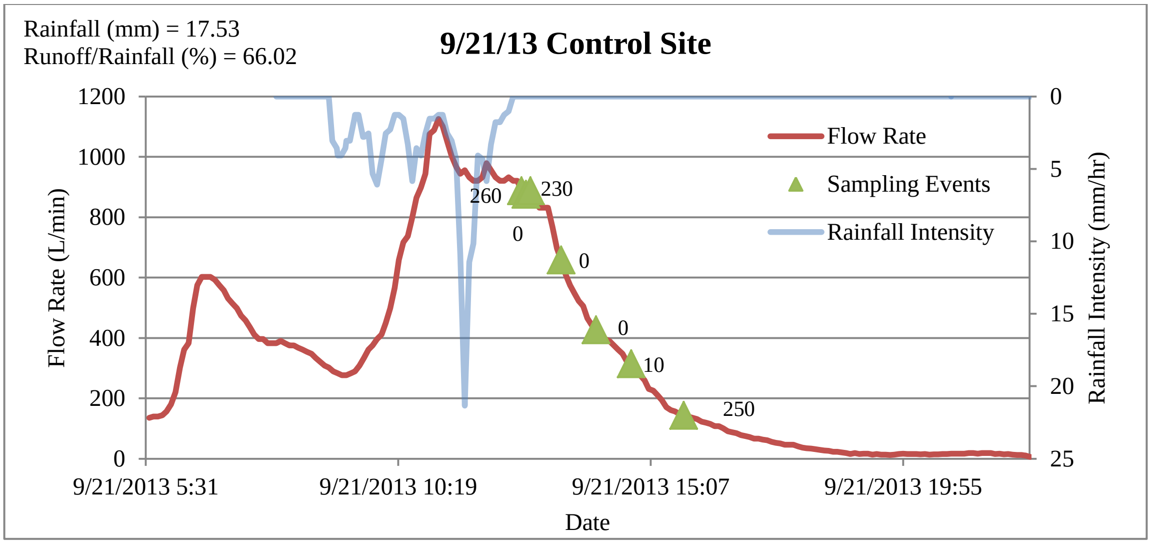

Figure 32. Hydrograph for sampling event on 9/21/13 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

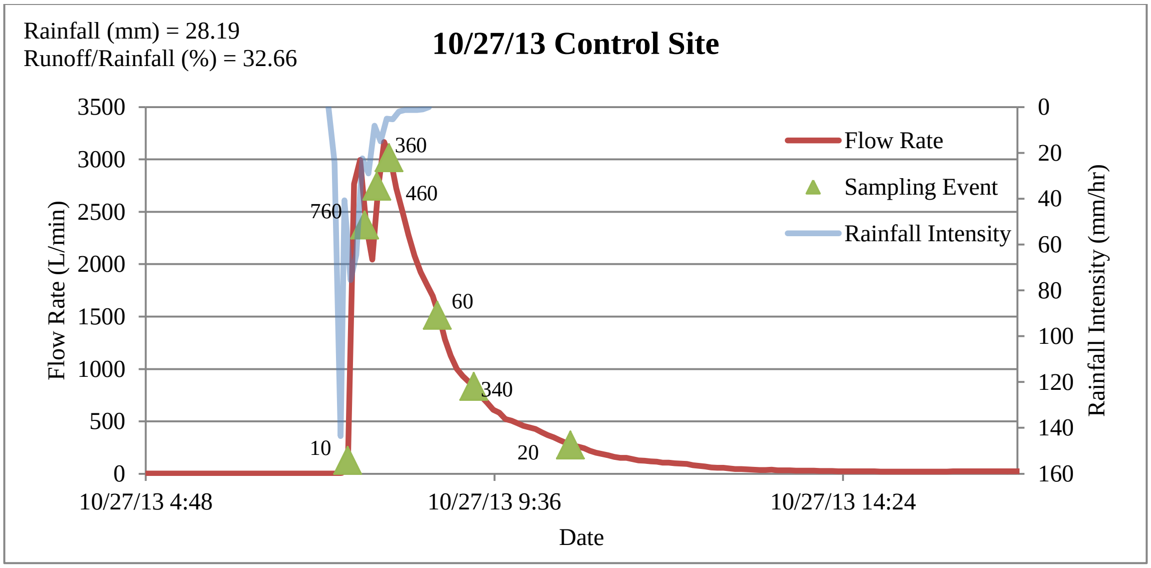

Figure 33. Hydrograph for sampling event on 10/27/13 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

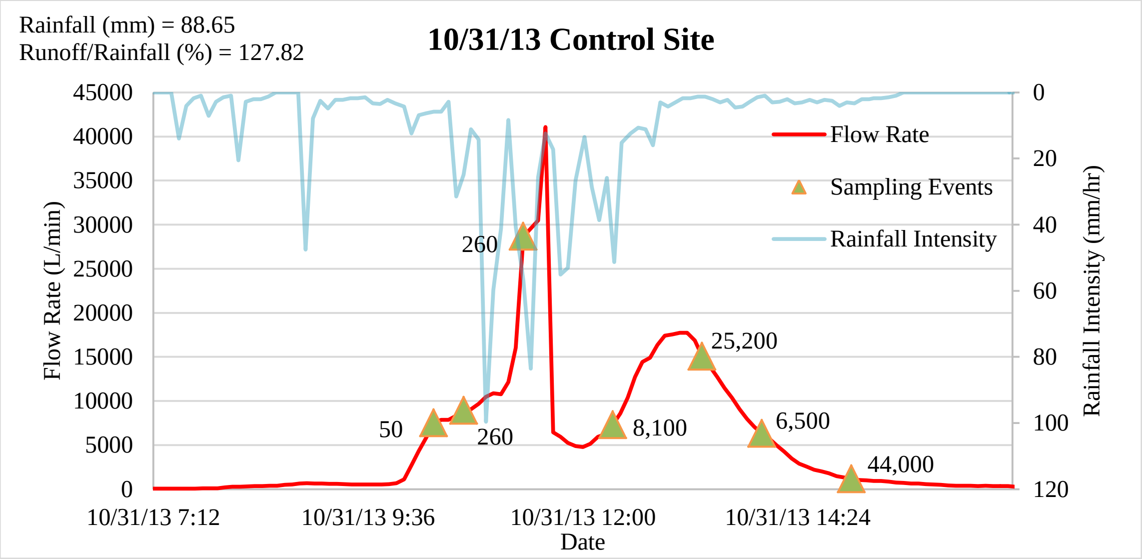

Figure 34. Hydrograph for sampling event on 10/31/13 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

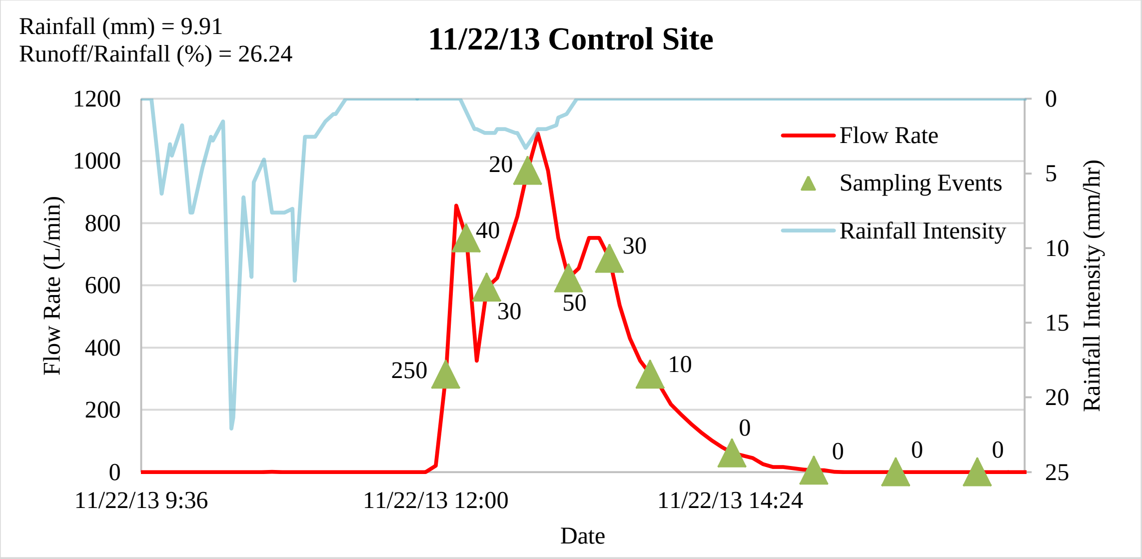

Figure 35. Hydrograph for sampling event on 11/22/13 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

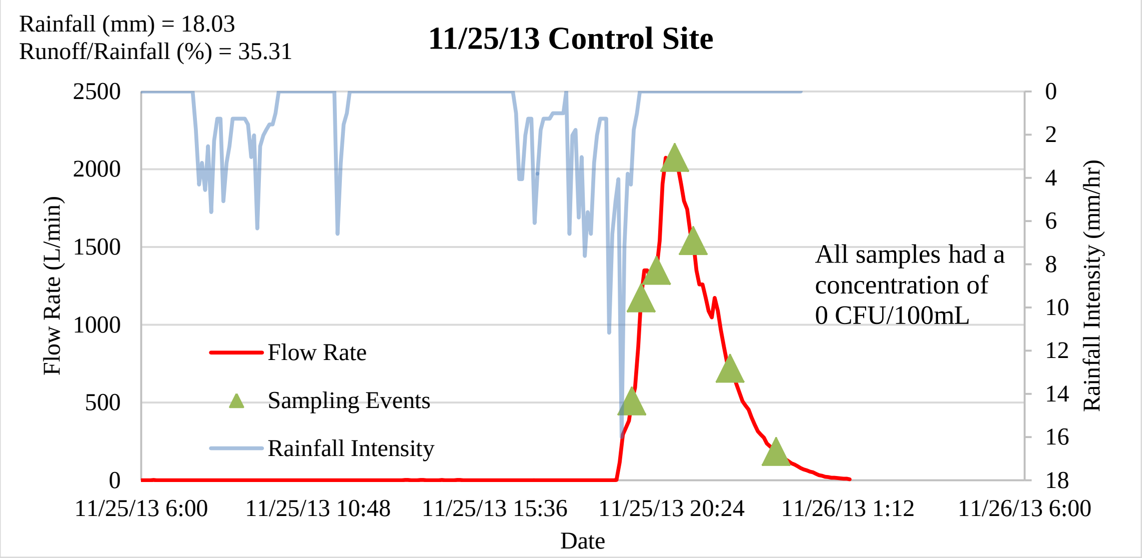

Figure 36. Hydrograph for sampling event on 11/25/13 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

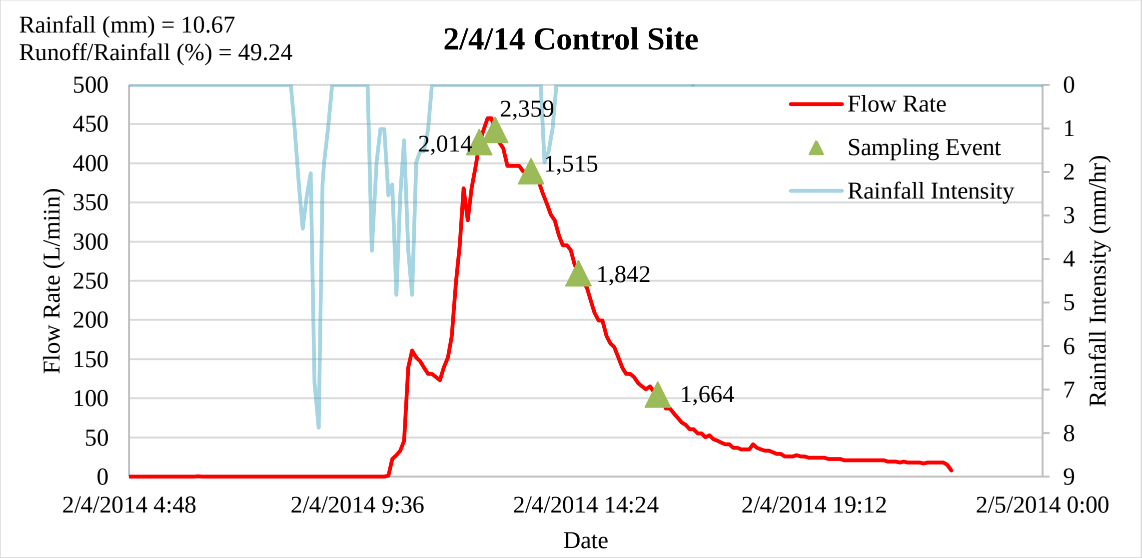

Figure 37. Hydrograph for sampling event on 2/4/14 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

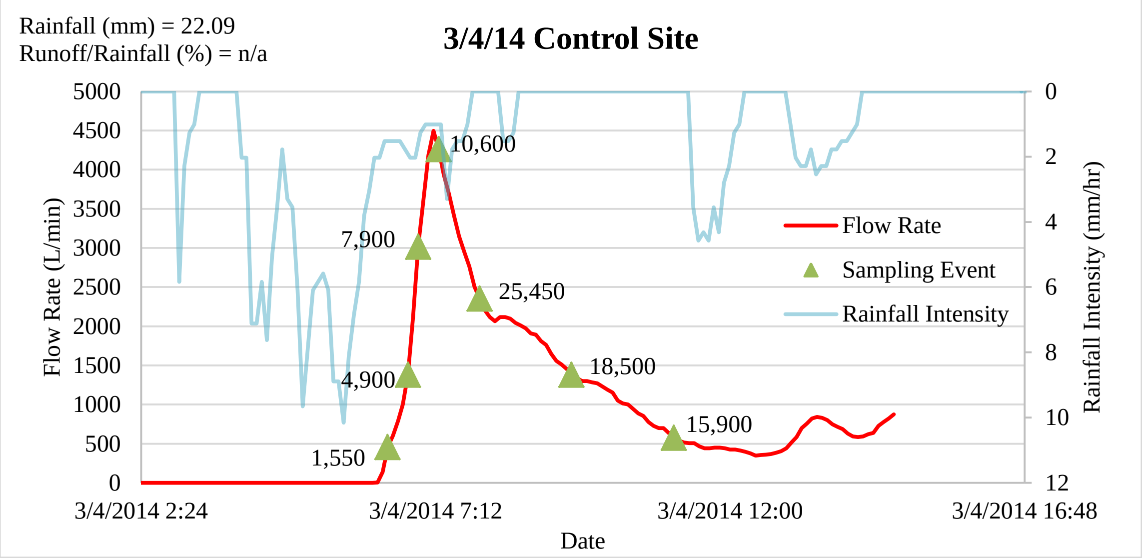

Figure 38. Hydrograph for sampling event on 3/4/14 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

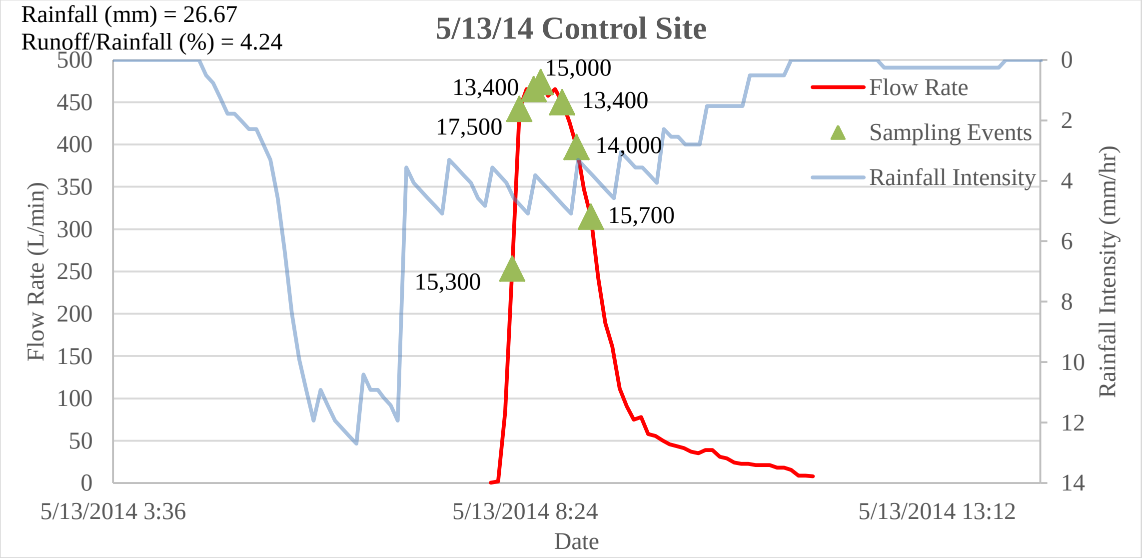

Figure 39. Hydrograph for sampling event on 5/13/14 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

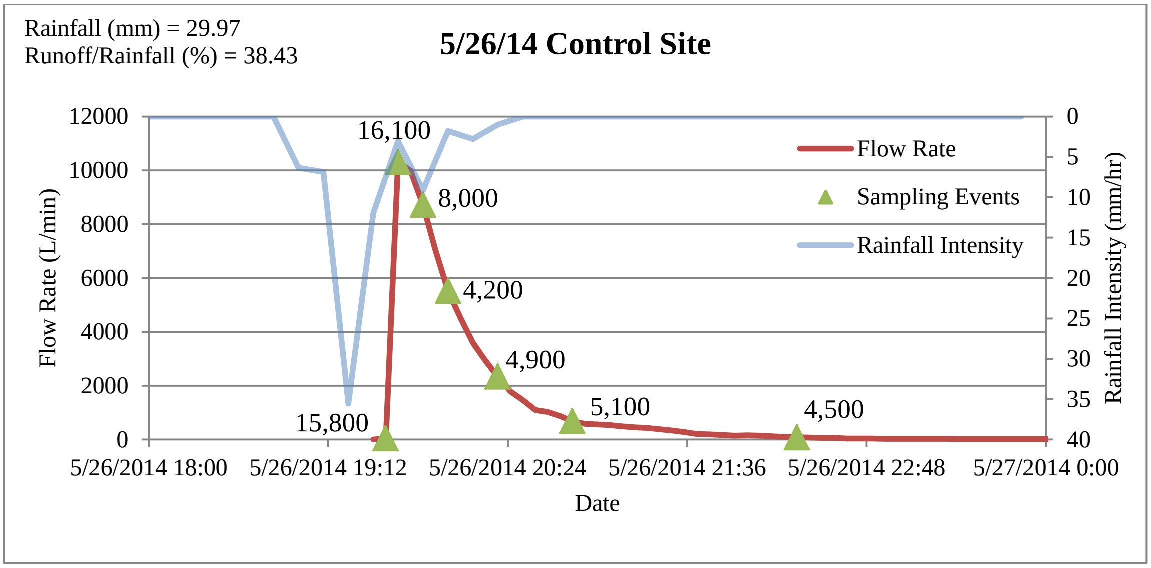

Figure 40. Hydrograph for sampling event on 5/26/14 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

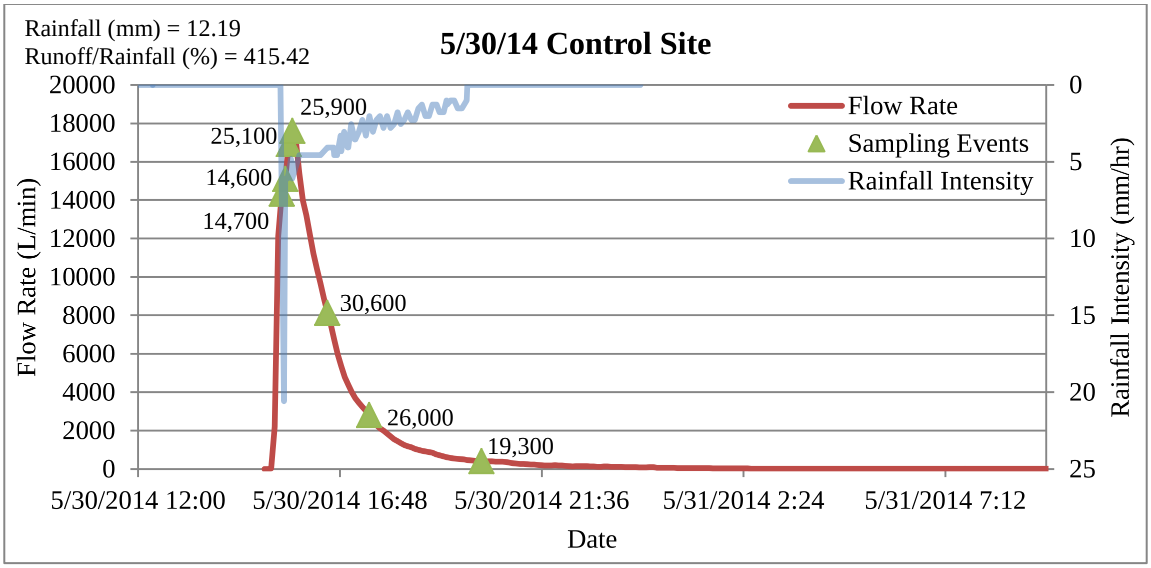

Figure 41. Hydrograph for sampling event on 5/30/14 at the control site. Numbers next to the sample times are the *E. coli* concentrations in CFUs/100 ml.

APPENDIX C

STATISTICAL ANALYSIS VARIABLES

Antecedent Moisture Content is the total amount of rainfall in the 7 days prior to the rainfall event.

Table 13. Statistical analysis correlation variables for each individual sampling event at the OSSF site.

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{0}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
5/10/13 11:40	17200	0.81	65.9	0	-
5/10/13 11:45	7800	94.43	65.9	0	-
5/10/13 11:50	3000	284.44	65.7	0	-
5/10/13 12:10	3800	383.10	65.8	0	-
5/10/13 12:30	4000	497.55	66.4	0	-
5/10/13 13:10	1500	614.26	66.8	0	-
5/10/13 14:25	4800	806.11	71.1	0	-
8/11/2013 16:10	9200	307.64	81.1	0.29	92
8/11/2013 16:40	5800	302.92	83	0.29	92
8/11/2013 17:10	5300	275.43	83.6	0.29	92
8/11/2013 17:30	4000	257.87	84.5	0.29	92
8/11/2013 17:50	4400	236.80	84.3	0.29	92
8/11/2013 18:10	210	216.68	84.6	0.29	92

Table 13. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
8/11/2013 18:30	5200	197.51	83.8	0.29	92
8/26/13 5:30	900	262.20	74.9	0.16	15
8/26/13 5:40	3400	1263.75	74.5	0.16	15
8/26/13 5:50	2900	2410.48	74.4	0.16	15
8/26/13 6:20	3000	5641.89	74.3	0.16	15
8/26/13 7:00	37000	6206.31	74.3	0.16	15
8/26/13 7:30	14700	4918.99	73.7	0.16	15
8/26/13 8:00	9700	3750.74	73.3	0.16	15
9/20/13 23:00	2285	121.43	77.3	0.15	25
9/20/13 23:15	1605	367.30	76.2	0.15	25
9/20/13 23:25	0	438.33	74.7	0.15	25
9/20/13 23:55	0	497.55	73.6	0.15	25
9/21/13 0:25	440	485.38	73.4	0.15	25
9/21/13 1:05	470	415.76	73.7	0.15	25
9/21/13 2:05	2500	326.90	74.0	0.15	25
9/21/13 13:05	1170	714.13	73.0	1.84	0
9/21/13 13:25	210	714.13	72.7	1.84	0
9/21/13 13:55	700	656.08	73.2	1.84	0
9/21/13 14:25	0	600.65	72.6	1.84	0

Table 13. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
9/21/13 14:45	30	554.27	73.7	1.84	0
9/21/13 15:45	170	444.07	73.3	1.84	0
9/21/13 16:05	1710	535.00	74.1	1.84	0
10/27/2013 7:42	0	16.35	62.8	0	35
10/27/2013 7:55	0	205.07	62.3	0	35
10/27/2013 8:35	0	270.98	61.0	0	35
10/27/2013 10:05	0	522.35	62.6	0	35
10/27/2013 11:05	3640	497.55	64.8	0	35
10/27/2013 11:45	430	449.86	66.1	0	35
10/27/2013 12:25	0	410.22	67.3	0	35
10/31/13 10:00	2320	239.57	72.2	1.45	4
10/31/13 10:50	1830	736.37	67.1	1.45	4
10/31/13 11:10	2200	2987.05	66.8	1.45	4
10/31/13 11:30	2160	12982.71	66.9	1.45	4
10/31/13 12:00	2180	35311.52	66.5	1.45	4
10/31/13 12:40	4400	112811.74	66.4	1.45	4
10/31/13 14:00	11600	147092.86	67.0	1.45	4
11/22/13 11:45	0	239.57	64.5	0.01	22
11/22/13 11:50	0	328.65	64.6	0.01	22

Table 13. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
11/22/13 11:55	0	471.02	64.6	0.01	22
11/22/13 12:00	0	490.74	64.5	0.01	22
11/22/13 12:05	0	471.02	64.4	0.01	22
11/22/13 14:05	0	552.50	52.6	0.01	22
11/25/13 22:45	0	1035.42	42.6	0.39	3
11/25/13 22:50	0	1035.42	42.7	0.39	3
11/25/13 22:55	0	1035.42	42.7	0.39	3
11/25/13 23:00	0	1006.00	42.7	0.39	3
11/25/13 23:05	0	1006.00	42.8	0.39	3
11/25/13 23:10	0	977.03	42.9	0.39	3
11/25/13 23:15	0	977.03	42.9	0.39	3
1/13/14 18:47	0	87.04	61.4	0.04	48
1/13/14 18:51	0	87.04	61.2	0.04	48
1/13/14 18:56	0	87.04	61.2	0.04	48
1/13/14 19:01	0	84.64	61	0.04	48
1/13/14 19:06	0	84.64	60.7	0.04	48
1/13/14 19:11	0	84.64	60.3	0.04	48
1/13/14 19:21	0	82.29	59.3	0.04	48
1/13/14 19:31	0	82.29	58.5	0.04	48

Table 13. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
1/13/14 19:41	0	82.29	58	0.04	48
2/2/14 18:25	0	228.64	41.3	0.05	20
2/2/14 18:44	959	236.80	41.4	0.05	20
2/2/14 19:19	11530	236.80	41.6	0.05	20
2/2/14 19:59	6488	228.64	41.8	0.05	20
2/2/14 21:29	15650	216.68	41.8	0.05	20
2/4/14 11:11	2382	224.61	49.1	0.47	2
2/4/14 11:25	2481	245.11	49	0.47	2
2/4/14 11:55	1664	266.57	50.1	0.47	2
2/4/14 12:45	2755	275.43	49.3	0.47	2
2/4/14 14:35	2613	279.91	49.2	0.47	2
3/4/14 5:22	4730	216.68	32.7	0.35	28
3/4/14 5:56	6730	356.96	32.6	0.35	28
3/4/14 6:56	5430	497.55	33.5	0.35	28
3/4/14 7:46	3830	663.19	33.5	0.35	28
3/4/14 9:26	6330	879.00	34.4	0.35	28
3/4/14 9:46	7530	862.51	35	0.35	28
3/4/14 10:06	6930	830.04	35.6	0.35	28
5/13/14 6:46	12400	1.02	67.3	0.11	69

Table 13. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
5/13/14 7:05	52000	346.78	67.4	0.11	69
5/13/14 7:30	33600	404.72	67.2	0.11	69
5/13/14 8:00	24200	426.97	67.2	0.11	69
5/13/14 9:10	25100	393.83	68	0.11	69
5/13/14 10:10	22750	356.96	71	0.11	69
5/13/14 11:30	13400	298.24	72.1	0.11	69

Table 14. Statistical analysis correlation variables for each individual sampling event at the control site.

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
5/10/2013 3:30	9500	n/a	79.4	0	-
5/10/2013 3:33	4000	n/a	79.3	0	-
5/10/2013 3:36	4500	n/a	79.6	0	-
5/10/2013 3:39	4200	n/a	79.7	0	-
5/10/2013 3:42	5900	n/a	79.7	0	-
5/10/2013 3:45	6300	n/a	79.8	0	-
9/20/13 21:05	0	189.25	79.4	0.15	25
9/20/13 21:35	0	396.81	77.8	0.15	25

Table 14. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^oF)	Antecedent Moisture Content (in)	Last Sampling Event (days)
9/20/13 21:55	0	1572.32	78.1	0.15	25
9/20/13 22:15	0	11387.54	78.2	0.15	25
9/20/13 22:30	160	8349.48	77.6	0.15	25
9/20/13 23:10	0	2133.46	77.1	0.15	25
9/21/13 0:10	0	697.05	73.4	0.15	25
9/21/13 12:40	260	886.68	73.2	1.84	0
9/21/13 12:45	0	875.51	73.4	1.84	0
9/21/13 12:50	230	886.68	73.3	1.84	0
9/21/13 13:25	0	658.19	72.7	1.84	0
9/21/13 14:05	0	426.61	73.2	1.84	0
9/21/13 14:45	10	314.26	73.7	1.84	0
9/21/13 15:45	250	143.44	73.3	1.84	0
10/27/2013 7:35	10	127.01	63.4	0	35
10/27/2013 7:49	760	2371.93	62.3	0	35
10/27/2013 7:59	460	2744.44	62.4	0	35
10/27/2013 8:09	360	3015.81	62.5	0	35
10/27/2013 8:49	60	1512.21	62.5	0	35
10/27/2013 9:19	340	831.58	63.2	0	35
10/27/2013 10:39	20	270.82	64.3	0	35

Table 14. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^oF)	Antecedent Moisture Content (in)	Last Sampling Event (days)
10/31/13 10:20	50	7521.71	68	1.45	4
10/31/13 10:40	260	8939.89	67.4	1.45	4
10/31/13 11:20	260	28663.99	67	1.45	4
10/31/13 12:20	8100	7297.60	66.4	1.45	4
10/31/13 13:20	25200	15056.33	67.1	1.45	4
10/31/13 14:00	6500	6332.37	67.2	1.45	4
10/31/13 15:00	44000	1171.86	66.8	1.45	4
11/22/13 12:05	250	314.18	64.4	0.01	22
11/22/13 12:15	40	752.23	59.8	0.01	22
11/22/13 12:25	30	593.72	59.1	0.01	22
11/22/13 12:45	20	968.93	55.7	0.01	22
11/22/13 13:05	50	623.83	54.1	0.01	22
11/22/13 13:25	30	686.43	53.3	0.01	22
11/22/13 13:45	10	314.18	52.6	0.01	22
11/22/13 14:25	0	61.19	52.8	0.01	22
11/22/13 15:05	0	5.68	53	0.01	22
11/22/13 15:45	0	0.17	52.7	0.01	22
11/22/13 16:25	0	0.17	51.9	0.01	22
11/25/13 19:20	0	508.12	42.9	0.39	3

Table 14. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (^{o}F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
11/25/13 19:35	0	1171.86	42.9	0.39	3
11/25/13 20:00	0	1348.99	42.8	0.39	3
11/25/13 20:30	0	2073.71	42.4	0.39	3
11/25/13 21:00	0	1539.35	42.5	0.39	3
11/25/13 22:00	0	718.93	42.8	0.39	3
11/25/13 23:15	0	184.46	42.9	0.39	3
2/4/2014 12:10	2014	426.61	49.9	0.47	70
2/4/2014 12:29	2359	441.95	49.4	0.47	70
2/4/2014 13:14	1515	389.54	48.3	0.47	70
2/4/2014 14:14	1842	259.04	49.6	0.47	70
2/4/2014 15:54	1664	104.35	49.8	0.47	70
3/4/2014 6:26	1550	457.57	32.8	0.35	28
3/4/2014 6:46	4900	1381.49	33.2	0.35	28
3/4/2014 6:56	7900	3015.81	33.5	0.35	28
3/4/2014 7:16	10600	4261.70	33.7	0.35	28
3/4/2014 7:56	25450	2353.11	33.5	0.35	28
3/4/2014 9:26	18500	1381.49	34.7	0.35	28
3/4/2014 11:06	15900	575.07	36.5	0.35	28
5/13/2014 8:18	15300	253.26	66.9	0.11	69

Table 14. Continued

Sampling Date and Time	Concentration (CFU/100mL)	Flow Rate (L/min)	Temperature (°F)	Antecedent Moisture Content (in)	Last Sampling Event (days)
5/13/2014 8:22	17500	441.95	66.9	0.11	69
5/13/2014 8:32	13400	465.49	66.9	0.11	69
5/13/2014 8:37	15000	473.48	67	0.11	69
5/13/2014 8:52	13400	449.72	67.3	0.11	69
5/13/2014 9:02	14000	396.81	67.6	0.11	69
5/13/2014 9:12	15700	314.26	68	0.11	69

Table 15. Statistical analysis correlation variables for the Total CFUs per sampling event at the OSSF site.

Date	Total CFUs (x 10^9)	Peak Rainfall Intensity (in/hr)	Peak Flow Rate (L/min)	Maximum Temperature (°F)	Last Sampling Event (days)
5/10/13	45.685	4.5	1419.15	80	---
8/11/13	4.099	0.32	312.39	85	92
8/26/13	232.363	2.01	6994.09	81	15
9/20/13	1.473	3.27	497.55	86	25
9/21/13	3.127	0.84	879.00	75	0
10/27/13	0.979	5.65	528.66	71	35
10/31/13	3,423.510	3.92	153668.10	78	4
11/22/13	0	0.87	552.50	75	22

Table 15. Continued

Date	Total CFUs (x 10^9)	Peak Rainfall Intensity (in/hr)	Peak Flow Rate (L/min)	Maximum Temperature (°F)	Last Sampling Event (days)
11/25/13	0	0.63	1035.42	46	3
1/13/14	0	1.63	322.02	72	48
2/2/14	27.495	0.86	240.94	70	20
2/4/14	9.608	0.31	284.44	51	2
5/13/14	4.596	0.5	426.97	77	69

Table 16. Statistical analysis correlation variables for the Total CFUs per sampling event at the control site.

Date	Total CFUs (x 10^6)	Peak Rainfall Intensity (in/hr)	Peak Flow Rate (L/min)	Maximum Temperature (°F)	Last Sampling Event (days)
9/20/13	269.221	3.27	11387.54	86	25
9/21/13	718.034	0.84	1125.964	75	0
10/27/13	824.243	5.65	3167.491	71	35
10/31/13	381,589.327	3.92	41069.58	78	4
11/22/13	24.682	0.87	1088.237	75	22
11/25/13	0	0.63	2073.713	46	3
2/4/14	1,794.642	0.31	457.5693	51	70
5/13/14	495.151	0.5	473.482	77	69