Variability of *Escherichia coli* (*E. coli*) concentrations in Whiteoak Watershed, Houston, Texas

Civil and Environmental Engineering Research Experience for Undergraduates
University of Houston

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August 5, 2008
# Table of Contents

Introduction ......................................................................................................................... 3  
Background and Theory ........................................................................................................ 3  
  History and Standards ....................................................................................................... 3  
  Watershed Description ...................................................................................................... 4  
  Organism ......................................................................................................................... 5  
  Sources of Bacteria ........................................................................................................... 6  
  Effect of Temperature and Rain ...................................................................................... 6  
Procedure ............................................................................................................................... 6  
  Sample Collection ........................................................................................................... 6  
  Laboratory Processing ....................................................................................................... 8  
  Analysis .............................................................................................................................. 8  
Results .................................................................................................................................. 9  
  Site Comparison .............................................................................................................. 11  
  Temperature .................................................................................................................... 12  
  Rain .................................................................................................................................. 12  
Discussion and Conclusions ............................................................................................... 15  
Acknowledgments ............................................................................................................... 15  
References ........................................................................................................................... 16

## Acronym Disambiguation

- Cfu = coliform forming unit (equivalent to MPN for practical purposes)
- CR = Contact Recreation
- CWA = Clean Water Act
- dL = deciliter = 100 milliliters
- MPN = most probable number (of bacteria)
- NCR = Non-Contact Recreation
- TCEQ = Texas Commission on Environmental Quality
- TMDL = Total Maximum Daily Load
- USGS = US Geological Survey
- WBW = Whiteoak Bayou Watershed
Abstract
The Clean Water Act requires the monitoring of all surface waters. The Texas Commission on Environmental Quality (TCEQ) is responsible for maintaining these waters at the level of quality required by Clean Water Act (CWA). The tributaries of Whiteoak Bayou Watershed, an urban watershed located in Houston, TX exceed the TCEQ standards for indicator bacteria concentration for contact recreation water bodies. Two tributaries of the watershed and Whiteoak Bayou itself were sampled for seven weeks in June and July of 2008 to assess variability in *Escherichia coli* concentrations. *E. coli* concentrations exceeded the standards for contact recreation purposes in 88.3% of the samples (n = 283). There was no association between *E. coli* concentration and water temperature ($R^2 = 0.0328$). Rain events appeared to have some correlation with spikes in bacteria concentrations, but the effects were not measurable.

Introduction
Bacterial contamination of surface waters is a significant health hazard. The U.S. Environmental Protection Agency has set maximum bacterial concentrations for different levels of water contact. All surface waters in Texas should meet the standards for contact recreation unless otherwise specified (Chief Engineer’s Office, Water Programs, TMDL Section, TCEQ 2008). The streams in Whiteoak Bayou Watershed significantly exceed the contact recreation standards for indicator bacteria. The Total Maximum Daily Loads (TMDLs) that can enter the streams without causing them to exceed the standards was recently evaluated (TMDL). The data in this study was collected in order to calibrate the model used to determine the TMDL. It has been evaluated here for daily and weekly variability and the effects of temperature and rain on indicator bacteria concentrations.

Background and Theory

History and Standards
The Federal Water Pollution Control Act was passed in 1948 with the goal of improving the quality of the surface waters within the United States. It was rewritten entirely in 1972 as the Clean Water Act (CWA) and amended since. Its missions have expanded to include the protection of aquatic wildlife and water recreation. The Clean Water Act emphasizes that it is the responsibility of state governments to ensure that standards made by the US Environmental Protection Agency are met (FWPCA 2008, FWPCA (CWA) 2008). Section 303(d) of the CWA requires all states to prepare a list of water bodies that do not meet each standard. These lists of impaired water bodies are called “303(d) lists” (TCEQ 2008).

The TCEQ is responsible for monitoring the quality of the surface water in Texas. It must take measures to improve the characteristics of the surface water that does not meet the CWA standards. The TCEQ then works to determine the Total Maximum Daily Load (TMDL) that can enter a water body without causing it to exceed the standards (FWPCA 2008, FWPCA (CWA) 2008). The next step is to determine the Best Management Practices (BMP) to improve water conditions.

The standards for surface waters depend on their intended use. The default for surface water in Texas is contact recreation (CR). Contact recreation includes any activity in which there is a high likelihood of ingesting water, such as swimming. Some
Water bodies have been classified as unsuitable for contact recreation because of high pollution levels from unavoidable sources. These must instead meet standards for noncontact recreation. Activities termed “noncontact recreation” include anything with insubstantial risk of ingesting the water, such as boating or fishing. Water bodies in Texas that are classified for contact recreation but do not meet its standards are placed on Texas’ Clean Water Act 303(d) List (UH 2007).

The TCEQ Surface Water Quality Standards for contact recreation in freshwater include a maximum geometric mean of *E. coli* of 126 cfu/dL. Single samples should not have concentrations higher than 394 cfu/dL. For noncontact recreation, the maximum geometric mean of *E. coli* is 605 cfu/dL. When insufficient *E. coli* data is available, the concentration of total fecal coliforms can be used to determine if the water body is suitable for recreation. For contact recreation, the maximum geometric mean is 200 cfu/dL and single samples should be less than 400 cfu/dL. For noncontact recreation purposes, the maximum geometric mean is 2000 cfu/dL (UH 2007).

**Watershed Description**

Whiteoak Bayou Watershed (WBW) is located in the San Jacinto River Basin in Houston, Texas. It flows into Buffalo Bayou, which ultimately flows into Galveston Bay and out into the Gulf of Mexico. The portion of the bayou in the WBW is 23 miles long. The drainage area of the WBW is 105 square miles. Brickhouse Gully (Segment 1017A), Cole Creek (1017B) and two other streams flow into Whiteoak Bayou (University of Houston and CDM).

The majority of the land in the Whiteoak Bayou Watershed is developed (30% high intensity development, 29% low intensity development). Twenty-four percent of the land is grassland, 14% is woody land, 2% is wetland, and 1% is open water. None of the land is cultivated or bare (University of Houston and CDM).

The climate is hot in the summer, with average temperatures in the eighties from June through September. The winters are mild, with averages in the fifties. The rainy season is inconsistent, with May and June receiving more rain on average than the other months (~5 in). The area is subject to Gulf Weather, with occasional hurricanes (Office of Homeland Security & Emergency Management 2008, UH and CDM 2008).

Whiteoak Bayou has historically exceeded CWA standards in the area of indicator bacteria (Figure 1). The tributaries of the Whiteoak Watershed were put on Texas’ Clean Water Act 303(d) List in 1992 for exceeding the bacteria standards. The goals of the 2008 TMDL for Buffalo and Whiteoak Bayou watersheds are to achieve the geometric mean required by the TCEQ and to keep the concentration of *E. coli* down to 395 MPN/dL three-quarters of the time (University of Houston and CDM).
Figure 1. Historical data of fecal coliform concentrations in Whiteoak Bayou (Chief Engineers Office, Water Programs, TMDL Section, TCEQ 2008).

The 2008 TMDL found that the *E. coli* concentration at Stations 11387, 16594, and 16593 exceeded the standard for a single sample approximately 95% of the time (Table 1). Their data also suggests that the bacteria density is highest at 11387, then 16594, and lowest at 16593 (UH and CDM 2008).

Table 1. Summary of Whiteoak Bayou *E. coli* data between 2001 and 2006. Provided by the City of Houston Health and Human Services Department and the Region 12 TCEQ Field Office (UH and CDM 2008).

<table>
<thead>
<tr>
<th>Station</th>
<th>Geometric Mean</th>
<th>% Exceeding Standard</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>11387</td>
<td>4481</td>
<td>96</td>
<td>50</td>
</tr>
<tr>
<td>16594</td>
<td>3333</td>
<td>95</td>
<td>38</td>
</tr>
<tr>
<td>16593</td>
<td>2845</td>
<td>95</td>
<td>38</td>
</tr>
</tbody>
</table>

**Organism**

*Escherichia coli* are a species of coliform, rod-shaped bacteria that are approximately 0.5 μm by 2 μm. They are commonly found in the intestinal tract and feces of warm blooded animals (Tchobanoglous & Schroeder 1987). *E. coli* are used as the indicator bacteria for non-tidal streams. This is based on a 1972 EPA study which found that, out of the bacteria considered, *E. coli* concentration had the strongest correlation with gastroenteritis in freshwater (USEPA 1986). Gastroenteritis is a disease that typically results in diarrhea and subsequent dehydration. It can be caused by viral infection or by bacteria. It is one among several diseases associated with water contaminated by human fecal matter. *E. coli* may die because of water temperatures and pHs to which they have not been acclimated, because of a lack of nutrition, or because of UV light (Tchobanoglous & Schroeder 1987).
Sources of Bacteria

Bacteria can enter water bodies from point and non-point sources. Point sources are usually deposited directly to streams through piping. They may include sanitary sewers that overflow and waste water treatment plant outflow. Non-point sources of pollution are diffuse and are usually brought to the water body with rain water runoff. These sources are usually unregulated. They may include septic systems, direct deposition from various animals, bacteria regrowth, and pollution from upstream sources (UH 2007).

Effect of Environmental Conditions

The amount of pollutants in streams is usually higher during and directly after rain events because of nonpoint-source pollution. The runoff picks up pollutants as it flows down to the streams. Urbanization exacerbates the issue because of two factors. First of all, in urbanized areas such as Whiteoak Bayou watershed, most of the surfaces the runoff traverses are impervious. Since very little of the runoff is absorbed by the ground, more of it ends up in the streams and it gets there faster. Because of this, the runoff can pick up more pollutants. Urbanization also causes more pollutants to be present on the surface. Organic fertilizer, on-site waste disposal, litter, and animal droppings contribute to increased organic and potentially pathogenic pollution (Walesh 1989).

Traister and Anisfeld found that the concentration of *E. coli* in the Upper Hoosic River, NY and VT, is effected by a number of environmental factors (2006). They found that bacterial levels vary seasonally, with the highest concentrations occurring during the summer. They monitored *E. coli* density throughout the day and found it was highest in the early morning. They also found that in streams in developed areas, the bacterial density was higher and the diurnal variability was greater (Traister & Anisfeld 2006). The results presented by Traister and Anisfeld suggest that the sampling done for this study may be capturing the highest possible bacterial densities. However, it is unlikely that the bacterial concentrations in Houston, TX would experience seasonal variation like that of New England.

Procedure

Sample Collection

Six sites were sampled in the Whiteoak Bayou Watershed from June 5 until July 23. Two sites were sampled at each segment. One of each pair had been used in the generation of the Buffalo and Whiteoak Bayous TMDL (Chief Engineers Office, Water Programs, TMDL Section, TCEQ 2008), the other is at the location of USGS flow gages. Station 11387 is on Whiteoak Bayou, 16594 is at Brickhouse Gully, and 16593 is at Cole Creek (Figure 2). Sample collection occurred between the hours of 8:30 and 14:00 each day.

At each site, water samples were taken and a water quality sonde was used to measure the standard chemical conditions. The YSI 6820 Multi-Parameter Water Quality Sonde is calibrated in the laboratory at Schlumberger for conductivity (1000 microSiemens per cubic centimeter), turbidity (0 NTU and 123 NTU), and pH (7 and 10). The same standards are used for post-calibration. At each site, the YSI Sonde is used to
find values for those parameters, as well as temperature and salinity. Water is collected in the cap of the Sonde, and then attached so all the probes are submerged. The readings and the time of sample collection are recorded in a log book. After field data is collected, post-calibration is done on the Sonde to ensure that it has maintained its accuracy through all of the readings.

Figure 2. Map of Whiteoak Bayou Watershed showing sampling locations.

Depth, width, and velocity of the stream are evaluated during sample collection. The depth of the water and the width of flow are measured using a 25’ Sokkia fiberglass measuring rod. Flow rate is measured using a Marsh Mc-Birney Flo-Mate. In the shallow channels at sites 16594 and 16594@USGS, the flow rate of the water may vary significantly across the width of the stream, so the flow rate is measured at five transects. This enables determination of the total flow rate across the stream. The flow rate and depth of the shallow channels are measured at five distances from the edge of the water, usually in two or three-foot increments.

Several measures are taken to ensure that the water samples taken are not contaminated. Plastic buckets are used to collect water samples from the sites. These buckets have previously been autoclaved and covered with foil to keep it sterile. The water is poured from the buckets into four 4 oz Nasco Whirl-Pak Bags. Each bag comes sealed with a 10 mg tablet of sodium thiosulfate. This is present to neutralize any chlorine that may be in the water. Since it does not directly affect the bacteria, it can be used if chlorine in the water is highly unlikely. The tables are necessary because chlorine would kill some of the bacteria in the water, interfering with an accurate assessment of how much was present when it was collected. The remaining water in the bucket is used
to fill a TSS bottle. This is sent to a different lab for measurement of total suspended solids.

On some days, a Field Blank is made by pouring autoclaved DI water into an autoclaved bucket, which is subsequently used to take a water sample. This is processed as a 1:1 dilution. In addition to the field blanks, replicate samples are made on some days. These replicates allow us to consider how consistent the bacteria concentration is at a site on a given day and how consistent our running of the data is. The QAQC requires a duplicate to be done on 10% of the sites.

**Laboratory Processing**

The water samples are processed in the laboratory to determine the population density of *E. coli*. Three replicates of 1:1, 1:10 and 1:100 dilutions are made using the water collected from each site. All dilutions are made in 120 mL IDEXX bottles containing sodium thiosulfate. One packet of IDEXX Colilert® reagent is emptied into each bottle. The bottle is shaken until no reagent is visible. This is poured into a Quanti-Tray®/2000, which is tapped to minimize bubbles. The trays are sealed in a Quanti-Tray® Sealer. If there is an insufficient amount of sample in one of the trays, the same dilution is made again and run. All of the sealed trays are incubated at 35.0°C for 24 to 28 hours.

Trays are read in two ways. First, the number of large and small wells containing yellow sample are counted. The yellow color indicates total coliforms. Each tray is then put under a fluorescent light in a dark box. The number of large and small fluorescing wells is counted. These numbers are used to determine the most probable number of all coliform bacteria and *E. coli* specifically.

A number of blanks are made each day. Every time a batch of samples is processed, a Lab Blank and a DI Blank are also run. For the Lab Blank, a 1:100 dilution bottle is run, adding only Colilert® reagent. The DI Blank is made by pouring autoclaved DI water directly into the bottle and adding reagent.

**Analysis**

All data were analyzed using Microsoft Excel. Ambient temperature data was provided by the National Weather Service. Rain data was from the Office of Homeland Security & Emergency Management. For analysis purposes, the day of a rain event and the following day were considered rain days. Four or more days following a rain event were classified as dry days. The days between were not considered. Rain events were grouped by the day on which they fell. Bacteria concentrations from Sites 11387-USGS and 11387-Whiteoak were compared with rain data from Whiteoak and Heights. Sites 16594-Brickhouse Gully and 16594-USGS used data from Brickhouse Gully and Costa Rica. For 16593-Cole Creek and 16593-USGS, the amount of rain from two locations were averaged. The 16593 sites are between White Oak Bayou @ Fairbanks N Houston and White Oak Bayou @ Pinemont. The geometric mean to consolidate a collection of points because bacterial concentrations are known to have a distribution similar to the lognormal. The 95% confidence interval is used to report the error in all analyses because it reflects how far the geometric mean may be from the true mean.
Results

Data was collected for seven weeks. The concentration of bacteria displayed a great deal of variability between days (Figures 3 and 4). For example, the 95% confidence intervals of the first two days of sampling at 11387-Whiteoak do not overlap. However, different sites frequently had high and low concentrations on the same weeks, with different magnitudes (Figure 5).

The indicator bacteria concentrations did not meet the TCEQ requirements for single sample standard and geomean standard. The concentration of total coliforms exceeded the requirements for contact recreation at all sites on 100% of the days (n = 254). It exceeded the standards for noncontact recreation 99.2% of the time (n = 254). The standards were met were at 16594 Brickhouse Gully and 16594 USGS on July 13. The concentration of E. coli exceeded the standards for contact recreation 88.3% of the time (n = 283). Over half of the occasions when the E. coli concentration met the standards were at Site 16593 USGS.

![Figure 3. Daily fecal coliform concentration at six sites in Whiteoak Bayou Watershed.](image)
Figure 4. Daily E. coli concentration at six sites in Whiteoak Bayou Watershed.

Figure 5. Geometric means of E. coli each week.
Site Comparison

Despite temporal variability, sites appear to follow the same trends of when they contain particularly high or low levels of indicator bacteria (Figure 5). Furthermore, two sites within the same segment consistently experience similar increases and decreases in bacteria levels. This makes sense because one flows into the other within a relatively short distance (Figure 2). Segment 11387 appears to have a higher density of bacteria than Sites 16594 and 16593. Segment 16593 appears to have a lower bacterial density than the other two segments (Figure 6).

Figure 6. The geometric mean of the E. coli concentration at each site. Error bars represent 95% confidence.

A student’s t-test was used to determine whether or not there was a difference between the sites. As shown in Table 2, there were no differences between sites within a segment. There was a difference between each of the Segment 16593 sites and the rest of the sites (Table 2). It appears from Figure 6 that Segment 16594 had lower levels of bacteria than Segments 11387 and 16594.

Table 2. Probability that the two data sets tested are the same using a t-test. Significant results are highlighted.
Temperature

During the months of June and July, the temperature ranged from 70°F to 100°F (National Weather Service). The water temperature at the sites tested was consistently between 20°C and 37°C when sampled. Ninety-nine percent of the data was between 25°C and 35.5°C. Within the narrow water temperature range, water temperature had no effect on bacteria concentrations. There was no association between the concentration of fecal coliforms and water temperature ($R^2 = 0.07$). There was no association between the concentration of *E. coli* and water temperature ($R^2 = 0.03$).

Rain

Rainfall did not appear to be strongly correlated with *E. coli* concentration (Figures 7, 8, 9). The *E. coli* concentration appears to decrease in late July, when there were only two small rain events. A spike occurred on July 23, which corresponds with a storm that came in then (Figure 8). This is not evident in Sites 11387 because the weather data for White Oak Bayou and Heights was incomplete and did not have data for July 23 (Figure 5). The effects of rain on the concentration of indicator bacteria were evaluated based on the time after the rain event and on the amount of rain that fell during a day.

![Graph](image-url)

Figure 7. Rain events and *E. coli* concentration in the area of White Oak Bayou and Heights.
Rain events are thought to effect bacterial concentrations for up to three days after they occur. The geometric mean of E. coli concentrations were determined for the day on which the rain event occurred and the following two days (Figure 10). Since only eighteen percent of the rain events in this study occurred before noon and sampling was usually completed before noon, most of the rain events could not affect the bacteria concentration that day. There was no difference between the E. coli concentrations on the day of rain events and the two days that follow (p > .5). There was a difference
between the *E. coli* concentrations on the rain day and the following two days versus three or more days after the rain event (n(wet) = 128, n(dry) = 98, P = 0.0394).

The effect of the amount of rainfall on the concentration of indicator bacteria was also considered. Approximately 13.6% of the *E. coli* concentration variability can be explained by rainfall concentration (n = 77, Figure 11). The histogram of the amount of rainfall was strongly skewed to the left, so the geometric mean (gm = 0.194 in.) was used to categorize rainfall. There was more bacteria on the days and locations of the larger rain events (gm(small) = 1370, gm(large) = 3352.192, P=0.0014).

![Figure 10. The geometric mean of *E. coli* concentration on the day of a rain event (1), the following two days (2, 3) and three or more days after the rain event.](image)

![Figure 11. The logarithm of the bacterial concentration that corresponds to varying amounts of rainfall.](image)
Discussion and Conclusions

The differences between the concentrations of indicator bacteria at different sites are most likely caused by where these streams are flowing into. Both Cole Creek and Brickhouse Gully flow into Whiteoak Bayou. From this we might expect that the highest bacteria concentrations would be in Whiteoak Bayou. Although the E. coli concentrations at the Whiteoak Bayou sites were not significantly different from those at Brickhouse Gully, they were higher than the concentrations at Cole Creek. The seemingly higher bacterial density at 16593-Cole Creek over 16593-USGS was probably caused by the wastewater treatment plant that is of and close to 16593- Cole Creek.

The similarly high and low bacterial concentrations at different sites suggest that the cause of the variability between days and weeks was not entirely local. This motivated consideration of temperature differences and rain events. Temperature was not correlated with indicator bacteria concentration ($R^2 = 0.03$). This unexpected lack of correlation can be attributed to the small range of temperature data. This could be remedied by doing similar sampling during the winter months.

Rain events did not appear strongly correlated with increases in bacterial concentrations, although some trends were visible (Figures 8 and 9). This was probably caused by a wash-off effect. When rain events occur in close temporal proximity, there is insufficient time for the E. coli to build up on land (Traister & Anisfeld 2006). Thus, the next rain events cannot wash as much bacteria into the streams. Despite the lower bacterial densities on rain days, the day on which it rains and the two days that follow have higher bacterial densities than “dry” days ($P = 0.0394$). This suggests that bacterial density begins to decrease after several days without rain runoff. Figures 7, 8, and 9 support this idea with lowered bacterial densities during the dry period in July.

The variability shown and discussed in this paper emphasizes the importance of taking multiple water samples when determining water quality. These samples should be taken in different streams within a watershed, during a dry period to determine baseline bacterial concentrations, and during or directly following a rain event to evaluate the spikes in bacterial density. It may be beneficial to take data during different seasons. If only a limited number of samples can be taken, they should be taken during the summer so that the exceedance of the standards is not underestimated.

Acknowledgments

The research study described herein was sponsored by the National Science Foundation under the Award No. EEC-0649163. The opinions expressed in this study are those of the authors and do not necessarily reflect the views of the sponsor. The Texas Commission on Environmental Quality sponsored the laboratory. I am tremendously grateful for the guidance of Hanadi Rifai and Anu Desai. It has been a pleasure to work with this research group: Yaa Amoah, Michael Cowert, Anuradha Desai, Matt Feaga, Alex Frimm, Lisa Grecho, Nathan Howell, Divagar Lakshmanan, Maria Modelska, Stephen Ray, Reba Scott and Zack Van Brunt.
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