Summer 8-13-2019

E. Coli in the Tanyard Creek Combined Sewage Overflow: a Spatial and Temporal Review Coordinated with Weather Patterns

Hannah-Leigh Crawford

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Abstract

**E. coli in the Tanyard Creek Combined Sewage Overflow: A Spatial and Temporal Review Coordinated with Weather Patterns**

By

Hannah-Leigh Crawford

August 2019

**INTRODUCTION:** Tanyard Creek is an urban creek in metro Atlanta contained in a large urban sub-watershed that sends stormwater to drain into the Chattahoochee River. The creek is considered impaired, with signage warning the public not to play, swim, or fish in creek. As an urban creek, it is subject to sewage overflow from one of the city of Atlanta’s combined sewer overflow (CSO) facilities, the Tanyard Creek CSO, as well as runoff from the surrounding commercial and residential areas, which may carry microbial contaminants into the creek. The creek is partly surrounded by an urban greenspace containing walking and biking paths and playgrounds, making it a candidate for remediation and future use for recreation. To understand the patterns of possible microbial contamination in this type of urban creek, the fecal indicator *Escherichia coli* was evaluated in the creek over time.

**AIM:** This research will determine the trends of *E. coli* in Tanyard Creek and if *E. coli* counts differ temporally (over the course of a year) and spatially (from sampling site to sampling site). Additionally, rainfall data from the National Weather Service will be used to determine if there is a relationship between rainfall amounts and *E. coli* counts at 24, 48, and 72 hours prior to sampling, as well as cumulatively.

**METHODS:** Water samples were collected weekly for roughly 47 weeks at 10 sites downstream from the Tanyard Creek CSO. The 10 sites were spaced along a half mile stretch of creek that included a concrete channel, a beaver dam, and a railroad bridge. All samples collected from the creek were then brought to the lab for analysis of microorganisms through membrane filtration for *E. coli* using BioRad RAPID'E. coli 2™ bacterial assay.

**RESULTS:** *E. coli* is present in Tanyard Creek at levels higher than the U.S. Environmental Protection Agency standards. This data indicates temporal trends; during the summer months (June-September) there are higher counts of *E. coli*. Also, *E. coli* from all sampling sites differed significantly by date. This data does not indicate spatial trends, as the *E. coli* from all dates by sampling site did not differ significantly. For all rainfall levels—24, 48, and 72 hours prior to sampling, as well as cumulatively, there was not a statistically significant relationship between rainfall and *E. coli* levels. These high levels of fecal indicator bacteria demonstrate that the creek is vulnerable to bacterial and viral contamination that may pose risks of waterborne disease. These risks might be mitigated if the creek is to be reclaimed in the future as a recreational urban greenspace, potentially requiring changes in urban stormwater and runoff management.
E. COLI IN THE TANYARD CREEK COMBINED SEWAGE OVERFLOW: A SPATIAL AND TEMPORAL REVIEW COORDINATED WITH WEATHER PATTERNS

by

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B.S., KENNESAW STATE UNIVERSITY

A Thesis Submitted to the Graduate Faculty
of Georgia State University in Partial Fulfillment
of the
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E. COLI IN THE TANYARD CREEK COMBINED SEWAGE OVERFLOW: A SPATIAL AND TEMPORAL REVIEW COORDINATED WITH WEATHER PATTERNS

by

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Monday, August 5, 2019
Author’s Statement Page

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____________________________
Hannah-Leigh Crawford
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CHAPTER 1 - INTRODUCTION

1.1 Background

Tanyard Creek is one of several urban creeks in the Atlanta area that eventually join larger creeks and streams to drain into the Chattahoochee River. There are several large urban sub-watersheds in the Atlanta area and they all have the potential to send large volumes of stormwater downstream to the Chattahoochee River, shown in Figure 1.1. As is the case with several Atlanta creeks, Tanyard is designated as being impaired and not fit for several uses, including recreation. There are large, brightly colored signs all around Tanyard Creek that warn to not play, swim, or fish in the creek due to contamination, shown in Figure 1.2. As this sign shows, Tanyard Creek is an urban creek and is subject to sewage overflows and runoff contaminants. However, the creek is not regularly monitored to determine how contaminated it might be by urban stormwater, runoff, or sewage.

In the United States, urban water pollution due to effluents originating from combined sewer facilities is considered a major source of water impairment and a significant human health concern (Tibbetts 2005). During dry conditions, the mixture of precipitation and sewage is channeled to a treatment plant before being discharged into waterways (Tibbetts 2005). During heavy precipitation, storm and wastewater exceeding a treatment plant’s processing capacity are discharged into local surface waters, a process known as a combined sewer overflow (CSO) (Tibbetts 2005). These overflows have several different possible health impacts, including spreading waterborne and vector borne disease. In fact, there are published articles regarding how Tanyard Creek (and other urban creeks affected by CSOs) is a prime mosquito (Culex quinquefasciatus) breeding habitat due to the favorable conditions presented by the combined sewage overflow (Calhoun et al. 2007, Nguyen et al. 2012). In Atlanta, *Culex quinquefasciatus* is the main urban vector of West Nile virus (Vazquez-Prokopec et al. 2010).

The United States Environmental Protection Agency (EPA) mandates the use of fecal indicator bacteria (FIB), like *Escherichia coli* (*E. coli*), as a method of detecting fecal contamination in water and assess the quality of drinking and recreational waters (U.S. EPA 2012). Fecal indicator bacteria (FIB) are bacteria that naturally live in the intestines of humans and animals; so the presence of them in drinking or recreational water indicates pollution (U.S. EPA 2012). Fecal indicator bacteria (FIB) have been used to determine if there is sewage contamination in water sources (rivers, creeks, lakes, etc.) to help protect the public from waterborne pathogens, including bacteria and viruses that spread through human and animal feces (Rose et al. 2015, Pandey et al. 2014).

Identifying and quantifying *E. coli*, an indicator of fecal contamination, can give us an idea of whether there is human and animal fecal pollution present in this creek. In an urban creek like Tanyard, urban runoff from buildings, streets, and impervious surfaces is a definite source of pollution. Urban runoff occurs during wet weather through rainfall events and during dry weather by waste flowing from urban landscapes into storm drains leading into the creek. Urban runoff carries contaminants into waterways: there can be sewage runoff from broken
pipes, animal waste from pets that defecate outdoors, industrial pollutants from accidental releases and illegal dumping, and pesticides applied to lawns, golf courses, and agriculture, creating health risks for those in contact with the waterways involved.

Sewage runoff has historically been a significant source of pollution in Atlanta's creeks due to the city originally having a combined sewer system with treatment plants that were regularly overwhelmed by heavy stormwater flows. In 1999, the city of Atlanta settled a Clean Water Act lawsuit by entering into a federal consent decree to improve its combined sewer system due to the sewer discharges that were in violation of the federal Clean Water Act and the Georgia Water Quality Act (Hunter & Sukenik 2007). Investments were required to make renovations, including separating the combined sewers into distinct sanitary sewer and stormwater lines and upgrading and constructing new treatment plants, as well as constructing off-line storage facilities to hold stormwater overflows. In July 2001, after years of studying and obtaining citizen’s input, the EPA and the state of Georgia’s Environmental Product Declaration (EPD) approved the city of Atlanta’s plan to eliminate water quality violations from combined sewer overflows (CSOs). The plan involved a combination of tunnels and separation of specific sewer areas. A revised plan was submitted to the EPA and EPD that would increase the water quality benefits of proposed sections of the plan and reduce the lengths of proposed CSO tunnels. The city of Atlanta’s plan for a storage and treatment system for sewer and stormwater involved capturing and storing combined sewer overflows (CSOs), storing them in large, underground tunnels in bedrock. After a rainfall event, the captured CSO volume is conveyed to a separate treatment system for removal of pollutants and ultraviolet disinfection before being discharged into waterways.

Before the consent decree and construction of stormwater control systems, Tanyard Creek received combined sewer overflows during heavy rains. The creek and surrounding park were routinely flooded with human fecal waste during rains. The city has since installed a stormwater control point, called the Tanyard Creek CSO. Now, the creek receives stormwater flows, but no longer receives combined sewer overflows. While urban streams like Tanyard Creek have improved since the 1999 consent decree and upgrades to city of Atlanta’s sewer systems, there are still sources of pollution from various types of urban runoff. Some types of sewage pollution from urban runoff are sewage runoff from broken pipes and animal waste from pets that defecate outdoors, and this type of fecal pollution can be detected by using E. coli as a fecal indicator bacteria. This research will look for patterns in the fecal indicator bacteria (FIB) E. coli in Tanyard Creek to determine whether fecal pollution is present, the magnitude of fecal pollution, and the spatial and temporal patterns.
1.2 Research Questions

What does *E. coli* in the Tanyard Creek Combined Sewage Overflow (CSO) look like spatially (from site to site) and temporally (January 2018-February 2019)? Also, how *E. coli* in the Tanyard Creek CSO relate to rainfall/meteorological events?
1.3 Research Aims and Hypotheses

This research will use the fecal indicator bacteria *Escherichia coli*, also called *E. coli*. Overall, the goals will be to explain the trends of *E. coli* in Tanyard Creek throughout a year (from January 2018-February 2019), observing if *E. coli* trends differ spatially (from site to site) and temporally (throughout the year). Also, this research will include rainfall data from the National Weather Service recorded at the Atlanta Hartsfield-Jackson International Airport for 24, 48, and 72 hours prior to each sampling date and will determine if there is a relationship between rainfall amounts and *E. coli* trends.

**Aim 1:** Compare *E. coli* levels present in all water samples of Tanyard Creek and how they change spatially (from site to site) and temporally (January 2018-February 2019).

**Hypothesis 1:** *E. coli* levels will indicate that the urban creek is considered to be impaired and not compliant with the Environmental Protection Agency’s Recreational Water Quality Standards.

**Aim 2:** Determine if there is a relationship between rainfall amounts in the Atlanta area and *E. coli* levels in Tanyard Creek after a rainfall event at four time periods: 24, 48, and 72 hours prior to sampling, as well as cumulatively.

**Hypothesis 2:** *E. coli* levels will be highest after significant rainfall events at each time period specified and the highest for cumulative rainfall.
CHAPTER II – LITERATURE REVIEW

2.1 *E. coli* as a Fecal Indicator Bacteria (FIB)

Indicator organisms are frequently used to assess the level of pathogens in different water sources and monitoring the levels of these indicator organisms is a typical method of quantifying potential pathogen loads (Pandey et al. 2014). The most commonly used indicator organisms for water-borne pathogen research are *Enterococci* and *Escherichia coli*, which are both fecal coliforms referred to as FIBs, or fecal indicator bacteria. For years, research scientists and public health researchers have evaluated water quality using *E. coli* levels in lakes, rivers, coastal waters, and estuaries (Pandey et al. 2014). Waterborne pathogens are carried in fecal pollution from animals and humans (Rose et al. 2015). Humans can be infected following exposure (often through ingestion) to contaminated drinking or recreational water (Olds et al. 2018).

2.2 Epidemiological Relationships

Currently, public health officials and scientists rely on exposure limits for assessing pathogen levels in water resources, which have been established to protect human health. The EPA defines acceptable recreational limits as those that will result in eight or fewer swimming-related gastrointestinal (GI) illnesses out of every 1,000 swimmers (U.S. EPA 1986). The current U.S. EPA fresh water quality criteria for *E. coli* is a geometric mean not exceeding 126 CFU/100 ml, or no samples exceeding a single sample maximum of 235 CFU/100 ml (U.S. EPA 2001), as shown in Table 2.1, from the EPA’s most recently published Recreational Water Quality Standards (RWQS) in 2012. Criteria were developed based on the U.S. EPA measurements of total and Highly Credible Gastrointestinal Illnesses (HCGI), which correlated with *E. coli* densities \( r = 0.804 \) in fresh recreational waters (Dufour 1984). Multiple studies have identified trends between indicator organisms in water and GI illness in humans, including vomiting, diarrhea, and fever (Cabelli 1983; Wade et al. 2006). The most common waterborne disease is gastrointestinal (GI) illness, and endemic occurrence in the community is difficult to quantify because most waterborne cases are sporadic and often not recognized as associated with water exposures (Rose et al. 2015). Studies estimate there are 11 to 19 million cases of GI illness from contaminated drinking water (Messner et al. 2006, Reynolds et al. 2008, Colford et al. 2006) and an estimated 90 million cases from exposure to recreational waters (DeFlorio-Barker et al. 2018) each year.
2.3 Combined Sewage Overflows & Urban Creeks

Human fecal contamination, i.e., untreated sewage, has the highest potential to cause disease because humans are the reservoirs for many human pathogens (Schoen et al. 2011, Olds et al. 2018). Stormwater systems have been found to be frequently contaminated by sanitary sewage as a result of infiltration of leaking sewage or illicit cross-connections, resulting in untreated sewage discharging directly into rivers and streams (Sercu et al. 2011, Sauer et al. 2011, Olds et al. 2018). Combined sewer systems are particularly vulnerable to overflows, as they collect runoff from impervious surfaces and convey sanitary sewage and stormwater to wastewater treatment plants (Olds et al. 2018).

The US Environmental Protection Agency (EPA) estimates 850 billion gallons of untreated sewage is discharged annually into US waterways by combined sewer overflows (CSOs) and up to 10 billion gallons from separated sewer overflows (SSOs) (U.S. EPA 2004, Olds et al. 2018).

2.4 Rainfall & Weather Patterns

Although there has been some research regarding relationships between rainfall and fecal coliforms, there are few studies containing a full year of data specifically for urban streams. One study is Chin et al. 2010, which used fecal coliforms to show that urban areas have impacts on stream pathogens, specifically that summer and rainfall raises pathogen levels in these urban streams.

Heavy rainfall has been linked with increased waterborne disease outbreaks (Curriero et al. 2001, Cann et al. 2013, Olds et al. 2018). Fecal pollution has been found to be widespread in the environment following rainfall events and/or snowmelt (Marsalek et al. 2010, Newton et al. 2013, Olds et al. 2018). Furthermore, under extreme precipitation events, sewer systems can become inundated with rainwater and cause sewer overflows (Passerat et al. 2011, McLellan et al. 2007, Olds et al. 2018)
CHAPTER III – METHODOLOGY

3.1 Research Areas

This study investigates two different research areas (RAs) linked to the research questions:

**RA 1:** Compare *E. coli* levels present in all water samples of Tanyard Creek and how they change spatially (from site to site) and temporally (January 2018-February 2019).

**RA 2:** Determine if there is a relationship between rainfall amounts in the Atlanta area and *E. coli* levels at Tanyard Creek after a significant rainfall event at four time periods: 24, 48, and 72 hours prior to sampling, as well as cumulatively.

3.2 Primary Data Collection

Water samples from Tanyard Creek were collected weekly for approximately a year from Tanyard Creek Park/Ardmore Park, which contains the part of Tanyard Creek that is just downstream of the Tanyard Creek CSO. Ardmore Park is located off of Collier Road and is part of the Atlanta BeltLine. All water samples collected from the creek were brought to the lab for analysis of *E. coli* for each of ten sampling sites along an approximately mile-long stretch of Tanyard Creek.

Tanyard Creek starts at the Tanyard Creek CSO facility, which is on Loring Drive NW, slightly north of Atlantic Station and where I-75 and I-85 meet north of the city—then, Tanyard Creek goes under I-75, flowing north roughly adjacent to I-75. Starting at the Tanyard Creek CSO and continuing approximately 1 mile downstream, the creek flows through a large open concrete channel that is mostly surrounded by development on both sides. As the creek flows through this channel, it flows by condominium housing, a green area with tennis courts, more suburban housing, and then flows into a green space called Tanyard Creek Urban Forest, where the main concrete channel ends (site 1) and there is a natural creek bed as the water flows toward 2. Then, the creek begins to look more natural as it flows into sites 3, 3A, 4, and 5. Once Tanyard Creek crosses under the railroad trestle bridge, it is considered to be in Tanyard Creek Park/Ardmore Park, where we have sites 6 and 6A along the Beltline trail adjacent to the creek. Then, when the Beltline trail crosses over Tanyard Creek, we have sampling sites 7 and 8 on the upstream and downstream sides of the pedestrian bridge, where our last samples are taken. After this, Tanyard Creek merges with Spring Valley Creek and Springlake Creek, then flows through Bobby Jones Golf Course and eventually flows into Peachtree Creek, a tributary of the Chattahoochee River.

In order to analyze *E. coli* counts from water samples, Membrane Filtration (MF) is used to estimate bacterial populations in water that is low in turbidity (U.S. EPA Water Treatability Database). This method is especially useful for large sample volumes or for many daily tests. Using the membrane filter technique, the water sample is passed through the membrane filter, which has a pore size of 0.45 µm, while the vacuum suction is turned on, pulling all debris,
organisms, etc. onto the membrane paper, shown in Figure 3.21 and Figure 3.22. Any organisms in the sample are concentrated on the surface of the membrane paper. The membrane paper, with its trapped bacteria, is then placed in a petri dish containing BioRad RAPID’E. coli 2™ chromogenic medium. This agar medium provides direct enumeration of E. coli in water samples—it is designed for the simultaneous detection and enumeration of E. coli and total coliforms in water. BioRad RAPID’E. coli 2™ medium is based on detection of β-D-glucuronidase (GLUC) and β-D-galactosidase (GAL) activities. Coliforms (GAL+/GLUC-) form green colonies, whereas for E. coli (GAL+/GLUC+), the combined GAL and GLUC (pink) enzyme activities result in purple colonies, shown in Figure 3.23. After filtration, plates inverted with their lids on and incubated at 44.5°C for 18-24 hours. Purple colonies (E. coli) are counted and expressed as colony forming units (CFU). When the results are read, and the total number of colonies exceeds 200 per membrane or the colonies are too indistinct for accurate counting, it was reported as “too numerous to count” (TNTC). Water was usually filtered at volumes between 5 mL and 25 mL, with some days the water being turbid enough that water samples would be filtered at 1 mL (while also adding sterile DI water to make sure that all of the water sample was caught on the membrane paper).
Since the water samples were not in the standard CFU per 100mL format, a conversion formula was used in order to have usable data for analysis.

**Equation for calculating CFU per 100mL from each sampling date’s CFU’s per smaller water sample volumes (converting and then normalizing to log base 10)**

\[
\frac{\text{add up all colony counts from each viable sample}}{\text{add up total volume of water samples for each viable sample}} \times 100 = \text{______ CFU per 100 mL}
\]

After calculating the CFU per 100mL, the data was normalized by taking \( \log_{10}(\text{CFU per 100mL}) \), and this is how the *E. coli* data was presented used in the data and statistical analyses.

**3.3 Secondary Data Collection**

Rainfall amounts were retrieved from the National Weather Service website using the “NOWData - NOAA Online Weather Data” retrieval search tool for mean rainfall amounts (in inches) from the Atlanta-Hartsfield Intl Airport area for 24-, 48-, 72-hours prior to sampling (as well as cumulatively) by searching in the “daily data for a month” category. Some of the data had “trace” amounts of rain, where there was not enough rainwater to measure, but not zero, either. The National Weather Service website states that, “‘trace’ is a small amount of precipitation that will wet a rain gage but is less than the 0.01-inch measuring limit.” These “trace” observations had to be removed (as well as their corresponding average *E. coli* count for that particular sampling date) from the data in order to conduct the linear regression analyses.

**3.4 Data Analysis**

Data analysis was conducted using Microsoft Excel to organize, sort, compile, and normalize the data. Graph Pad Prism 5 as well as Microsoft Excel were used to make graphs. For multiple sites and dates, Graph Pad Prism calculated an average count of *E. coli* for each site.
Box and whisker plots are used to understand the variability, spread, and trends of *E. coli* as well as the central tendency. The means of each box and whisker plot are indicated by a “+” in the box for each sampling date or site. The primary focus of the data analysis will be the comparison of *E. coli* amounts for sites by date (temporally) and for dates by site (spatially). Graph Pad Prism was also used to make dual y-axis graphs showing *E. coli* counts on the left y-axis and rainfall amounts on the right y-axis for the four time periods being investigated. Red lines were added to the graphs indicating the minimum level at which the EPA considers *E. coli* levels to be impaired, which is approximately 2.1 log$_{10}$CFU/100mL. This was added to as a reference to compare the level of impairment Tanyard Creek experiences to that of the standard for primary contact in recreational waters. For all graphs with dates on the x-axis, colored vertical lines were added to denote the different seasons for trends Microsoft Excel was used to make scatterplots with a line of best fit, equation, and R$^2$ value to explore potential linear relationships for 24-, 48-, 72-hour, and cumulative rainfall amounts and how each related to the corresponding average *E. coli* count for each particular sampling date.

### 3.5 Statistical Analysis

Statistical analysis was conducted using Graph Pad Prism to perform two one-way analysis of variance tests to generate ANOVA tables in order to assess relationships and potential significance ($\alpha = 0.05$) among *E. coli* counts between all sampling sites by date and then between all dates by site. The $p$-values for each ANOVA test is outline in a red box in the table output. Also, the Microsoft Excel Data Analysis Toolpak was used for correlation and the linear regression analysis to assess potential relationships and significance ($\alpha = 0.05$) between rainfall amounts and average *E. coli* counts for 24, 48, and 72 hours prior to sampling as well as cumulatively. The correlation coefficient and $p$-value for each time period analyzed are outlined in a red box in the table output.
CHAPTER IV – RESULTS

4.1 *E. coli* Trends Spatially and Temporally

Figure 4.11 All sites by date (with mean *E. coli* log$_{10}$CFU per 100mL)

*Figure 4.11* shows the temporal trends of *E. coli* in Tanyard Creek for all sampling sites by date. There is a spike in *E. coli* levels during the summer season (June-September), while the spring season (March-May) also shows very high levels of *E. coli*, both seasons with the majority of data averaging much higher than the EPA’s recreational water quality standard for an acceptable level of *E. coli* of 126 CFU/100mL, or 2.1 log$_{10}$CFU/100 mL, indicated by the horizontal red line. High levels of *E. coli* were also observed in the fall after Hurricane Michael (10/7/2018-10/16/2018), also higher than the EPA’s recreational water quality standard. There is substantial variability in the mean and median *E. coli* from week to week. In general, the *E. coli* levels tend to range between 1 and 4 log$_{10}$CFU/100 mL, and are above the EPA’s recreational water quality standard for a majority of sampling dates.
Table 4.11. ANOVA Table investigating temporal relationship (sites by date)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (between columns)</td>
<td>157.7</td>
<td>47</td>
<td>3.356</td>
<td>24.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Residual (within columns)</td>
<td>54.59</td>
<td>393</td>
<td>0.1389</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>212.3</td>
<td>440</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.11 shows the output from the one-way ANOVA test that was conducted to determine if there were significant differences in average *E. coli* counts from all sampling sites by date. This ANOVA test was conducted with 95% confidence (\( \alpha = 0.05 \)), which means that a \( p \)-value less than 0.05 is statistically significant. \( P < 0.0001 \), which means that average (arithmetic mean) *E. coli* counts differ significantly by date.

Figure 4.12 All dates by site (with mean *E. coli* log\(_{10}\)CFU per 100mL)

Figure 4.12 shows the spatial trends in *E. coli* from site to site for all sampling dates at Tanyard Creek. The plot shows that all sites are impaired, with their mean and median *E. coli* levels all being higher than the EPA’s recreational water quality standard. Although there is not much difference in mean and median *E. coli* from site to site, the plot shows that site 4 may have slightly higher means than other sites, but it is not statistically significant. Site 4 is the location of a beaver dam, which causes the flow of the creek to slow in this area.
Table 4.12. ANOVA Table investigating spatial relationship (dates by site)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment (between columns)</td>
<td>7.163</td>
<td>9</td>
<td>0.7959</td>
<td>1.672</td>
<td>0.0934</td>
</tr>
<tr>
<td>Residual (within columns)</td>
<td>205.1</td>
<td>431</td>
<td>0.4760</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>212.3</td>
<td>440</td>
<td>0.4760</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.12 shows the output from the one-way ANOVA test that was conducted to determine if there were significant differences in average E. coli counts from all dates by site (spatially). This ANOVA test was conducted with 95% confidence (α = 0.05), which means that a p-value less than 0.05 is statistically significant. P=0.0934, which means that average E. coli counts do not differ significantly by site.

4.2 E. coli Trends Related to Weather Patterns

Figure 4.21a All sampling dates with average E. coli and *24-hour rainfall

*24-hour rainfall refers to the rainfall for an entire 24 hours before the sampling date

Figure 4.21a shows E. coli counts (average of all sites on each date) and 24-hour rainfall by sampling date. 24-hour rainfall refers to the rainfall for an entire 24 hours before the sampling
date. There are two lines on the graph depicting the trends in *E. coli* throughout the year, in purple, and another line on the graph (in orange) depicting trends in 24-hour rainfall prior to the sampling date. *E. coli* counts are highest in the spring and fall, as shown in Figure 4.11, with the same red line marking the standard for maximum allowable *E. coli* for recreational waters by the EPA. Visual inspection suggests that 24-hour rainfall does not seem to follow the same trends as *E. coli* levels. Rainfall has a number of zero values; *E. coli* was always measurable at every site.

Figure 4.21b Scatterplot and line of best fit for 24-hour rainfall and average *E. coli* with equation, R² value, correlation coefficient *r*, and *p*-value

![Scatterplot and line of best fit](image)

Table 4.21 Correlation and Linear Regression Analysis Output for 24-hour rainfall

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Correlation Statistics</th>
<th>24 hour rainfall (inches)</th>
<th>Average E. coli log₁₀ CFU/100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R</td>
<td>0.308127922</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Square</td>
<td>0.094942816</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted R Square</td>
<td>0.071736222</td>
<td>24 hour rainfall</td>
<td>1</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.576361916</td>
<td>Average E. coli log₁₀ CFU/100 mL</td>
<td>0.308127922</td>
</tr>
<tr>
<td>Observations</td>
<td>41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th>Regression</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Significance F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>1.359068199</td>
<td>1.359068199</td>
<td>4.091199883</td>
<td>0.0500002097</td>
</tr>
<tr>
<td>Residual</td>
<td>39</td>
<td>12.95552925</td>
<td>0.332193058</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>14.31459745</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Coefficients**

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.718042398</td>
<td>0.097596501</td>
<td>27.84979351</td>
<td>2.37671E-27</td>
<td>2.520634842</td>
<td>2.915449955</td>
<td>2.915449955</td>
</tr>
<tr>
<td>24 hour rainfall</td>
<td>0.382877805</td>
<td>0.189293125</td>
<td>2.022671472</td>
<td>0.0500002097</td>
<td>-3.6814E-06</td>
<td>0.765759291</td>
<td>0.765759291</td>
</tr>
</tbody>
</table>

**Figure 4.21b** is a scatterplot showing 24-hour rainfall and average *E. coli*. **Table 4.21** shows the correlation and linear regression analysis. This table shows that the correlation coefficient for
24-hour rainfall and average *E. coli* is 0.308. Also, this table shows the linear regression analysis which confirms that there is not a statistically significant relationship between 24-hour rainfall prior to sampling and average *E. coli* because the $P=0.05$.

**Figure 4.22a All sampling dates with average *E. coli* and *48-hour rainfall***

*48-hour rainfall refers to the rainfall for an entire 48 hours before the sampling date*

**Figure 4.22a** shows *E. coli* counts (average of all sites for that date) and 48-hour rainfall. 48-hour rainfall refers to the rainfall for an entire 48 hours before the sampling date. There are two lines on the graph depicting the trends in *E. coli* throughout the year, in purple, and another line on the graph (in pink) depicting trends in 48-hour rainfall prior to the sampling date. *E. coli* counts are highest in the spring and fall, as shown in **Figure 4.11**, with the same red line marking the EPA recreational water standard. Visual inspection does not suggest that 48-hour rainfall and *E. coli* levels follow the same trends.
Figure 4.22b Scatterplot and line of best fit for 48-hour rainfall and average *E. coli* with equation, $R^2$ value, correlation coefficient $r$, and $p$-value

![Figure 4.22b Scatterplot and line of best fit](image)

$y = 0.1277x + 2.7017$

$R^2 = 0.0197$

$r = 0.1401$

$p$-value = 0.353

**Table 4.22 Correlation and Linear Regression Analysis Output for 48-hour rainfall**

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Correlation Statistics</th>
<th>48 hour rainfall (inches)</th>
<th>Average $E. coli$ log$_{10}$ CFU/100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R</td>
<td>0.140188445</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Square</td>
<td>0.0196528</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted R Square</td>
<td>-0.002627818</td>
<td>48 hour rainfall</td>
<td>1</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.608765181</td>
<td>Average $E. coli$ log$_{10}$ CFU/100 mL</td>
<td>0.140188445</td>
</tr>
<tr>
<td>Observations</td>
<td>46</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>Significance F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>0.326886368</td>
<td>0.326886368</td>
<td>0.882058116</td>
<td>0.352705969</td>
</tr>
<tr>
<td>Residual</td>
<td>44</td>
<td>16.30613202</td>
<td>0.370595046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>16.6330839</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Coefficients**

<table>
<thead>
<tr>
<th></th>
<th>Standard Error</th>
<th>t Stat</th>
<th>$P$-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.7017766562</td>
<td>0.094941554</td>
<td>28.45573417</td>
<td>5.68863E-30</td>
<td>2.510384433</td>
<td>2.893068691</td>
<td>2.510384433</td>
</tr>
<tr>
<td>48 hour rainfall</td>
<td>0.127680817</td>
<td>0.135949325</td>
<td>0.939179491</td>
<td>0.352705969</td>
<td>-0.146307043</td>
<td>0.401688678</td>
<td>-0.146307043</td>
</tr>
</tbody>
</table>

**Figure 4.22b** is a scatterplot showing 48-hour rainfall and average *E. coli*. **Table 4.22**, which shows the correlation and linear regression analysis, shows that the correlation coefficient for 48-hour rainfall and average *E. coli* is 0.140. Also, this table shows the linear regression analysis which confirms that there is not a statistically significant relationship between 48-hour rainfall prior to sampling and average *E. coli* because $P=0.353$. 

---

Table 4.2
Figure 4.23a All sampling dates with average *E. coli* and *72-hour rainfall*
*72-hour rainfall refers to the rainfall for an entire 72 hours before the sampling date*

Figure 4.23a shows average *E. coli* counts and 72-hour rainfall. 72-hour rainfall refers to the rainfall for an entire 72 hours before the sampling date. There are two lines on the graph depicting the trends in *E. coli* throughout the year, in purple, and another line on the graph (in black) depicting trends in 72-hour rainfall prior to the sampling date. *E. coli* counts are highest in the spring and fall, as shown in Figure 4.11, with the same red line marking the level of impairment accepted for recreational waters by the EPA. Visual inspection suggests that 72-hour rainfall and *E. coli* levels do not follow the same trends.
Figure 4.23b Scatterplot and line of best fit for 72-hour rainfall and average *E. coli* with equation, $R^2$ value, correlation coefficient $r$, and $p$-value

![Scatterplot with line of best fit](image)

Table 4.23 Correlation and Linear Regression Analysis Output for 72-hour rainfall

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Correlation Statistics</th>
<th>72 hour rainfall (inches)</th>
<th>Average <em>E. coli</em> log$_{10}$ CFU/100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R</td>
<td>0.014302327</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Square</td>
<td>0.000204557</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted R Square</td>
<td>-0.025431224</td>
<td>72 hour rainfall</td>
<td>1</td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.629510904</td>
<td>Average <em>E. coli</em> log$_{10}$ CFU/100 mL</td>
<td>-0.01430233</td>
</tr>
<tr>
<td>Observations</td>
<td>41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>$F$</th>
<th>Significance $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>0.003162174</td>
<td>0.0003162174</td>
<td>0.007979338</td>
<td>0.929279091</td>
</tr>
<tr>
<td>Residual</td>
<td>39</td>
<td>15.45511638</td>
<td>0.396295297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>15.45867876</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>P-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.797864585</td>
<td>0.115447121</td>
<td>24.2350313</td>
<td>4.02615E-25</td>
<td>2.564350742</td>
<td>3.031378427</td>
<td>2.564350742</td>
</tr>
<tr>
<td>72 hour rainfall</td>
<td>-0.018830754</td>
<td>0.210806636</td>
<td>-0.089327138</td>
<td>-0.029279091</td>
<td>-0.445227421</td>
<td>0.407565914</td>
<td>-0.445227421</td>
</tr>
</tbody>
</table>

Figure 4.23b is a scatterplot showing 72-hour rainfall and average *E. coli*. Table 4.23 shows the correlation and linear regression analysis. This table shows that the correlation coefficient for 72-hour rainfall and average *E. coli* is -0.014. Also, this table shows the linear regression analysis which confirms that there is not a statistically significant relationship between 72-hour rainfall prior to sampling and average *E. coli* because $P=0.929$. 

28
Figure 4.24a All sampling dates with average *E. coli* and *cumulative rainfall*

*Cumulative rainfall refers to the aggregate amount of rainfall collected 24, 48, and 72 hours before sampling.

Figure 4.24a shows average *E. coli* counts and cumulative rainfall. Cumulative rainfall refers to the aggregate amount of rainfall collected 24, 48, and 72 hours before sampling. There are two lines on the graph depicting the trends in *E. coli* throughout the year, in purple, and another line on the graph (in blue) depicting trends in cumulative rainfall prior to the sampling date. *E. coli* counts are highest in the spring and fall, as shown in Figure 4.11, with the same red line marking the level of impairment accepted for recreational waters by the EPA. Visual inspection suggests that cumulative rainfall and *E. coli* levels do not follow the same trends.
Figure 4.24b Scatterplot and line of best fit for cumulative rainfall and average *E. coli* with equation, $R^2$ value, correlation coefficient $r$ and $p$-value

![Scatterplot and line of best fit for cumulative rainfall and average E. coli](image)

$$y = 0.1694x + 2.6318$$

$R^2 = 0.0669$

$r = 0.259$

$p$-value = 0.0823

Table 4.24 Correlation and Linear Regression Analysis Output for cumulative rainfall

<table>
<thead>
<tr>
<th>Regression Statistics</th>
<th>Correlation Statistics</th>
<th>Cumulative rainfall (inches)</th>
<th>Average E. coli log$_{10}$ CFU/100 mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R</td>
<td>0.256127643</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R Square</td>
<td>0.065601369</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted R Square</td>
<td>0.044836955</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.602942257</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Observations</td>
<td>47</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.258650682</td>
<td>1</td>
</tr>
</tbody>
</table>

**ANOVA**

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>$SS$</th>
<th>MS</th>
<th>$F$</th>
<th>Significance $F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>1</td>
<td>1.148536152</td>
<td>1.148536152</td>
<td>3.159317149</td>
<td>0.082253498</td>
</tr>
<tr>
<td>Residual</td>
<td>45</td>
<td>16.35927146</td>
<td>0.363539366</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>17.50780761</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coefficients</th>
<th>Standard Error</th>
<th>t Stat</th>
<th>$P$-value</th>
<th>Lower 95%</th>
<th>Upper 95%</th>
<th>Lower 95.0%</th>
<th>Upper 95.0%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.633668164</td>
<td>0.107178731</td>
<td>24.57267531</td>
<td>1.02893E-27</td>
<td>2.417799118</td>
<td>2.849537209</td>
<td>2.417799118</td>
</tr>
<tr>
<td>Cumulative rainfall</td>
<td>0.168846225</td>
<td>0.094768645</td>
<td>1.777446806</td>
<td>0.082253498</td>
<td>-0.022427624</td>
<td>0.359320074</td>
<td>-0.022427624</td>
</tr>
</tbody>
</table>

**Figure 4.24b** is a scatterplot showing cumulative rainfall and average *E. coli*. **Table 4.24** shows the correlation and linear regression analysis. This table shows that the correlation coefficient for cumulative rainfall and average *E. coli* is 0.259. Also, this table shows the linear regression analysis which confirms that there is not a statistically significant relationship between cumulative (24, 48, and 72 hours altogether) rainfall prior to sampling and average *E. coli* because the $p$-value is shown to be 0.0822.
CHAPTER 5 – DISCUSSION & CONCLUSION

5.1 Overall Discussion Overview

The purpose of this study was to evaluate the spatial and temporal patterns of \textit{E. coli} in Tanyard Creek and to determine if \textit{E. coli} levels are related to rainfall events. As stated earlier, \textit{E. coli} is a very common bacteria that is found in the digestive system of humans and warm-blooded animals, making it an indicator of the presence of fecal contamination from humans and animals. Some possible sources of fecal contamination in an urban creek similar to Tanyard Creek include: agricultural runoff, runoff from impervious surfaces (roads, highways, etc.) due to roadkill, wildlife that uses the creek as part of their natural habitat (deer, birds, squirrels, etc.), and wastewater treatment plant overflows. Heavy precipitation may cause microorganisms like \textit{E. coli} to be washed into creeks, rivers, streams, lakes, or ground water (Olds et al. 2018, U.S. EPA 2012). The types of illnesses and diseases acquired from contact with contaminated water can cause gastrointestinal (GI) illness, as well as skin, ear, respiratory, and wound infections (U.S. EPA 2012). Some of the most commonly reported symptoms are stomach cramps, diarrhea, nausea, vomiting, and a low-grade fever (U.S. EPA 2012). When \textit{E. coli} levels exceed the permissible level in recreational water, it results in the closing of beaches, ponds, lakes, and other swimming and fishing areas (U.S. EPA 2012, Wade et al. 2008). This acceptable level of \textit{E. coli} in recreational water is determined by risk analysis based on statistics to protect human health, and is shown in Table 2.1 (U.S. EPA 2012). At this time, Tanyard Creek is not considered to be a recreational water area.

5.2 Discussion of Research Questions

The aim of this study was to investigate spatial and temporal trends in \textit{E. coli} and to determine if there were relationships between \textit{E. coli} levels and rainfall 24, 48, and 72 hours prior to sampling, as well as cumulatively. Overall, the data showed that the levels of \textit{E. coli} in Tanyard Creek are much higher than the permissible level for recreational waters designated by the EPA, indicating that Tanyard Creek is not fit for primary contact recreation at this time. Temporally, \textit{E. coli} in Tanyard Creek does show some trends. First, \textit{E. coli} in Tanyard Creek differs significantly when comparing all mean \textit{E. coli} levels between all sites by date. Next, \textit{E. coli} levels differ throughout the year, with summer having the highest \textit{E. coli} levels, spring the second highest, followed by fall and winter, respectively. Lastly, \textit{E. coli} levels throughout the entire year show a pattern of still being impaired with respect to the EPA’s recreational water quality standards. Spatially, \textit{E. coli} in Tanyard Creek does not differ significantly when comparing all mean \textit{E. coli} levels for all dates by site. All sampling sites at Tanyard Creek show a mean \textit{E. coli} level higher than the permissible level of \textit{E. coli} in recreational waters. For all rainfall time periods—24, 48, and 72 hours prior to sampling, as well as cumulatively, there was not a statistically significant relationship between average \textit{E. coli} and rainfall before sampling. This may have been due to not having enough statistical power in the analysis. Across all
sampling dates, E. coli levels show substantial variation, but tend to cluster between 1 and 4 log_{10} CFU/100 mL.

5.3 Study Strengths and Limitations

This main strength of this study is that data had been gathered for an entire year; so, there was a year’s worth of data to analyze, and this was beneficial for observing trends for different seasons of the year.

The main limitation in this study was that plates that had E. coli colonies in quantities too numerous to count (TNTC) were not helpful at all because it is not quantitative data that can be used in making graphs and conducting statistical analyses. Another limitation was that rainfall data from the National Weather Service had observations that were ‘trace’, when the rainfall was wet enough to wet the rain gauge, but still less than the 0.01-inch mark. This requires that the data be analyzed to take into account zero values.

5.4 Implications of Findings

Although Tanyard Creek is not considered a recreational water area and has signage warning not to swim in the creek, in reality it may be used as a recreational area by users of the park. The park is a popular recreation area heavily used for walking and running the trails. Many people bring dogs, which they sometimes allow to get in the creek. People are also occasionally spotted in the creek itself. Since this study has found Tanyard Creek to be impaired throughout an entire year, which could potentially lead to illness and disease, actions must be taken to mitigate the contamination caused by storm and sewer overflows.

5.5 Environmental Impact

The current EPA recommendations for body-contact recreation (swimming, wading, etc.) is fewer than 200 CFU per 100 mL and for fishing it is fewer than 1000 CFU per 100 mL (U.S. EPA 2012). The entire year of E. coli data presented in this study consistently shows that Tanyard Creek exceeds these recommendations, further indicating the magnitude of impairment of this urban creek.

5.6 Interventions and Solutions

The best short-term solution for Tanyard Creek would be preventing it from becoming even more contaminated than it is currently. First, this could be achieved through contacting the local government agency responsible for the pollution and ensuring that the EPA’s water quality standards are being met. Next, the trash and physical pollution in the creek needs to be cleaned out and disposed of properly. This could be accomplished by incorporating the community via local clean-up groups, high school extracurricular activities, and even college-level programs like experience courses, capstones, or thesis projects.
The best long-term solution for the contamination and pollution in Tanyard Creek would be updating the city of Atlanta’s combined sewer overflow disinfection systems. However, this would need substantial funding from the local governments for a complete remodeling of the combined sewer overflows (CSO) with the best equipment for disinfecting water before releasing it into public waterways.

5.7 Recommendations

The water in Tanyard Creek directly comes from the Tanyard Creek CSO. However, the city of Atlanta’s Department of Watershed Management does not publish release dates or volumes for the water being discharged from CSOs for public domain. It would benefit the public to have this data readily available for analysis in research projects similar to this one because it would allow more questions to be asked and answered relating to trends in water flow, rainfall, and *E. coli* levels.

5.8 Next Steps

Next steps for this project at Tanyard Creek are incorporating MacConkey agar medium into the membrane filtration assay to determine if antibiotic-resistant bacteria is present in the creek and its magnitude and collecting data from the stream flow logger installed between sites 6A and 7 to determine if there are relationships between stream flow volumes, rainfall, and *E. coli* levels.

5.9 Conclusions

The data in this study indicates that there is a trend among the water samples on a temporal level. During the hottest months of the year, the summer, from June-September, are when the highest levels of *E. coli* are observed, and they were much high than the EPA’s recreational water quality criteria. When data was presented on a spatial level, there were not statistically significant differences in the levels of *E. coli* observed between sampling sites, but all sites had average *E. coli* levels that were very high and above the EPA’s recommended recreational water quality criteria. In conclusion, Tanyard Creek is impaired due to combined sewer overflows (CSOs). Further studies are needed to investigate how stream flow volume from flow loggers and data from the CSO could impact *E. coli* levels and to identify trends in antibiotic-resistant bacteria in Tanyard Creek, as well as determining if rainfall and stream flow volume are related to the presence and magnitude of antibiotic-resistant bacteria.
REFERENCES


Pandey et al.: Contamination of water resources by pathogenic bacteria. AMB Express 2014 4:51.


Hannah-Leigh Crawford  
MPH Thesis


**Websites & Images:**

