

Investigating Sources of *E. coli* Using Emerging Contaminants in Choccolocco Creek, AL

by

Eleanore Alexandra Larson

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
December 10, 2022

Keywords: *E. coli*, emerging contaminants, principal component analysis, water quality

Copyright 2022 by Eleanore A. Larson

Approved by

Dr. Ann S. Ojeda, Chair, Assistant Professor, Geosciences

Dr. Stephanie Rogers, Assistant Professor, Geosciences

Dr. Matthew Waters, Associate Professor, Crop, Soil, and Environmental Sciences

Abstract

Pathogen loading, specifically high *Escherichia coli* (*E. coli*) concentrations, is a major cause for poor surface water quality worldwide. Many rivers and streams are used recreationally, and contamination poses a communal health risk. This study focused on a mixed-use watershed with agricultural, urban, and rural areas that has a history of poor water quality in central Alabama. Our goal was to track fecal contamination within the watershed and determine if anthropogenic input played a significant role in *E. coli* loading. To test this, we collected water samples at nine sites and quantified *E. coli*, pharmaceutical and personal care products (PPCPs), major and minor metals, nutrients, and other water quality parameters over 6-months. We utilized the multi-proxy dataset with geospatial context to assess the relationships between land-use and water contamination.

PPCPs were frequently detected over the recreational period, including acetaminophen (13% of samples), caffeine (50%), salicylic acid (25%), sucralose (38%), and Bisphenol A (13%). We found a strong, environmentally significant correlation between *E. coli* concentrations and the total concentration of all PPCPs ($r = 0.5075$; $p\text{-value} = 0.0448$) and the total number of compounds ($r = 0.5339$; $p\text{-value} = 0.0331$). Other significant variables that were correlated with *E. coli* included water temperature, pH, and average total phosphorous. Finally, the multiproxy data was interpreted in a geographic context to understand controls from land-use and the built environment. Sample sites were categorized into pristine, agricultural/rural development, and urban development and enrichment factors were calculated for each site. The agricultural/rural development sites had elevated total PPCP concentrations (3.58, 2.28, and 1.78 ng/L) compared to the pristine site (1) which may be due to a high density of septic systems and low density of municipal sewer infrastructure in the region. Malfunctioning septic systems could contribute to the increased *E. coli* and PPCP in these areas and our combined results support that idea pointing to a significant anthropogenic loading of *E. coli*, particularly in agricultural/rural regions of the watershed. In a larger context, our results suggest that malfunctioning septic systems may play a largely unrecognized role in contamination of surface water in mixed land-use watersheds.

Acknowledgements

First, I would like to express deep appreciation to my committee chair Dr. Ann Ojeda for her support of me during this thesis. Her mentorship has inspired me through all the challenges we faced in research and life. Her fervor for research and impacting the community is evident in every facet of her work. I am grateful for my time learning and growing under her guidance.

Secondly, I would like to thank my committee members, Dr. Stephanie Rogers and Dr. Matt Waters for their guidance throughout this project. Their collaboration helped shape the project and provided new perspectives on our work.

This project and its dissemination would not be possible without the financial support of the following grant programs: Alabama Water Resource Research Institute, AU Geology Advisory Board, and AU College of Sciences and Mathematics. Thank you for your support.

I must acknowledge the entire Ojeda lab group for their contributions and support of this project. Sidney Millner was a hard-working field technician and friend. Her help made fieldwork fun and lab work manageable. Thank you Dr. Natalia Malina for being a wealth of knowledge in the lab and assisting in the development of the methodology for this project. Her many skills were indispensable for the lab running smoothly.

Finally, thank you to my friends who felt like family. After a long day of work, coming home to Arielle and Michael helped me unwind and find balance. My favorite felines, Roary, Juniper, and Pongo, provided endless entertainment and love. Within the department, I could not have asked for better officemates. Caitlyn and Mallory were always quick to lend a helping hand or moral support whenever there were challenges. Thank you for all the fun times and laughs we shared.

I dedicate this thesis to the loved ones I lost during these last two years. Aunt Regina, Uncle Dan, Aunt Sheila, Grandma Ruth, & Nana Sandy – thank you for always cheering me on.

Table of Contents

Abstract 2
Acknowledgements 3
1. Introduction 9
2. Methodology 15
 2.1 Study Site 15
 2.2 Water sampling 15
 2.3 Water Quality Parameters 16
 2.4 Pathogens 16
 2.5 Trace Metals 17
 2.6 Emerging Contaminants 17
 2.7 Statistical Analysis 19
 2.8 Geographic Data 20
 2.9 Weather Data 21
3. Results 21
 3.1 Trends in *E. coli* Across the Watershed 21
 3.2 Correlations Between *E. coli* and Other Variables 22
 3.3 Principal Component Analysis of All Data 25
 3.4 Assessing Holistic Variable Dynamics Across Site Groupings 28
 3.5 Emerging Contaminants Classification 30
 3.6 Detection Frequency 32
 3.7 Principal Component Analysis of Emerging Contaminants 34
 3.8 Heat Map of Source Specific Variables 38
4. Discussion 40
 4.1 Relationships between *E. coli* and Water Quality Parameters 40
 4.2 Trends Across Watershed Groups 42
 4.3 Potential Sources of *E. coli* 44
 4.4 Limitations 46
5. Conclusion 47

List of Tables

Table 1. Pearson and Spearman correlative statistics between measured variables and <i>E. coli</i> .	14
Table 2. Principal component analysis calculated from <i>E. coli</i> and water quality variables	24
Table 3. Classification of the EC compounds based on sources and use	30
Table 4. Principal component analysis calculated from <i>E. coli</i> and EC	34

List of Figures

Figure 1. Study site map of Choccolocco Watershed	13
Figure 2. Box and whisker of <i>E. coli</i> concentration.....	20
Figure 3. Principal component analysis of <i>E. coli</i> and all variables	25
Figure 4. Principal component scores of <i>E. coli</i> and all variables.....	26
Figure 5. Principal component biplot of <i>E. coli</i> and all variables.....	27
Figure 6. Heat map of <i>E. coli</i> and water quality variables.....	29
Figure 7. Box and whisker plot of EC compounds and frequency	32
Figure 8. Principal component analysis of <i>E. coli</i> and EC	35
Figure 9. Principal component scores of <i>E. coli</i> and EC	36
Figure 10. Principal component biplot of <i>E. coli</i> and EC.....	37
Figure 11. Heat map of <i>E. coli</i> and source-specific variables.....	38

List of Abbreviations

ANOVA	Analysis of Variance
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
CaRT	Classification and Regression Tree
CI	Confidence Interval
DEA	Diethanolamine
DEET	Diethyltoluamide
DI	Deionized
DIA	Deisopropylatrazine
DO%	Dissolved Oxygen percentage
EC	Emerging Contaminants
<i>E. coli</i>	<i>Escherichia Coli</i>
EPA	Environmental Protection Agency
fDOM	fluorescent Dissolved Organic Matter
FIB	Fecal Indicator Bacteria
GC-MS	Gas Chromatography – Mass Spectrometry
MLR	Multiple Linear Regression
ORP	Oxidation Reduction Potential
OUST	Sulfometuron, methyl
PCA	Principal Component Analysis
PC#	Principal Component 1, 2, 3,...
PPCP	Pharmaceuticals and Personal Care Products
SIM	Selected Ion Monitoring

SPE	Solid Phase Extraction
SPW	Sample Point Watersheds
TCEP	Tris(2-carboxyethyl) phosphine
TCPP	Tris(2-chloroisopropyl) phosphate
UV	Ultraviolet

1. Introduction

Fecal waste contamination is a threat to water quality and public health. Wastewater can include bacteria, protozoa, and viruses that could cause sickness (Naidoo and Olaniran, 2013). Diseases and sickness caused by fecal contamination is especially dangerous for young children, pregnant women, and the elderly. Frequently, fecal waste contamination is evaluated by measuring fecal indicator bacteria (FIB) like *Escherichia coli* (*E. coli*). *E. coli* ingestion can cause abdominal sepsis, septicemia, urinary tract infections, and newborn meningitis (Jang et al., 2017).

As part of the Clean Water Act, state agencies like the Alabama Department of Environmental Management (ADEM) and the Environmental Protection Agency (EPA) set thresholds for the concentration *E. coli* in recreational water to be protective of human health. When a waterbody does not meet these criteria, steps are taken to reduce *E. coli* loading in the watershed. For example, if a surface waterbody routinely does not meet water quality criteria, then the waterbody is placed on the 303(d) list of impaired waterbodies and a Total Maximum Daily Load (TMDL) is developed to account for and manage pollutant loading. The TMDL allocates pollutant loading, for example *E. coli*, from point-sources (e.g., wastewater treatment plants, stormwater discharges, contained feeding operations) and non-point sources (e.g., agricultural or urban runoff). Based on the TMDL, strategies are developed and implemented to reduce pollutant loading from these sources. However, tracking the source of *E. coli* in water systems is difficult because it is not a source-specific marker for fecal waste. *E. coli* can originate from multiple point and non-point sources of pollution within a watershed. For example, human sources of *E. coli* include leaky sewer lines and/or malfunctioning septic tanks and wastewater treatment plants (Buerge et al., 2006; Cogger 1988; Paul et al., 2004; Richards et al., 2016; Watkinson et al., 2007). Wildlife, cattle and farm animals, and naturalized *E. coli*, or *E. coli* naturally occurring in the

sediment, also contribute to water contamination (An and Breindenbach, 2005; Coleman et al., 2013; Hansen et al., 2020; Ishii 2006; Somarelli et al., 2007). Together, these factors make tracking wastewater input difficult using *E. coli* and other FIB.

Chemical source tracking is an improved method to track the source of fecal waste in water (Staley et al., 2016). Chemical source tracking measures a suite of chemical compounds and relates them to FIB concentrations to understand sources of FIB in a watershed. Chemical tracers can include traditional water quality parameters such as dissolved oxygen, pH, conductivity, nitrate, and ammonium or more complex organic compounds like emerging contaminants (EC) that are highly source specific. There is strong evidence that these emerging contaminants including caffeine, ibuprofen, and acetaminophen, are reliable human proxies and can be used to distinguish sources of human versus animal waste (Buerge et al., 2006; Jones-Lepp, 2006; Kumar et al., 2019; Leal-Bautista et al., 2011; Luo et al., 2014). Other ECs include herbicides and pesticides like atrazine and metolachlor. These compounds are often detected in surface waters closely associated with agricultural lands (Brovini et al., 2021; Carazo-Rojas et al., 2018; de Souza et al., 2020; Glinski et al., 2018; Mukhopadhyay et al., 2022; Wilkinson et al., 2022).

As discussed previously, establishing the source of *E. coli* in water bodies is especially important because it is often an indicator of sewage-impacted waters. When functioning properly, the infrastructure to manage sewage as wastewater collection systems (e.g., sewer pipelines), wastewater treatment plants (WWTPs) and onsite wastewater treatment systems, most frequently as septic systems, effectively transport and treat fecal waste. However, malfunctioning collection or treatment systems can be a source of untreated or undertreated water in the environment (Buerge et al., 2006; Cogger 1988; Paul et al., 2004; Richards et al., 2016; Watkinson et al., 2007).

Previous studies have attempted to establish markers for distinguishing different sources of human waste in surface water. The water quality of WWTP effluent is regulated through the National Point Discharge Elimination System (NPDES). The monthly discharge reports contain information about *E. coli* concentrations and other regulated water quality variables in the effluent. The concentration of ECs is not measured or reported in municipal discharge monitoring reports (City of Oxford DMR, 2021), but WWTPs process high volumes of wastewater that are laden with ECs. Several studies have evaluated the efficacy of WWTP processes in EC attenuation. For example, WWTPs eliminate ~99% of caffeine and acetaminophen, however, the influent concentrations are so high that these compounds are still detectable in the effluent in the ng/L to ug/L range (Buerge et al., 2006; Lin et al., 2010). This creates a spike in overall EC concentrations in the surface water to which the effluent is discharged (Tran et al., 2018).

Untreated or undertreated human waste from a malfunctioning septic system, sewer overflows, or leaking sewer mains is more difficult to track. Septic systems are classified as a non-point source of pollution since they do not require a NPDES permit and are maintained and managed by the resident. The fate and transport of pollutants like microorganisms and ECs to surface water is subject to sorption and biodegradation, before eventual discharge to surface water (Sui et al., 2015). Even so, residential septic systems can have a unique chemical fingerprint (Richards et al., 2016; Richards et al., 2017). The anaerobic environment typically causes the bioconversion of nitrogen (N) to ammonium nitrogen ($\text{NH}_4\text{-N}$) (Lusk et al., 2018). High concentrations of phosphates in sewage are a result of household cleaners and fecal materials (Lusk et al., 2018). Similarly, septic systems are often treated with copper sulfate, a common pesticide, herbicide, and fungicide used to kill encroaching tree roots in septic systems. For these reasons, elevated *E. coli* concentration with elevated $\text{NH}_4\text{-N}$, phosphorous (P) and copper (Cu) in surface

water could indicate that the source is septic tank discharge, as they have been shown to be enriched in septic tank effluent (Richards et al., 2016). Other parameters including total suspended solids, turbidity, and artificial sweeteners like sucralose have also been shown to have high enrichment factors in septic tank effluent (Richards et al., 2017).

Sewer collection systems can introduce pollutants to surface water through leaky pipes or sewer system overflows (SSOs). SSOs are the more apparent mechanism for water impairment, and these events are tracked and reported through state agencies like ADEM. Even so, the pollutant loading is poorly understood primarily due to the intermittent nature and sampling difficulties surrounding SSO events (Petrie, 2021). Leaking sewer systems are even more difficult to track, but the fate and transport considerations (sorption and biodegradation) mechanisms are similar to septic systems. In contrast, sewer collection systems carry much higher volumes of wastewater and therefore higher loading potentials compared to leaking septic systems. Therefore, leaking sewer mains likely create a diffuse source to the surface water through groundwater discharge (Tran et al., 2013).

Variations in loading, fate, and transport create more difficulties in untangling the socioenvironmental and physiochemical controls on EC occurrence. ECs also transform in the surface waterbody through sorption, biodegradation and photodegradation in natural systems (Lin et al., 2010). However, the complexity and uniqueness of the socioenvironmental settings (e.g., water treatment technologies allowing for different degrees of attenuation, EC source variation and concentrations, fluctuations in seasonal sunlight that control photodegradation) make it difficult to model and extrapolate results from one study or study site to another.

More recently, statistical models have been introduced to understand relationships between EC concentrations, FIB, and other socio-environmental factors controlling their occurrence.

ANOVA tests and Pearson/Spearman correlations can be used to define significant relationships between environmental factors, EC concentrations, and *E. coli* (Carpenter and Helbling, 2018; Zhang et al., 2022). Techniques like principal component analysis (PCA) reduces the complexity of variables while maintaining the variability within the dataset. PCA can reveal variables that covary (Karpuzcu et al., 2014, Zhang et al., 2022), pointing to site-specific or watershed level sources of water quality impairment. For example, Karpuzcu et al. (2014) showed that herbicides and pesticides may aggregate in one principal component indicating a strong, agricultural source signature, and pharmaceuticals may aggregate in another principal component indicating an urban wastewater signature. Another useful statistical technique is classification and regression tree (CART) analysis. CART relies on nonlinear modelling based on multiple predictor variables where dependent variables are split into categories based on their influence on the dependent variable. This model can elucidate multi-variable relationships between environmental factors, EC concentrations, and *E. coli* concentrations (Verhougstraete et al., 2015; Gregor et al., 2002). Overall, the statistical approach has become popular because it is not dependent on a fully formed fate and transport model for ECs. Instead, statistical models use empirical data to generate significant relationships that can then be applied to reduce pollutant loading for a specific watershed.

This work examined a region with multiple potential point and non-point sources of surface water fecal pollution in a subtropical watershed in the Southeastern US. There were two research questions to be answered:

Research Question (1) What is the relationship between human waste proxies and *E. coli* in Choccolocco Creek?

Research Question (2) Can certain proxies, in conjunction with spatial data, allow us to distinguish between *E. coli* from wastewater treatment plants and *E. coli* from onsite septic tank systems?

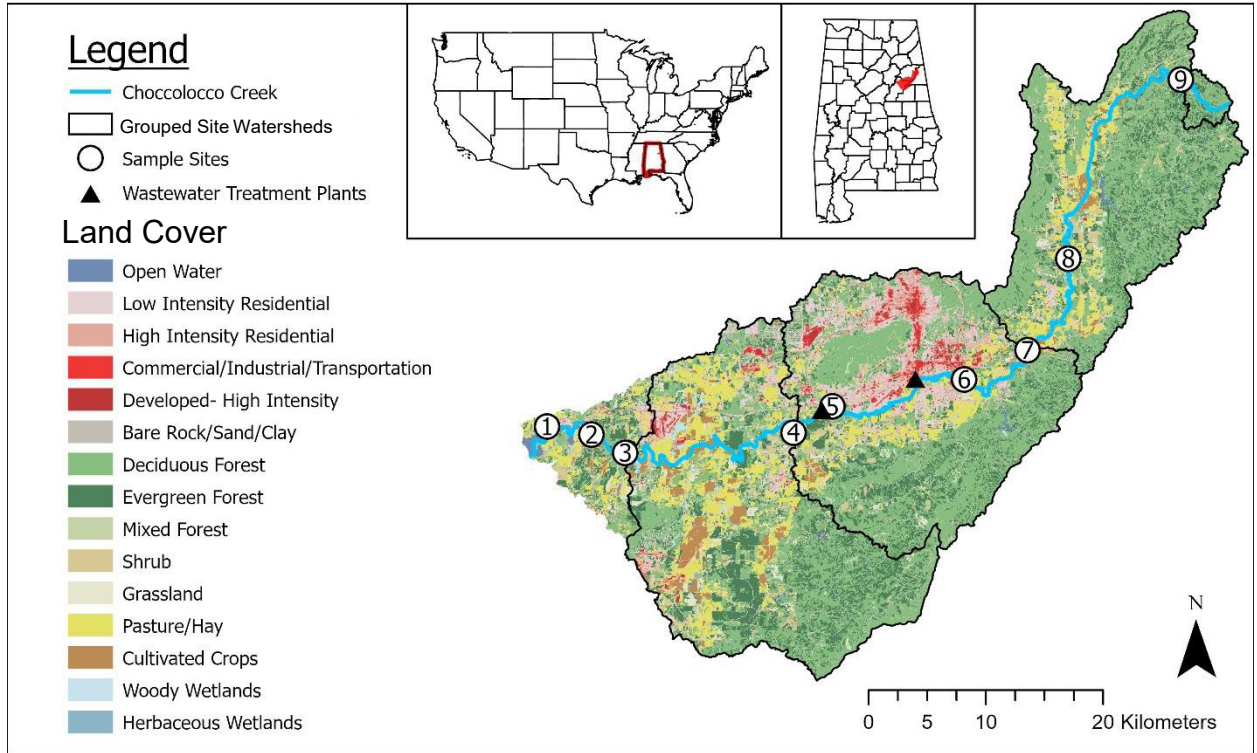


Figure 1. Map of Choccolocco Creek Watershed including sample sites. The blue line represents Choccolocco Creek which flows from the northeast (site 9) to the southwest (site 1) where it converges with the Coosa River. The black lines outline the grouped site watershed boundaries. The black triangles are the two wastewater treatment plants that discharge into the creek.

Here, a multiproxy approach was taken to understand relationships between land use, water quality parameters, ECs, and *E. coli*. Correlations and PCA between human proxies and *E. coli* contamination were used to identify potential fecal waste sources. In this region, other studies have focused on quantifying ECs, but with no emphasis on ECs as human proxies for *E. coli* contamination (Okweye et al., 2021 and Bradley et al., 2019). Chemical source tracking across a watershed differentiates between potential sources of *E. coli* and these data serve as a model for pulling apart complexities in contaminant sources for future watershed health research.

2. Methodology

2.1 Study Site

In Alabama, the total mileage of river impairment due to *E. coli* is greater than any other contaminant category including heavy metals, runoff nutrients, and toxic compounds (ADEM, 2016). This study examines Choccolocco Creek, a river in northeastern Alabama (**Figure 1**) where several potential sources of pathogens have been documented. The Choccolocco Creek watershed can be found in the counties of Calhoun, Clay, Cleburne, and Talladega. The study region contains both a high density of residential septic tank systems that are susceptible to failure (Jordan, 2022) and two wastewater treatment plants (WWTP) in Anniston, AL and Oxford, AL that discharge effluent directly into Choccolocco Creek (NPDES PERMIT NO. AL0058408 & AL0022195). There is a third WWTP in Talladega County that discharges into one of Choccolocco Creek's tributaries, Eastaboga Creek (NPDES PERMIT NO. AL0054658). Over 1,000 farms exist in the counties of Talladega and Calhoun (U.S. Census of Agriculture, 2017), and livestock on these farms is within the Choccolocco Creek Watershed and can contribute to the *E. coli* loading within the creek. The upper Choccolocco Creek lies in Calhoun County and estimates show that there are about 2 million chickens and about 20,000 cows in this region (U.S. Census of Agriculture, 2017). The lower Choccolocco lies in Talladega County with about 850,000 chickens and 13,500 cows (U.S. Census of Agriculture, 2017; USDA, 2020).

2.2 Water sampling

Nine sites were sampled monthly from April to September of 2021 (**Figure 1**, numbered circles) along Choccolocco Creek. Sample sites were selected based on accessibility and to achieve spatial distribution along the creek. Samples were collected following EPA guidelines

for surface water sampling to maintain sample integrity (U.S. Environmental Protection Agency, 2007). Collection was made from the center of the creek using a Nasco Sampling Swing Sampler (Whirl-Pak, Madison, WI). The sampler cup was cleaned with bleach, DI water, and creek water between each site. Water was collected in amber glass gallon jugs that were stored in coolers on ice until they were transported back to the lab and stored at 4°C.

2.3 Water Quality Parameters

Water quality measurements including turbidity, pH, Oxidation Reduction Potential (ORP), dissolved oxygen, conductivity, and fluorescent Dissolved Organic Matter (fDOM) content were made at each site using a YSI and an EXO² sonde (Xylem, Yellow Springs, OH). All equipment was washed with DI water three times in between sample sites and gloves were worn during collection. Nitrate and ammonia concentrations were measured using an ion-selective electrodes (Hach, Loveland, CO). Calibrated ranges for the electrodes were 1 to 100 mg/L ammonium-nitrogen and nitrate-nitrogen. Total nitrogen and total phosphorous concentrations were measured following the Boyd procedure (Gross and Boyd, 1998).

2.4 Pathogens

E. coli enumeration was performed using Coliscan Easygel® kits. At each site 2-mL of water from the collected sample was added to the Coliscan polymer, poured into the petri dish, and let incubate for 48 hours at 35°C before counting coliforms. Coliscan Easygel® kits have been shown to be accurate measures of colonies when compared to more advanced laboratory analysis (Stepenuck et al., 2011). Enumeration was performed in triplicate to ensure a representative count.

The Alabama Department of Environmental Management (ADEM) sets water quality standard for *E. coli* in rivers. Choccolocco Creek is categorized by ADEM as a Public Water Supply (PWS) and Fish and Wildlife (F&W) waterbody (ADEM, 2020). For these classes of

stream, the geometric mean at least five samples during a 30-day period must not exceed 126 cfu/100 mL and a single sample must not exceed 298 cfu/100 mL. In this study, the sampling scheme (single monthly samples) did not allow for calculating a 30-day geometric mean, and therefore, it is not possible to identify sites that violate that criterion. Instead, single sample threshold was used and the geometric mean over six months was calculated for the 126 cfu/100mL threshold.

2.5 Trace Metals

Trace metal concentrations including that of copper, beryllium, sodium, magnesium, aluminum, potassium, calcium, vanadium, chromium, manganese, iron, cobalt, nickel, zinc, arsenic, selenium, strontium, molybdenum, silver, cadmium, antimony, barium, titanium, lead, thorium, and uranium were measured for each water sample at the Auburn Geosciences ICPMS lab following a modified version of U.S. EPA method 6020. Approximately 60-mL of water was filtered and acidified to a pH of 2 using 1.0M hydrochloric acid. Samples were stored in polypropylene bottles for metals analysis. Total metal concentrations are presented in **Appendix Table 3**.

2.6 Emerging Contaminants

Caffeine and ibuprofen were analyzed in the Ojeda lab at Auburn (**Appendix Table 1 & Table 2**). These compounds were extracted from water samples using Sep-Pak® C18 Solid Phase Extraction (SPE) cartridges and then analyzed using an Agilent Gas Chromatography Mass Spectrometer (GC-MS, 8890 GC-5977 MSD). Samples were filtered using a 0.45 µm polyvinylidene difluoride filter for each sample and brought to a pH of 2 using 1.0M hydrochloric acid to provide the highest percentage recovery for target analytes (Togola et al., 2007). For SPE, the C18 cartridges were conditioned with 15mL of dichloromethane, 15mL of methanol, and

15mL of ultrapure water at a pH of 2. Next, one liter of each water sample was passed through the cartridge with the vacuum set to ~850mbar. The cartridge was then left to dry for about 60 minutes. After drying, the target compounds were eluted with 7mL of dichloromethane in a test tube before being evaporated by a gentle stream of nitrogen gas. Samples were then reconstituted with 100 μ l of dichloromethane, and polar functional groups were derivatized with 100 μ l of N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) for 30 minutes at 60 °C. Finally, the sample solution was analyzed using the GC-MS following these parameters: capillary column an HP5/MS (30 m \times 0.25 mm \times 0.25 μ m film thickness), 1 μ l injection in splitless mode at 305 °C with ultrapure Helium at a constant flow of 1.4 mL/minute. Initially, the oven temperature was held at 100 °C for 1 minute. Then, at a rate of 4 °C /minute the temperature increased to 300 °C. Samples were analyzed using both a scan mode (50-550 total ion chromatogram) and a selected ion monitoring mode (SIM) to improve precision. SIM ions for ibuprofen include m/z 160, 73, 206 and for caffeine include m/z 194, 109, 67.

A calibration curve to quantify the concentration of pharmaceuticals in water samples has been completed using two methods: direct injection of the pharmaceutical stock solutions, and analysis after solid phase extraction and reconstitution. These calibration curves serve to constrain the analytical uncertainty as we can use their slope and standard error to assess the recovery of our extraction methods (**Appendix Figure 1 & Figure 2**).

Four sets of samples from four sites (3, 4, 7, and 9) were sent to Eurofins Eaton Analytical laboratory for analysis using EPA methods 527, 539, and 542. One site from each of our defined geographic site groupings was included, along with our control site near the headwaters. Sites were selected based on high preliminary *E. coli* concentrations, and sent for testing for the months May, July, August, and September. Eurofins returned concentrations for over 90 human-specific

emerging contaminants (ECs) and serves as quality control for analysis done in the Ojeda Lab (Appendix Table 1 & Table 2).

2.7 Statistical Analysis

Statistical Analysis was done using GraphPad Prism (v9.2, San Diego, CA, USA) software. First, a D'Agostino-Pearson normality test was done on all variables to determine whether the data was normally distributed. This test computes skewness and kurtosis and then calculates how the dataset differs from a Gaussian distribution. Variables with normally distributed data were analyzed using Pearson correlation tests and non-normal variables in Spearman correlation tests. All correlations were performed in relation to *E. coli* and evaluated as significant at the $\alpha = 0.05$ level.

Principal component analysis was utilized to detect variable relationships within the high-dimensional data that we collect from the water samples (Jolliffe et al., 2016). PCA uses linear combinations of the input data to generate principal components (Tabachnick and Fidell, 2007). The first principal component (i.e., PC1) is calculated so that it accounts for the most variance in the data set. The second principal component (i.e., PC2) is calculated similarly, but with the added mathematical condition that it is uncorrelated (orthogonal) to the first principal component. Then, the process continues until the number of principal components is equal to the original number of variables, and the sum of the variances of all of the principal components equals the sum of the variances of all of the variables. Interpretable results from PCA are: 1) the eigenvalues for each principal component as the variance explained by each principal component, 2) the loadings of each variable within each principal component which explains the variables that contribute most strongly to each principal component, and 3) aggregates of variables within a principal component biplot (e.g., PC1 versus PC2). The number of principal components selected for interpretation

often follows Kaiser's rule so that eigenvalues are greater than 1. Tabachnick and Fidell (2007) suggest that loading cutoffs be classified as 0.32 (poor), 0.45 (fair), 0.55 (good), 0.63 (very good) or 0.71 (excellent). This study chose to use 0.50 as a loading threshold for meaningful interpretations.

2.8 Geographic Data

Septic system permit data was gathered for each county within the Choccolocco Creek watershed: Calhoun, Clay, Cleburne, and Talladega. Septic system location was provided by the Alabama Department of Public Health for all systems permitted from January 1, 2000, to August 11, 2020. Some addresses included multiple permits; these addresses were assumed to only have one septic system. The newest approval/repair permits were appended to the septic system point data to have one point representing the system.

Land use data was gathered from the United States Geological Survey 2019 National Land Cover Database. The percentage of urban, forested, and hay/cropland was calculated from cell count for each category using the Summarize Categorical Raster tool in ESRI ArcGIS Pro (v2.9, Redlands, CA).

Sample point watersheds (SPW) were created with the ArcGIS Pro Hydrology toolkit. Flow accumulation and flow direction were calculated. Based on these measurements, pour points within 30-meter distances were used to delineate watershed boundaries for each sample point (Jordan, 2022). These individual SPWs were then combined to create grouped site watersheds (GSW). There are four total site groupings. First is site 9 acting as the control. The second group is comprised of sites 8, 7, and 6 as the Upper Choccolocco. The third group is the Middle Choccolocco made of sites 5 and 4, and finally site 3 and lower represent the Lower Choccolocco.

Heat maps were created in GraphPad Prism to evaluate changes in measurements across sites. Variable data was normalized using the standard equation: $x = \frac{x - x_{min}}{x_{max} - x_{min}}$, and x_{min} were derived from average values across all sites in order to scale the data between 0 and 1.

2.9 Weather Data

Weather data was gathered from the PRISM Climate Group database (Northwest Alliance for Computational Science & Engineering, Corvallis, OR). Data included average daily temperature (°C) and precipitation (mm) on one day, three day, and seven day intervals before the sample collection date. One day indicates precipitation measured on the day of sampling, three day indicates the precipitation measured for the day of sampling plus the two days prior and so on for seven day. The data for each site was gathered from one of PRISM's 4-kilometer grid cells using latitude and longitude measurements.

3. Results

3.1 Trends in *E. coli* Across the Watershed

The mean of the triplicate measurements was taken to represent concentration for each sampling session. Of these concentrations, 17 samples (30%) exceeded 298 cfu/100 mL. Sites 8, 5, and 4 had three exceedances followed by sites 7 and 3 with two exceedances, and finally 9, 6, 2 and 1 with one each. The geometric mean was taken using the arithmetic mean calculations over the six-month sampling period. Of these values, sites 4 and 5 exceeded the monthly exceedance of 126 cfu/100 mL. These trends are illustrated in overall average concentrations of *E. coli* through the study as well (**Figure 2**). Site 8 and site 4 show higher average concentrations of *E. coli* compared to other sites with the lowest average at site 1 near the confluence of Choccolocco Creek and the Coosa River. Temporal trends in *E. coli* were highly variable across the watershed. Early spring months of April and May showed higher concentrations of *E. coli*

compared to the summer month of July and August (**Appendix Table 3**). Together, this data supports the ADEM 303(d) listing of Choccolocco Creek as an impaired waterbody for *E. coli* and provides a basis on which to use human proxies to delineate sources of *E. coli* in the watershed.

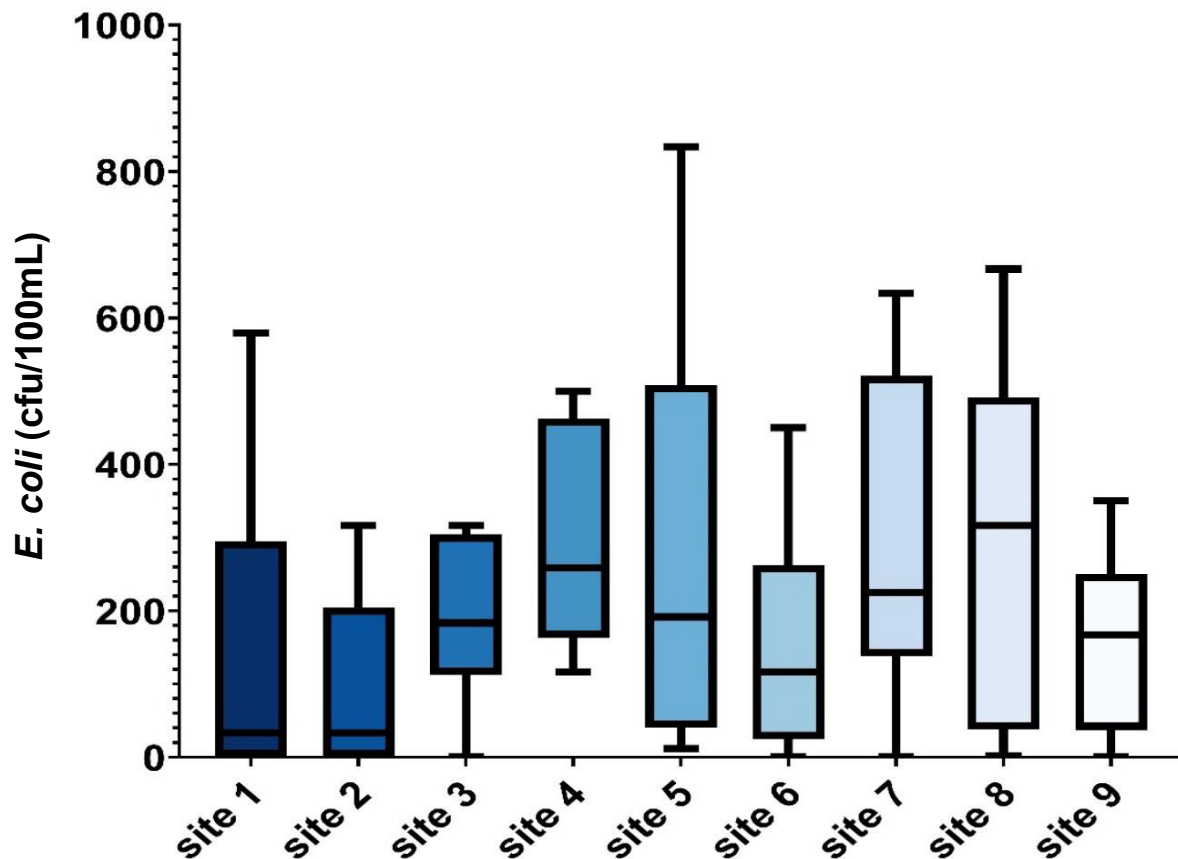


Figure 2. *E. coli* concentration box and whisker plot. The x-axis displays the site number, and the y-axis is *E. coli* concentration in cfu/100mL. The sites have been color coded throughout the results plots (upstream lightest blue and downstream darkest blue). This plot was created using *E. coli* averages for the months of April-September 2021.

3.2 Correlations Between *E. coli* and Other Variables

All measured variables were evaluated in relation to *E. coli* to determine correlative relationships. Statistically significant relationships ($p < 0.05$) were recorded for seven of the variables measured (**Table 1**). For non-normal data distributions, Spearman correlations yielded two inverse relationships: one day precipitation and nitrate-nitrogen. These relationships were

inverse based on 54 pairs of datapoints. For normally distributed data, Pearson correlations yielded five significant relationships: pH, water temperature, average total phosphorous, total EC concentration, total number of EC compounds. Only water temperature and pH illustrate inverse relationships with *E. coli* within their respective ranges (17.39 – 33.55 °C and 6.10 – 9.22 pH).

Table 1. Pearson and Spearman correlative statistics between measured variables and *E. coli*. Each variable is reported with the number of pairs, r value, p value, average measurement, and range of measurements across the dataset. Asterisks indicate the significance of the relationship from most to least. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

Parameter	# of pairs	Spearman r	P value	Average	Range
One Day Precipitation (mm)	54	-0.3709	0.0058**	1.48	0 – 15.63
NH ₃ -N (mg/L)	54	-0.4117	0.0020**	0.0320	0 – 0.19
Parameter	# of pairs	Pearson r	P value	Average	Range
Avg Total P (µg/L)	54	0.2729	0.0459*	57.1	16.99 – 210.2
pH	54	-0.4391	0.0009***	7.44	6.10 – 9.22
Water Temp. (°C)	54	-0.3586	0.0077**	23.3	17.39 – 33.55
Total EC concentration (ng/L)	16	0.5075	0.0448*	798	0 – 2784
Total number of EC compounds	16	0.5339	0.0331*	6.81	0 – 15

3.3 Principal Component Analysis of All Data

PCA of water quality variables and *E. coli* was calculated as shown in **Table 2**. The scores for PC1 and PC2 were significant following the Kaiser rule. PC1 had an eigen value of 3.30, PC2 a value of 2.02, and PC3 with a value of 1.33. Of the 10 variables assessed in the PCA, only two did not meet the loading criteria within the first two principal components. Both DO% and Cu were only significant until the third principal component. PC2 had high negative loadings for *E. coli* and average total phosphorous. The rest of the variables had high negative loadings in PC1.

Table 2. Principal component scores calculated from PCA of water quality variables.				
Variables		PC1 (33.04%)	PC2 (20.22%)	PC3 (13.35%)
Water Quality Parameters				
	<i>E. coli</i>	0.35	-0.63	0.36
	pH	-0.60	0.67	0.08
	Water Temp.	-0.72	0.15	-0.19
	Conductivity (SPC)	-0.79	-0.25	0.39
	Dissolved Oxygen (DO%)	-0.20	-0.40	0.54
Species in Water				
	Nitrate – N (mg/L)	-0.74	-0.14	0.24
	Avg Total Nitrogen (µg/L)	-0.72	-0.44	-0.11
	Ammonium – N (mg/L)	-0.65	0.14	0.00
	Avg Total Phosphorus (µg/L)	0.00	-0.78	-0.36
	Copper (ppb)	-0.42	-0.30	-0.72

PC1 accounts for 33.04% of variability in the dataset and PC2 for 20.22%. Combined, PC1 and PC2 explain 53.26% of total variance in the dataset. **Figure 3** illustrates the loading for the PCA. On the x-axis is PC1 and on the y-axis is PC2. The water quality variable loadings are shown in blue and *E. coli* loading in black. The criteria for variables to be included is a loading score with

an absolute value greater than 0.5. *E. coli* covaries weakly with total phosphorous. There is a group of variable loadings orthogonal (unrelated) to *E. coli* including nitrate, total nitrogen, and specific conductance. pH and *E. coli* meet at a large angle, indicating a negative correlation.

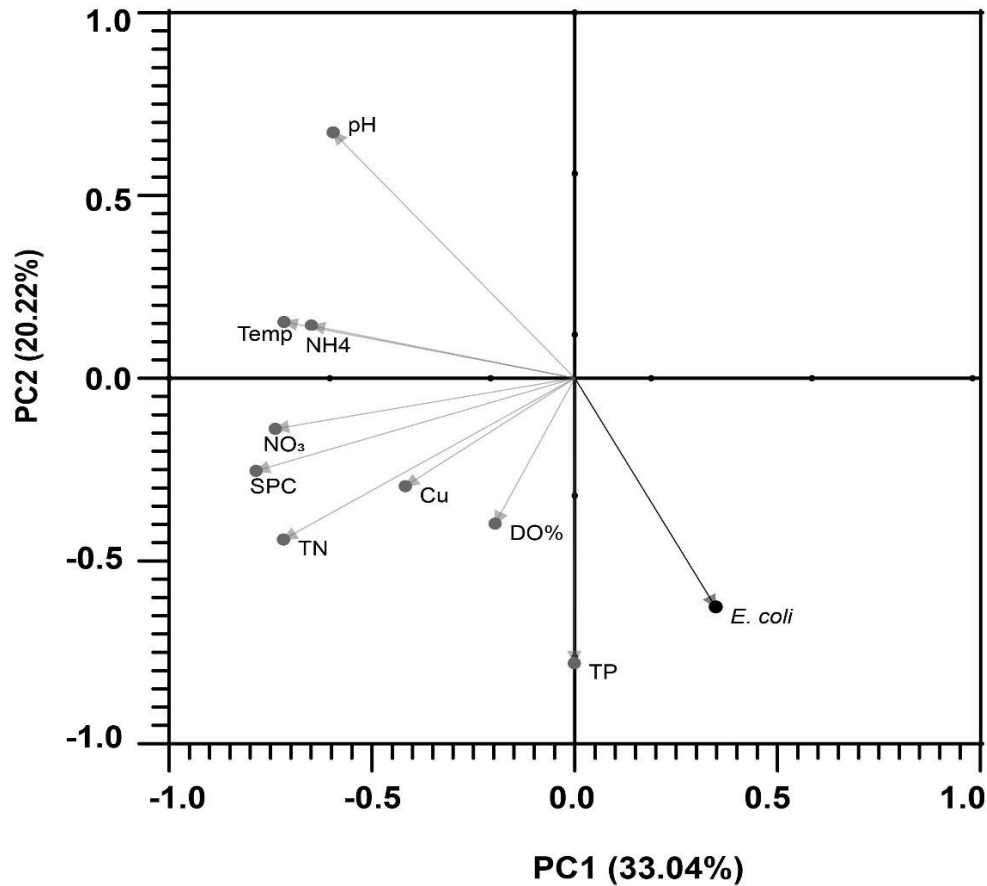


Figure 3. PCA of *E. coli* with water quality variables. PC1 on the x-axis and PC2 on the y-axis. Both axes display the percent each principal component contributes to the variability of the dataset.

Figure 4 shows the distribution of PC scores based on the PCA of the water quality variables. The scores are organized by site location across the six-month sampling period. The water quality/chemistry tends follow the sample site location along PC1 from site 9 (positive PC1) to downstream sites (more negative values of PC1). Sites 9, 8, 7, and 6 aggregate together in the PC scores, and moving downstream to sites 5 to 1 at the confluence of Choccolocco Creek

and the Coosa River, aggregation of sites is less apparent. This could be due to the mixing of source and dilution effects as river widens leading to greater variability in the water chemistry.

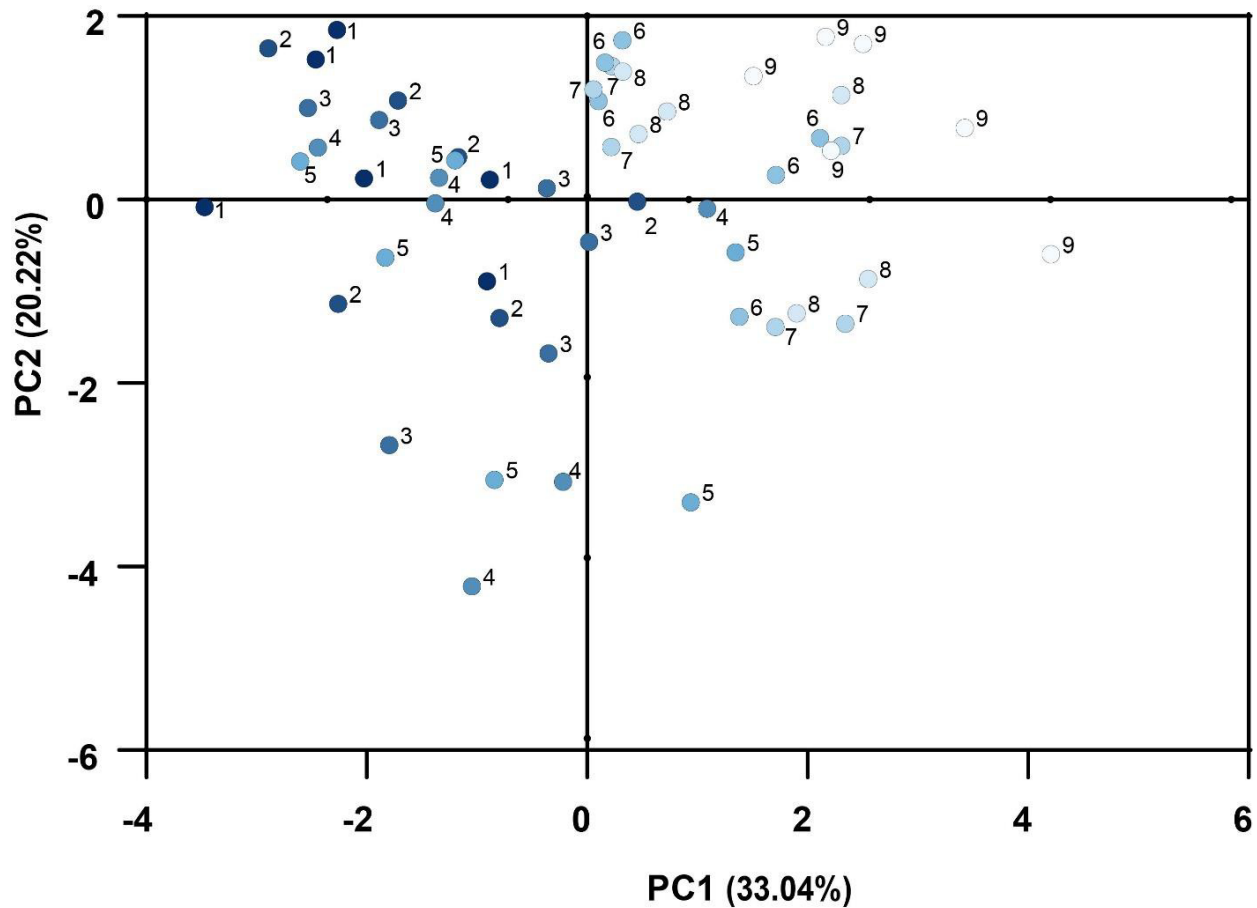


Figure 4. PC scores of *E. coli* with water quality variables by site. The sites are plotted along PC1 and PC2 on the x and y axes. Both axes display the percent each principal component contributes to the variability of the dataset.

A biplot was created overlay the variable loadings and the PC scores (**Figure 5**). In the negative PC1 positive PC2 quadrant, pH loads alongside the lower Choccolocco sites 1, 2, and 3. Temperature and ammonium nitrogen load in the same quadrant near the sites 1, 4, and 5. The aggregation of Lower Choccolocco sites lies opposite to the loading of *E. coli* in the positive PC1, negative PC2 direction. Nitrate-nitrogen, specific conductance, total nitrogen, and dissolved oxygen loadings are in the negative direction for PC1 and PC2. Sites 1 through 5 are

scattered in this quadrant as well. In the opposite quadrant of positive PC1 and PC2 there is an aggregation of sites 6 through 9. Total phosphorous loading lies directly in the negative PC2 direction.

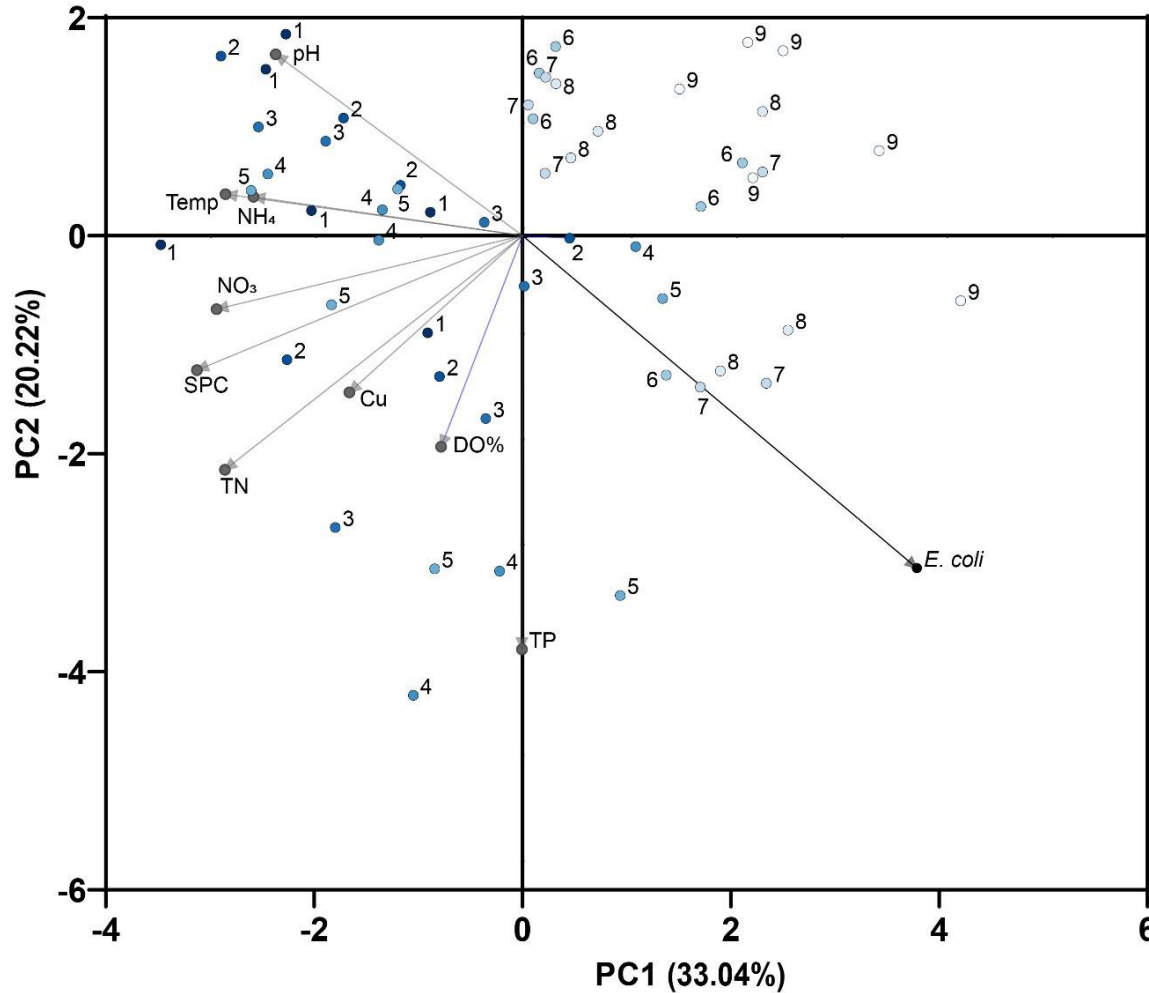


Figure 5. PCA biplot of loadings and site scores. PC1 on the x-axis and PC2 on the y-axis. Both axes display the percent each principal component contributes to the variability of the dataset.

3.4 Assessing Holistic Variable Dynamics Across Site Groupings

Site location plays an integral role in the assessment of contamination source(s) in Choccolocco Creek. For each site, potential *E. coli* inputs were identified. For example, site 9 is in the Talladega National Forrest near the headwaters of the creek and served as a control site due

to the lack of septic tank systems and human influence. Land use for site 9 is 98% forested and 1% developed (**Appendix Table 7**), implying *E. coli* loading at this site could be from wild animal or naturalized sources. The rest of the sites and their watersheds were divided into three groups characterized by geographic location and land use. The sites 6-8 watershed is 79% forest, 12% hay/cropland, and 4% developed. They are downstream of the headwaters and were named the Upper Choccolocco group. Sites 4-5 have a watershed made of 55% forested, 29% developed, and 9% hay/cropland. These sites are located just downstream of the two wastewater treatment plants and characterized by more development and were named the Middle Choccolocco group. The final grouping is sites 1-3 which have a watershed with 60% forested, 21% hay/cropland, and 10% developed. These sites are further downstream from the wastewater treatment outfalls and near the confluence with the Coosa River. They are characterized by the widest cross sections of the river and greatest volume of water resulting in a dilution of all upstream discharge, and they have been named the Lower Choccolocco group.

A heat map was used to visualize changes of the measured variables across site groupings. **Figure 6** illustrates the relative magnitude of each variable across the groups. There were strong correlations calculated in section 3.2 between *E. coli* and pH, water temperature, and the number and concentration of EC compounds.

Site 9, near the creek headwaters, is shown to have a lower magnitude of all variables but DO%. Moving downstream, site 7 had increased concentrations of *E. coli* with moderately high water temperatures and conductivity. Further downstream at site 4, all variables are elevated. Notably, site 4 (a part of the Middle Choccolocco grouping) had the greatest magnitude for both number of EC compounds and total concentration of EC compounds along with high *E. coli*.

Finally, site 3 nearest the confluence showed a decrease in *E. coli* and elevated water temperatures, conductivity, and pH.

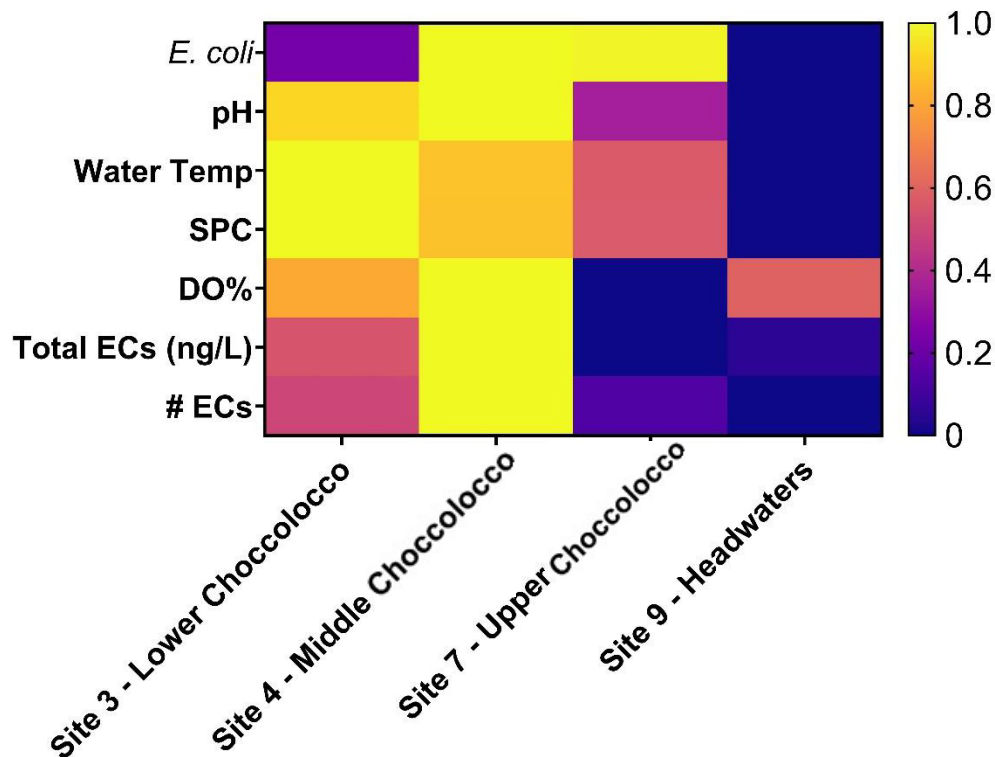


Figure 6. Heat Map of *E. coli*, water parameters, and EC compound data across four groupings. Data has been normalized across all sites in order to scale the data between 0 and 1.

The heat map provides a visualization of variables that tend to be elevated to certain geographic regions. Most notable is site 4, which is elevated in all water quality variables, *E. coli*, and ECs. To examine potential sources at a higher resolution, particularly around site 4, EC compounds were broken down into subcategories.

3.5 Emerging Contaminants Classification

The ECs originate from various sources including products in the home, at construction site, or in used in agricultural applications. The ECs were organized into three categories based on the intended use of the compound: pharmaceutical and personal care products (PPCP),

herbicides/pesticides, and industrial/home (**Table 3**). The PPCP category was further broken down into over the counter, prescription, and medical office subcategories to illustrate the accessibility and more specific source of the compounds. For example, caffeine, ibuprofen, and sucralose are more widely accessible and ingested than a prescription drug like primidone. Similarly, DEET and salicylic acid are personal care products some people use every day in their homes and outside, while iohexol is only used as an x-ray contrast agent in certain medical settings. By classifying these compounds, their abundance in each category can be used to identify trends in anthropogenic input across the watershed.

Table 3. Classification of the EC compounds based on sources and use. Three categories for EC include Pharmaceuticals and Personal Care Products (PPCP), herbicides/pesticides, and industrial/home.			
Classification	Source	Compound	Definition/Use
Pharmaceutical & Personal Care Products	<i>Over the counter</i>	1,7-Dimethylxanthine	Caffeine Degradate
		Acetaminophen	Analgesic
		Caffeine	Stimulant
		Ibuprofen	Analgesic
		Lidocaine	Analgesic
		Naproxen	Analgesic
		Phenazone	Analgesic
		Sucralose	Sugar Substitute
		Theobromine	Caffeine Degradate
	<i>Prescription</i>	Atenolol	Hypertension drug
		Carbamazepine	Anti-Convulsant
		Estradiol	Estrogenic Hormone
		Flumequine	Antibiotic
Metformin		Antihyperglycemic drug	
Primidone		Anti-Convulsant	
Sulfamethoxazole		Antibiotic	
Theophylline (semi-quantitative)		Anti-Asthmatic	
Trimethoprim	Antibiotic		
<i>Personal Care</i>	DEET (Deethylatrazine)	Insect repellent	
	Ethylparaben	Preservative	
	Salicylic Acid	Antiseptic	
<i>Medical office</i>	Iohexol	X-ray Contrast Agent	
Herbicide & Pesticides	<i>Herbicide</i>	Atrazine	Weed killer
		Bromacil	Weed killer
		DEA	Atrazine degradate
		DIA (Deisopropylatrazine)	Atrazine degradate
		Metolachlor	Weed killer
		OUST (Sulfometuron, methyl)	Weed killer
	<i>Pesticide</i>	Thiabendazole	Controls disease
Industrial & Home	<i>Manufacturing/Home</i>	BPA	Plastics
		TCEP	Flame Retardant
		TCPP	Flame Retardant

3.6 Detection Frequency

The classifications from **Table 3** help to understand the dominant sources of human contamination. For analysis and interpretations, parent and daughter compounds of atrazine (atrazine, DEA, and DIA) and caffeine (caffeine and theobromine) were summed. There was a total of 32 EC compounds detected in the water samples. Of those compounds, 14 of them were

detected 25% of the time (**Figure 7**). These frequently detected compounds included 9 compounds from the PPCP category, 3 compounds from herbicide/pesticide category, and 2 compounds from industrial/home category. The PPCP category also has some of the most frequently detected compounds with caffeine in 50% of samples, lidocaine in 44%, and sucralose and iohexol in 38%. Sucralose, iohexol, and salicylic acid also have the highest measured concentrations for a single sample in the dataset. Notably, atrazine is detected 44% of the time in the herbicide/pesticide category along with DEA 31% and Metolachlor 25% of the time. TCCP and TCEP are detected 25% and 31% respectively within the industrial/home category. Detection frequency and concentration for all EC compounds can be found in **Appendix Table 1**.

Water samples were collected for analysis in the Ojeda Lab. We prepared to measure ibuprofen and caffeine for every sample collected. This data was collected (**Appendix Table 2**), but it was omitted from data analysis. There are two primary reasons for its omittance. The first was that the detection limit for Ojeda lab samples was not low enough for water samples compared to those of Eurofins Analytical. Our minimum detection level using GC-MS methods was around 15-20 ng/L while LC-MS methods for ECs commonly have detection limits ranging from 0.01 to 10 ng/L (Boix et al., 2016; Valsecchi et al., 2015). The second is that we were only collecting data for two EC compounds (ibuprofen and caffeine) while Eurofins collects data for over 90 potential contaminants. The collection of just two compounds does not capture the variety of contaminants and their potential sources. For these reasons, the Ojeda lab EC data was excluded from analysis in favor of the Eurofins EC data.

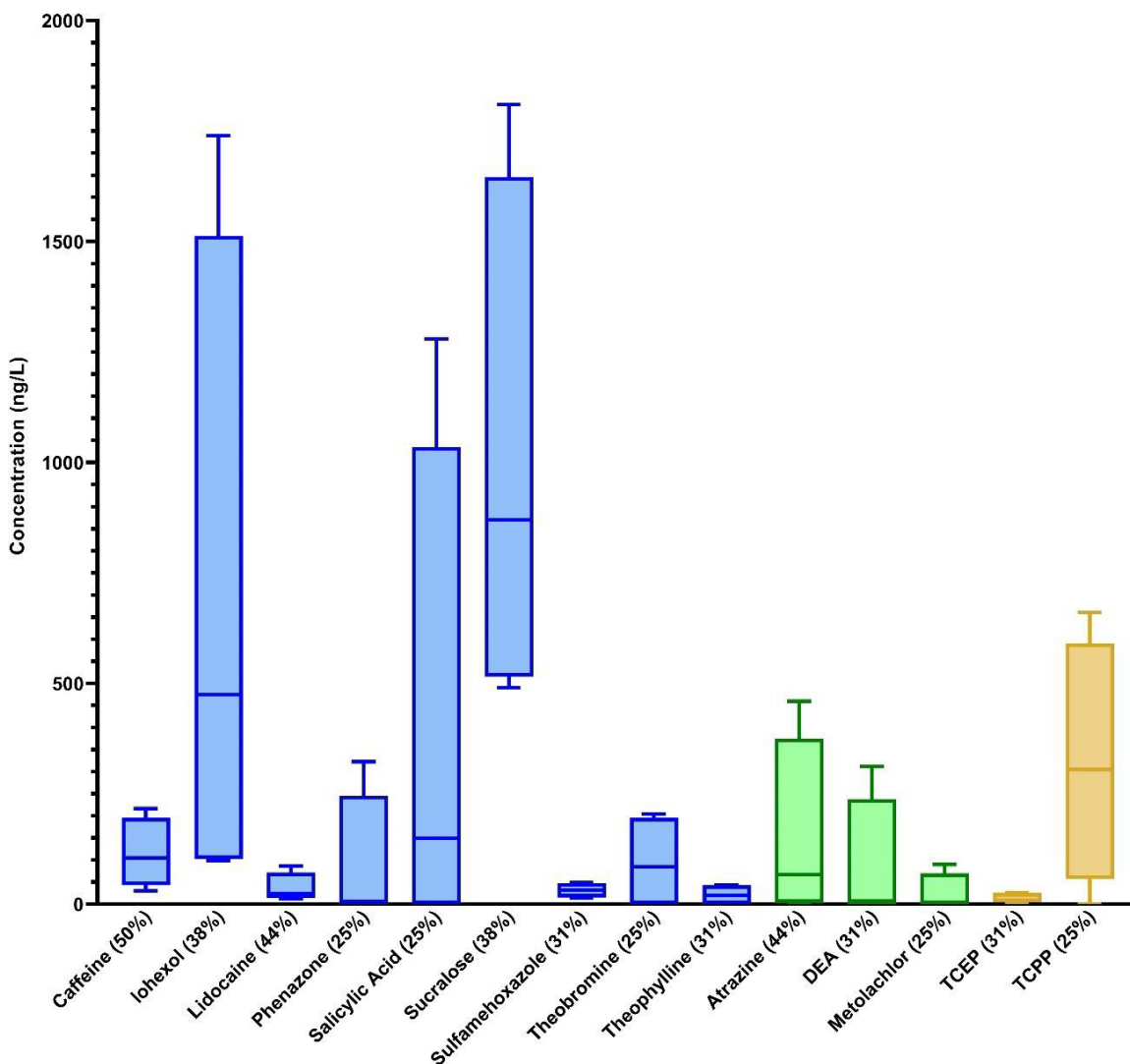


Figure 7. A box and whisker plot of EC compounds and their detection frequencies from four sites (9, 7, 4, 3) across four months in 2021 (May, July, August, September) with a detection frequency of 25% or greater. PPCP are represented in blue, herbicides/pesticides in green, and industrial/home in yellow.

3.7 Principal Component Analysis of Emerging Contaminants

PCA for *E. coli* and EC compounds was calculated as shown in **Table 4**. The first PC accounts for 30.02% of total variability, while PC2 and PC3 account for 20.56% and 13.19%, respectively. These principal components met the criteria set by the Kaiser rule with eigen values

of 4.20, 2.88, and 1.85 for PC1, PC2, and PC3. 12 of the 14 variables assessed met the score criteria. Salicylic acid and metolachlor were the only two compounds with scores with an absolute value less than 0.5 for the first three PCs.

Table 4. Principal component scores calculated from PCA of EC compounds. Variables are organized into five categories including water quality parameter, herbicide/pesticides, over the counter pharmaceuticals, prescription pharmaceuticals, and industrial/home. Scores that meet the criteria are bolded.

Variables		PC1 (30.02%)	PC2 (20.56%)	PC3 (13.19%)
Water Quality Parameter				
	<i>E. coli</i>	0.587	-0.160	0.565
Herbicide/Pesticides				
	Atrazine (+)	0.209	0.073	0.794
	Metolachlor	-0.142	-0.005	0.482
	Thiabendazole	0.451	0.172	-0.208
Over The Counter Pharmaceuticals				
	Caffeine (+)	0.630	0.637	-0.056
	Lidocaine	0.598	0.179	-0.486
	Salicylic Acid	-0.022	-0.001	0.289
	Sucralose	0.262	-0.839	-0.135
Prescription Pharmaceuticals				
	Iohexol	0.459	-0.865	-0.023
	Sulfamethoxazole	0.770	-0.040	-0.270
	Theophylline	0.869	0.068	-0.134
	Trimethoprim	0.496	-0.777	0.045
Industrial/Home				
	TCEP	0.711	0.138	0.426
	TCPP	0.675	0.546	0.063

The PCA of EC compounds and *E. coli* is illustrated in **Figure 8**. Once again, only variables with loading scores with an absolute value greater than or equal to 0.5 were included in the figure. All variable loadings go in the positive PC1 direction. *E. coli* covaries weakly with sulfamethoxazole. The rest of the EC compounds do not appear to aggregate within their respective categories. For example, the pharmaceuticals including ibuprofen, lidocaine, and trimethoprim would covary separately from the herbicides/pesticides like atrazine and bromacil.

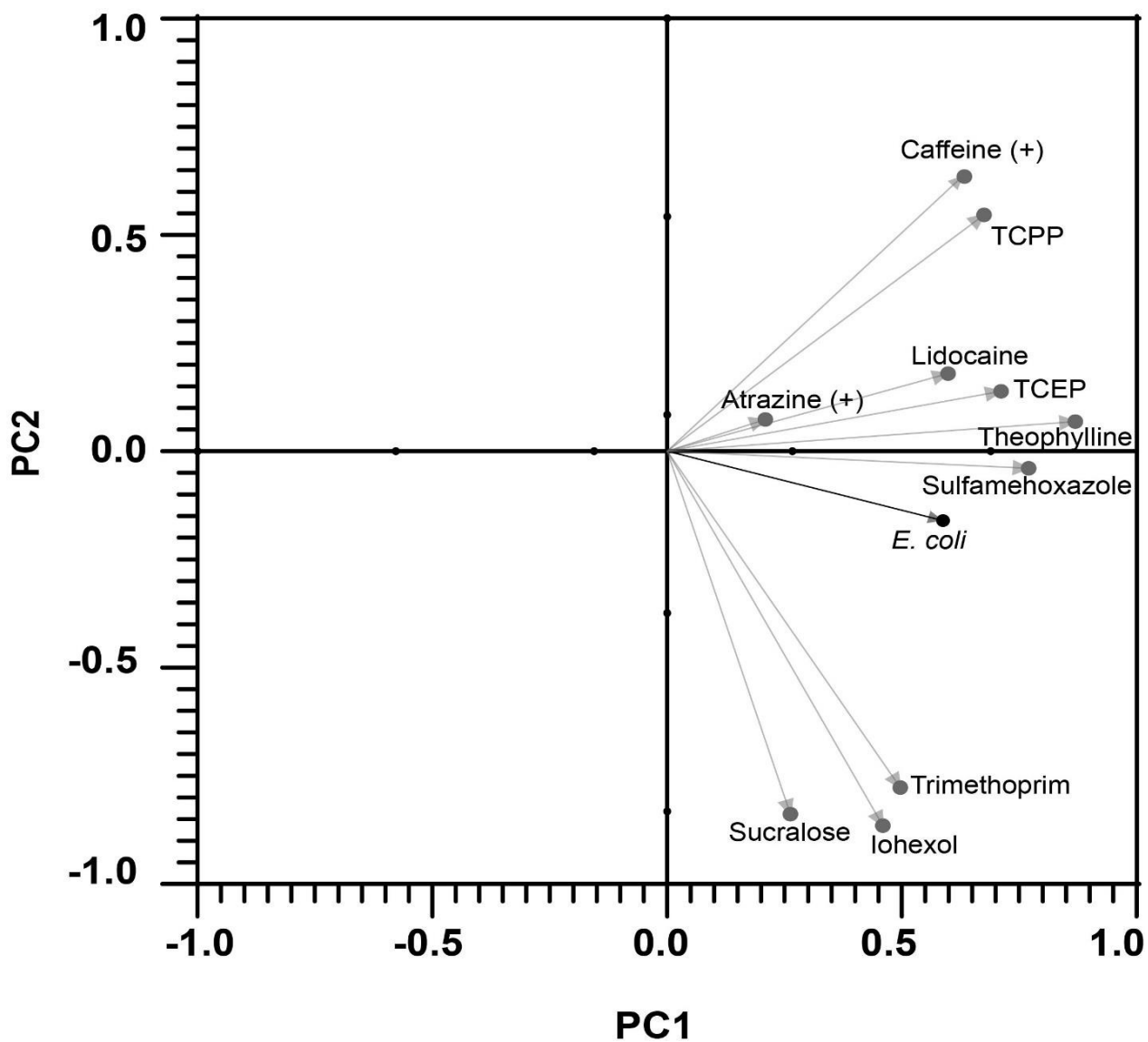


Figure 8 PCA loadings for *E. coli* and ECs. The x-axis is PC1 and the y-axis is PC2. Both axes display the percent each principal component contributes to the variability of the dataset.

The PC scores for EC were plotted in **Figure 9**. Sites 3 falls in the negative PC2 direction. Sites 7 and 9 aggregate moderately in the negative PC1, positive PC2 direction.

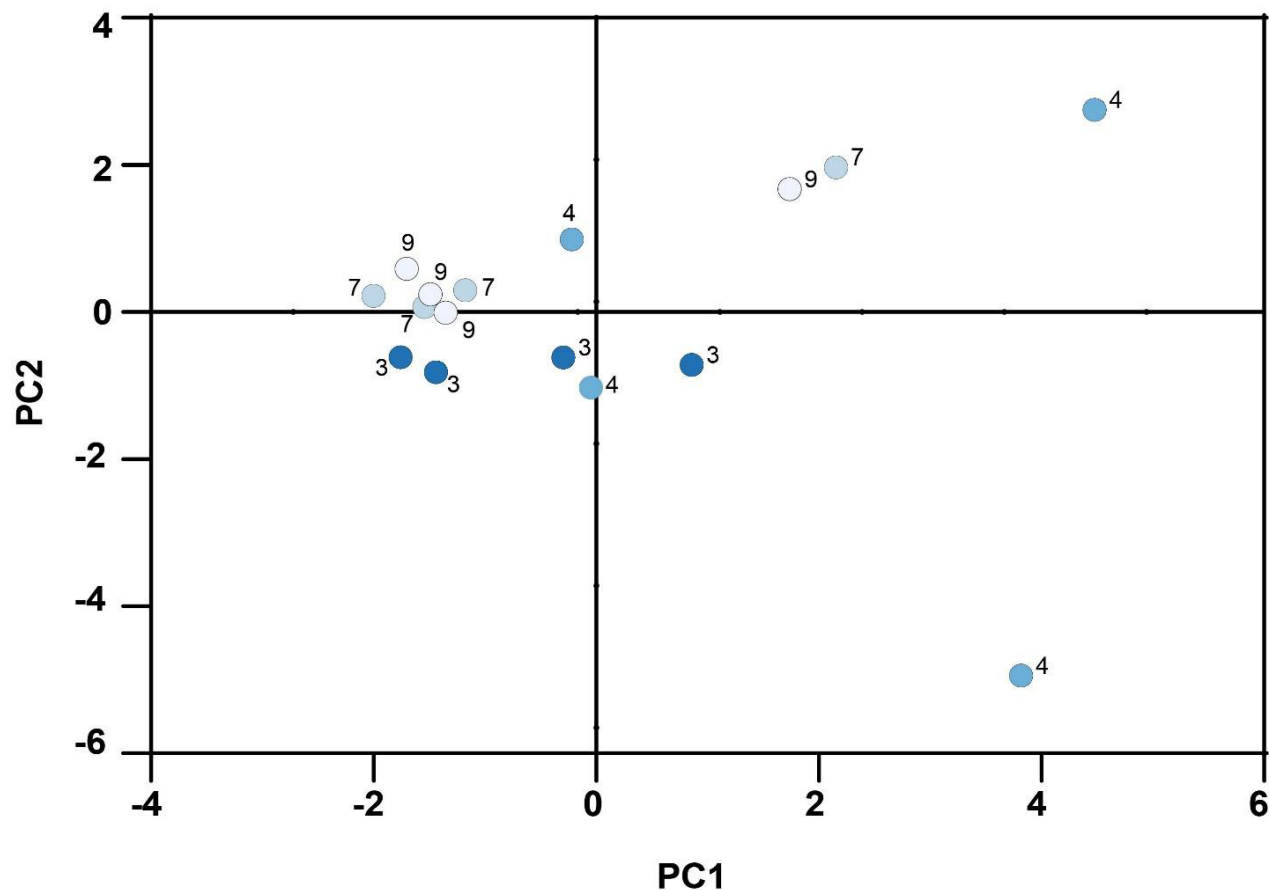


Figure 9 PCA scores of the sites along PC1(x-axis) and PC2 (y-axis). Both axes display the percent each principal component contributes to the variability of the dataset.

Figure 10 shows the biplot of the loadings and the PC scores. Most of the loading vectors point away from the site scores on the biplot except for two very different site 4 scores. For the site 4 vector with a positive PC2 and PC1 score, caffeine and its degradants and TCPD contribute to its variability. Along these same vectors, there is a site 9 and site 7 score. The negative PC2 score site 4 is influenced by iohexol and trimethoprim.

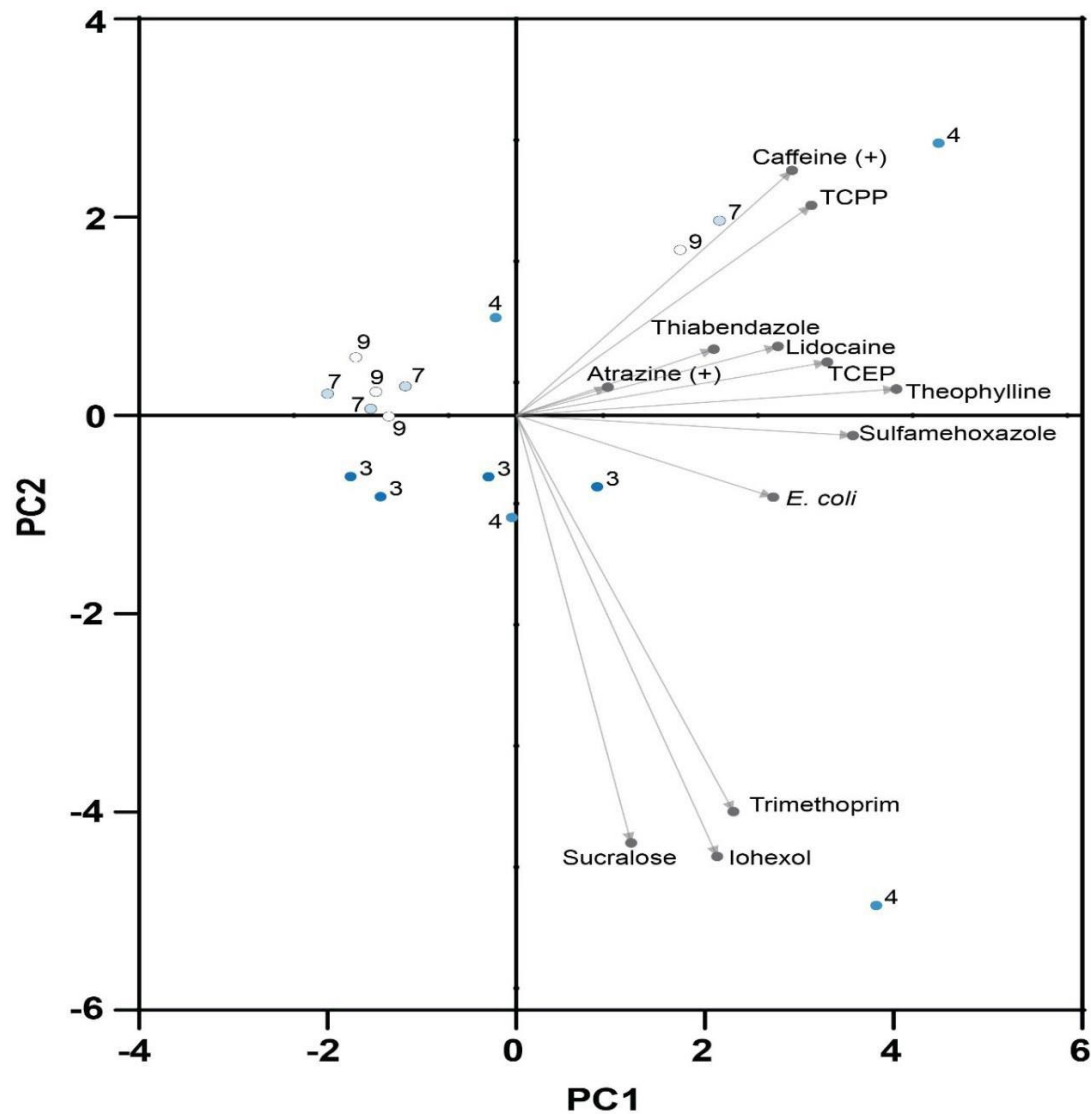


Figure 10. PCA biplot of loadings and site scores for *E. coli* and ECs. PC1 on the x-axis and PC2 on the y-axis. Both axes display the percent each principal component contributes to the variability of the dataset.

3.8 Heat Map of Source Specific Variables

Figure 11 shows a heat map where the EC compounds organized into categories to focus on source specific markers and geographic distribution. *E. coli*, septic system density, total average nitrogen and phosphorous, copper, land cover percentage, and EC concentrations were used to evaluate water contamination and potential loading sources across the four groupings. Starting at the Headwaters of site 9, the percent forested land dominates with no other variables in high

magnitude. At the Upper Choccolocco group, site 7, there is elevated *E. coli* and herbicide/pesticide compounds along with moderate elevation for hay/cropland percentage. Middle Choccolocco (site 4) is a part of the urban group and once again is elevated in many of these variables. Notably, developed land percentage, *E. coli*, septic system density, copper, PPCP compounds, and industrial/home compounds all have high normalized values for this site. Finally, the Lower Choccolocco at site 3 furthest downstream shows elevated concentrations in nitrogen, copper, and percent hay/cropland.

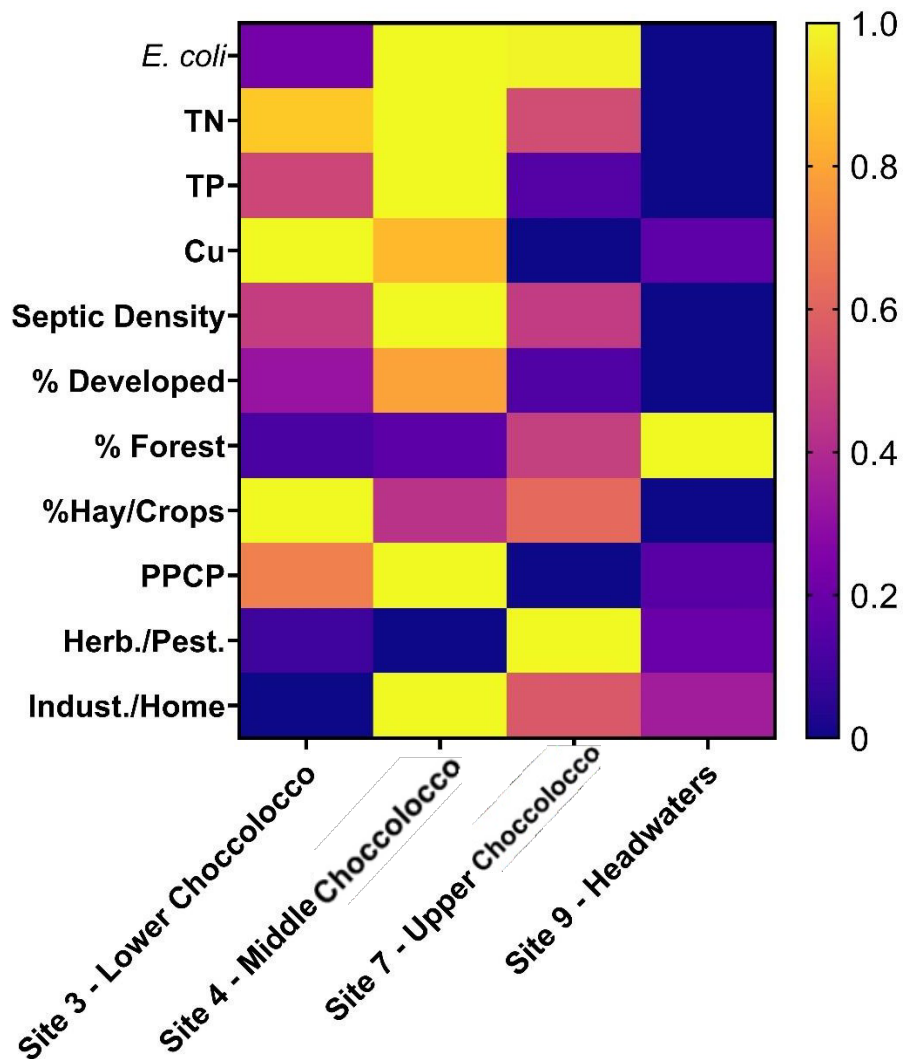


Figure 11. Heat map of *E. coli* and source specific data across the four groups. Data has been normalized across all sites to scale the data between 0 and 1.

4. Discussion

4.1 Relationships between *E. coli* and Water Quality Parameters

Data over a 6-month period shows *E. coli* had strong relationships with water quality and environmental variables. The correlations calculated (**Table 1**) correspond with past research that reported *E. coli* concentration was highly correlated to pH, turbidity, DO%, and temperature (Lawrence et al., 2012; Shamsudin et al, 2016, Vialle et al., 2011). This study's correlation

calculations showed a strong negative relationship between pH and *E. coli* while Shamsudin et al., 2016 and Vialle et al., 2011 found a positive relationship. The range of our pH data is 6.10 – 9.22 which is relatively high maximum value for a river system compared to a relatively wider range of 5.5–7.0 in other studies. It should be noted that site 1 was the only site with consistently elevated pH measurements of 9.0 or above. Sites 3-9 fell within the average pH range for a river system. It has been reported that *E. coli* does not grow in wastewater with a pH greater than 9.2 (Parhad and Rao, 1974), suggesting that this dataset is near the upper limit of survivability of *E. coli* in the water. Contextualizing this relationship is necessary if pH is being used to understand *E. coli* in the environment.

Lawrence et al. 2012 and Shamsudin et al. 2014 reported a significant positive relationship between water temperature and *E. coli* while this study showed a strong negative relationship. *E. coli* bacteria is a thermotolerant bacteria that can survive in a wide range of conditions (Petersen, 2020). Lawrence et al., 2012 reported that *E. coli* densities during an eight-year study were higher during the warm season and suggested that colder months showed lower densities because lower water temperatures inactivate *E. coli* or inhibit its growth. In contrast, we suggest the negative relationship between *E. coli* and water temperature could be explained by solar ultra-violet (UV) light that inactivates *E. coli* (Barcina et al., 1986; Whitman et al., 2008). The influence of UV light in the creek can be controlled by environmental factors including stream width, tree cover, and cloud cover. Choccolocco Creek widens considerably from the headwater site to its confluence with a major drainage basin. There are no direct measurements of UV light for Choccolocco Watershed, but we observed decreased *E. coli* concentrations during the warmer months and generally lower concentrations in the wider regions of Choccolocco Creek (**Appendix Table 3**).

Overall, the relationship observed between *E. coli* and these variables are likely related to the survivability of bacteria in the stream.

Weather variables like precipitation can control *E. coli* distributions. In a seven-year study, Tornevi et al. (2014) found that FIB increased exponentially two days after rainfall concomitant with increases in turbidity, suggesting that runoff as a result of heavy precipitation was a driver for elevated FIB. Similarly, Pandey et al. (2012) found that predictions of *E. coli* in streams improved when correlations to landcover were supplemented with 30-day rainfall data. Jardé et al., 2018 found that rainfall served as a mechanism of transport for contamination in soil to surface waters. In this study, cumulative precipitation one day, three days, and seven days prior to the sampling event was included as potential explanatory variables to evaluate the role of runoff that contribute to *E. coli* concentrations in the creek. Of these variables, the 1-day precipitation showed significant negative correlation to *E. coli*. Based on a hydrograph from USGS monitoring at site 6, our sampling took place primarily during baseflow (**Appendix Figure 5**). This suggest that runoff due to precipitation is not significantly impacting the samples collected.

4.2 Trends Across Watershed Groups

There are trends between the source-specific variables and *E. coli* across the watershed. The heat maps show relative changes in the variables moving downstream (**Figure 3** and **Figure 5**). The headwaters (site 9) showed low contamination of ECs and other indicator species (e.g., nitrate, phosphorus) as well as low *E. coli* and EC concentrations. The headwaters are located in the Talladega National Forest, and therefore it is expected to be a relatively pristine control site.

The Upper Choccolocco site has elevated concentrations of *E. coli* along with total nitrogen and industrial/home compound concentrations. It has the highest concentrations of herbicide/pesticide ECs and a high percentage of hay/cropland and forested land cover. This site

group is upstream of wastewater treatment outfall but there are septic systems located within the sites' watersheds (**Figures 1 & 11**). One indication that septic systems may be contributing to the contamination is the elevated home/industrial compound concentrations, but there is not elevated PPCP concentrations or Cu which were previously observed in septic effluent (Richards et al. 2016). Otherwise, there is a strong case for agricultural *E. coli* input in this region with elevated nitrogen and herbicide/pesticides with predominantly agricultural land cover.

The Middle Choccolocco site has higher concentrations of *E. coli* as well as higher concentrations of total nitrogen and total phosphorus. This site group is downstream of the wastewater treatment outfalls, and therefore we presume that PPCP concentrations are likely to originate from WWTP effluents with additional inputs from other anthropogenic sources like leaking septic systems or leaking sewer mains. Indeed, this site group watershed has a high septic system density (Jordan, 2022 and **Appendix Figure 4**), but the extent and condition of the sewer collection system in the region was not assessed in this study. This site had the highest concentration of both PPCP and industrial/home compound concentrations along with elevated levels of Cu. Richards et al. 2015 suggested that Cu could be enriched in regions affected by septic effluent. The high concentrations of copper and septic density at could be seen as evidence of *E. coli* contamination with a septic system signature, but Cu is most elevated further downstream at site 3 where there is a lower septic density. The wastewater treatment plant outfall just upstream of site 4 could explain this increase in Cu and ECs at these sites. Additionally, Cu is not elevated in site 7 upstream of all wastewater treatment outfall, which has high septic density and high normalized values *E. coli*. Cu concentrations could be a result of septic input as previously discussed (copper sulfate and pipes), but there is the possibility of erosion of earth materials as well. Ore minerals including chalcopyrite, pyrrhotite, and sphalerite were historically mined in the

state of Alabama (Espenshade, 1963). There is an old copper mine approximately 20 miles southeast of site 8 in Choccolocco Creek. This mining region lies within the Mad Indian Group near Morrison Crossing. This geologic group contains schists mined for copper mineral ores. It is unlikely that this rock could erode into the river causing higher concentrations of copper as the watershed has a different bedrock composition. Choccolocco Creek watershed lies in the Valley and Ridge Province of Alabama and includes districts of the Coosa Valley and Weisner Frontal Ridges. The creek runs through areas of Cambrian to Ordovician aged rock including the Knox Group, Upper and Middle Cambrian Series, and the Lower Cambrian series (Geological Survey of Alabama, 2021). These groups are composed of dolomite, limestone, sandstones, and shales (Planert et al., 1989).

The Lower Choccolocco shows decreased concentrations of *E. Coli* in comparison to Upper and Middle Choccolocco. This site is elevated in total nitrogen and phosphorous and PPCPs. It has the greatest agricultural land cover and highest copper concentrations. This site is downstream of two wastewater treatment outfalls and represents the widest parts of the creek. The location of the site and the mixed signal of both agricultural and human waste indicators, which creates a heterogeneous signature in the heat map. The normalized values are lower than site 4, which is likely the result of dilution downstream c (**Figure 11**).

4.3 Potential Sources of E. coli

Two potential sources, WWTPs and SSOs, can be eliminated as major drivers of *E. coli* within this dataset. There are no records of WWTP citations for exceeding *E. coli* thresholds for their respective NPDES permits from ADEM. Additionally, there were 4 SSO reports from April 1, 2021, to October 1, 2021. However, the dates of the SSOs reported in the area do not align with the sampling dates for this study, and there was a minimum of seven days between the closest SSO

and sampling date. Therefore, the source(s) of *E. coli* over the sampling period in this watershed are likely to be diffuse.

The heat map (**Figures 6 & 11**) and the correlative relationships (**Table 1**) have illustrated there is a strong link between *E. coli* and EC compounds. These relationships support our hypothesis and suggest that there is a human-component to *E. coli* concentrations in the Choccolocco Creek.

The next step in the analysis was to discern patterns and relationships between the categories of ECs and *E. coli* that could lead to narrowing the potential diffuse source(s) of *E. coli*. PCA of ECs was calculated (**Table 4**) to connect land use and specific sources to contamination. PCA of the EC compounds in this study did not yield well defined variable loading aggregations related to compound class that indicate sources, nor were there clear site delineations when visualizing PC scores that indicate sites that experience similar profiles of ECs over the season. Karpuzcu et al, (2014) found that atrazine, metolachlor, and acetochlor covaried together in the PCA loading plots to create a distinct “agriculture” EC signature. Whereas in this study, loadings of herbicides and pesticides were distributed in different quadrants of the biplot and did not covary. This suggests that there is not a strong “agriculture” signature that is sustained throughout the watershed, and that there is potential mixing of these chemicals from various sources. One reason for this discrepancy is that the previous study included 120 samples in their PCA whereas we were only able to examine 16 samples. We would suggest any future research to focus on collecting more data over time, specifically EC and *E. coli* concentrations. This would likely lead to more accurate analysis and delineation of site characteristics based on sources.

4.4 Limitations

The first and perhaps largest limitation of this study was the water sampling design. Samples were taken once a month for a six-month period at each of nine sites. Of these samples, only four samples from four sites were sent off for endocrine disruptor analysis at Eurofins Analysis, yielding 16 datapoints for EC analysis. The first PCA calculated using water quality data was able to examine 54 datapoints, but for PCA analysis of ECs, the number of pairs had to be reduced down to 16. While this is still a relatively large-scale study that examines an entire watershed compared to previous studies that examine between 2 and 5 sites (Richards et al., 2016 and Jardé et al., 2018), previous research by Lawrence et al. (2009) and Karpuzcu et al. (2014) evaluated relationships among water quality variables and *E. coli* using hundreds or thousands of datapoints versus our 54 and 16 datapoints.

Along with an increase of samples over time, it would have benefited our study to increase the samples geographically. For example, EC loading from septic systems in site 4 cannot be clearly distinguished from WWTP effluent nor leaking sewer lines. Investigating the tributaries to Choccolocco Creek within this portion of the watershed may have helped to delineate these sources. Still, this work guide future sampling efforts to this critical section of the Creek.

Finally, this study relies on the assumption that the non-point sources of *E. coli* in this system are relatively uniform over space and time in the watershed. For example, this study did not account for changes in the naturalized *E. coli* community, nor evaluate how that community changed throughout the watershed. Additionally, there is also no way to ground-truth contributions from malfunctioned septic systems and leaky sewer lines in the region because this data does not exist. Future work should be directed at filling these data gaps to ensure watershed assessments of non-point sources of pollution are more accurately represented.

5. Conclusion

The data in this study indicated there was a human component to *E. coli* contamination in Choccolocco Creek due to the significant relationship observed between *E. coli* and EC compounds. Further assessing ECs in their subcategories did not yield source delineation in the data that would point to specific source(s). Therefore, we cannot say with certainty whether contamination is coming from a predominantly septic source or wastewater treatment source. Further investigations that target Choccolocco Creek tributaries should be prioritized so that the WWTP influence can be delineated from septic influence, and therefore a more precise interpretation of EC and *E. coli* relationships can be made.

Watersheds with a history of elevated *E. coli* measurements may be impacted by diffuse fecal contamination at a higher rate, but pinpointing the source of fecal contamination proves difficult. Assessing contamination takes an understanding of the total environment its multicollinear variables on a local scale.

References

- ADEM (2020) Alabama's Water Quality Assessment and Listing Methodology
ADEM Water Quality Branch (2016) 2016 Integrated Water Quality Monitoring and Assessment Report. 346–346
- An Y-J, Breindenbach GP (2005) Monitoring E. coli and total coliforms in natural spring water as related to recreational mountain areas. *Environmental Monitoring and Assessment* 102:131–137. <https://doi.org/10.1007/s10661-005-4691-9>
- Barcina I, Arana I, Iriberry J, Egea L (1986) Influence of light and natural microbiota of the Butrón river on E. coli survival. *Antonie van Leeuwenhoek* 52:555–566. <https://doi.org/10.1007/BF00423416>
- Boix C, Ibáñez M, Fabregat-Safont D, Morales E, Pastor L, Sancho J, Sánchez-Ramírez JE, Hernández F, (2016) Analytical methodologies based on LC–MS/MS for monitoring selected emerging compounds in liquid and solid phases of the sewage sludge. *MethodsX*, 3, 333–342. <https://doi.org/https://doi.org/10.1016/j.mex.2016.04.010>
- Bradley PM, Journey CA, Berninger JP, et al (2019) Mixed-chemical exposure and predicted effects potential in wadeable southeastern USA streams. *Science of The Total Environment* 655:70–83. <https://doi.org/10.1016/j.scitotenv.2018.11.186>
- Brovini EM, de Deus BCT, Vilas-Boas JA, et al (2021) Three-bestseller pesticides in Brazil: Freshwater concentrations and potential environmental risks. *Science of The Total Environment* 771:144754–144754. <https://doi.org/10.1016/j.scitotenv.2020.144754>
- Buerge IJ, Poiger T, Müller MD, Buser HR (2006) Combined sewer overflows to surface waters detected by the anthropogenic marker caffeine. *Environmental Science and Technology*. <https://doi.org/10.1021/es052553l>
- Carazo-Rojas E, Pérez-Rojas G, Pérez-Villanueva M, et al (2018) Pesticide monitoring and ecotoxicological risk assessment in surface water bodies and sediments of a tropical agro-ecosystem. *Environmental Pollution* 241:800–809. <https://doi.org/10.1016/j.envpol.2018.06.020>
- Carpenter CMG, Helbling DE (2018) Widespread Micropollutant Monitoring in the Hudson River Estuary Reveals Spatiotemporal Micropollutant Clusters and Their Sources. *Environmental Science & Technology* 52:6187–6196. <https://doi.org/10.1021/acs.est.8b00945>
- City of Oxford Water Works & Sewer Board (2021) Discharge Monitoring Report of Tull C Allen.
- Cogger C (1988) On-site Septic Systems: The risk of groundwater contamination. *Journal of Environmental Health* 51:12–16
- Coleman BL, Louie M, Salvadori MI, et al (2013) Contamination of Canadian private drinking water sources with antimicrobial resistant Escherichia coli. *Water Research* 47:3026–3036. <https://doi.org/10.1016/j.watres.2013.03.008>
- de Souza RM, Seibert D, Quesada HB, et al (2020) Occurrence, impacts and general aspects of pesticides in surface water: A review. *Process Safety and Environmental Protection* 135:22–37. <https://doi.org/10.1016/j.psep.2019.12.035>
- Espenshade, G. H., & U.S. Govt. Print. Off. (1963). Geology of some copper deposits in North Carolina, Virginia, and Alabama. In *Bulletin*. <https://doi.org/10.3133/b11421>
- Glinski DA, Purucker ST, Van Meter RJ, et al (2018) Analysis of pesticides in surface water, stemflow, and throughfall in an agricultural area in South Georgia, USA. *Chemosphere* 209:496–507. <https://doi.org/10.1016/j.chemosphere.2018.06.116>

- Gregor J, Garrett N, Gilpin B, et al (2002) Use of classification and regression tree (CART) analysis with chemical faecal indicators to determine sources of contamination. *New Zealand Journal of Marine and Freshwater Research* 36:387–398. <https://doi.org/10.1080/00288330.2002.9517095>
- Gross A, Boyd CE (1998) A Digestion Procedure for the Simultaneous Determination of Total Nitrogen and Total Phosphorus in Pond Water. *Journal of the World Aquaculture Society* 29:300–303. <https://doi.org/10.1111/j.1749-7345.1998.tb00650.x>
- Hansen S, Messer T, Mittelstet A, et al (2020) Escherichia coli concentrations in waters of a reservoir system impacted by cattle and migratory waterfowl. *Science of The Total Environment* 705:135607–135607. <https://doi.org/10.1016/j.scitotenv.2019.135607>
- Ishii S, Sadowsky MJ (2008) Escherichia coli in the environment: Implications for water quality and human health. *Microbes and Environments*. <https://doi.org/10.1264/jsme2.23.101>
- Jang J, Hur HG, Sadowsky MJ, et al (2017) Environmental Escherichia coli: ecology and public health implications—a review. *Journal of Applied Microbiology*. <https://doi.org/10.1111/jam.13468>
- Jolliffe, IT (1982) A Note on the Use of Principal Components in Regression. *Journal of the Royal Statistical Society. Series C (Applied Statistics)*, 31(3), 300–303. <https://doi.org/10.2307/2348005>
- Jolliffe IT, Cadima J (2016) Principal component analysis: A review and recent developments. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*. <https://doi.org/10.1098/rsta.2015.0202>
- Jones-Lepp TL (2006) Chemical markers of human waste contamination: Analysis of urobilin and pharmaceuticals in source waters. *Journal of Environmental Monitoring* 8:472–478. <https://doi.org/10.1039/B512858G>
- Jordan M (2022) Evaluating the impact of onsite wastewater treatment systems on watershed contamination, Choccolocco Creek Watershed, AL
- Karpuzcu ME, Fairbairn D, Arnold WA, et al (2014) Identifying sources of emerging organic contaminants in a mixed use watershed using principal components analysis. *Environmental Science: Processes & Impacts* 16:2390–2399. <https://doi.org/10.1039/C4EM00324A>
- Khan FM, Gupta R, Sekhri S (2021) Superposition learning-based model for prediction of E. coli in groundwater using physico-chemical water quality parameters. *Groundwater for Sustainable Development* 13:100580–100580. <https://doi.org/10.1016/j.gsd.2021.100580>
- Kumar M, Chaminda T, Honda R, Furumai H (2019) Vulnerability of urban waters to emerging contaminants in India and Sri Lanka: Resilience framework and strategy. *APN Science Bulletin* 9:
- Lawrence SJ (2012) Escherichia coli Bacteria Density in Relation to Turbidity, Streamflow Characteristics, and Season in the Chattahoochee River near Atlanta, Georgia, October 2000 through September 2008 — Description, Statistical Analysis, and Predictive Modeling. 81–81
- Leal-Bautista RM; H-Z Galdy; Jaime M, Norma A; Cuevas R, Germán; Velázquez Oliman, Guadalupe, Oliman V (2011) Pathogens and Pharmaceuticals Pollutants as Indicators of Contamination at the Northeastern Aquifer of Quintana Roo. *Tropical and Subtropical Agroecosystems* 13:211–219
- Lin AYC, Lin CA, Tung HH, Chary NS (2010) Potential for biodegradation and sorption of acetaminophen, caffeine, propranolol and acebutolol in lab-scale aqueous environments. *Journal of Hazardous Materials*. <https://doi.org/10.1016/j.jhazmat.2010.07.017>
- Luo Y, Guo W, Ngo HH, et al (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*. <https://doi.org/10.1016/j.scitotenv.2013.12.065>

- Lusk M, Toor GS, Obreza T (2018) Onsite Sewage Treatment and Disposal Systems: Phosphorus. *Institute of Food and Agricultural Sciences, Onsite Sewage Treatment and Disposal Systems*. <http://edis.ifas.ufl.edu/ss549>.
- Mukhopadhyay A, Duttagupta S, Mukherjee A (2022) Emerging organic contaminants in global community drinking water sources and supply: A review of occurrence, processes and remediation. *Journal of Environmental Chemical Engineering* 10:107560–107560. <https://doi.org/10.1016/j.jece.2022.107560>
- Naidoo S, Olaniran AO (2014) Treated Wastewater Effluent as a Source of Microbial Pollution of Surface Water Resources. *International Journal of Environmental Research and Public Health* 11:249–270. <https://doi.org/10.3390/ijerph110100249>
- Parhad NM, Rao NU (1974) Effect of pH on Survival of *Escherichia coli*. *Journal (Water Pollution Control Federation)* 46:980–986
- Paul M, Wolf L, Fund K, et al (2004) Microbiological Condition of Urban Groundwater in the Vicinity of Leaky Sewer Systems. *Acta hydrochimica et hydrobiologica* 32:351–360. <https://doi.org/10.1002/aheh.200400539>
- Planert M, and Pritchett JL (1989) Geohydrology and Susceptibility of Major Aquifers to Surface Contamination in Alabama; Area 4. *Water Resources Investigation Report* 88-4133: <https://doi.org/10.3133/wri884133>.
- Okweye P, Garner K, McCullers K, et al (2021) The Presence of Contaminants of Emerging Concern (CECs) and Volatile Organic Compounds (VOCs) in Northern Alabama Aquatic Ecosystems. *Journal of Environmental Science and Engineering B* 10: <https://doi.org/10.17265/2162-5263/2021.03.001>
- Pandey PK, Soupir ML, Haddad M, Rothwell JJ (2012) Assessing the impacts of watershed indexes and precipitation on spatial in-stream *E. coli* concentrations. *Ecological Indicators*, 23, 641–652. <https://doi.org/https://doi.org/10.1016/j.ecolind.2012.05.023>
- Petersen F, Hubbart JA (2020) Physical Factors Impacting the Survival and Occurrence of *Escherichia coli* in Secondary Habitats. *Water* 12: <https://doi.org/10.3390/w12061796>
- Petrie B (2021) A review of combined sewer overflows as a source of wastewater-derived emerging contaminants in the environment and their management. *Environ. Sci. and Pollut. Research* 28:32095–32110. <https://doi.org/10.1007/s11356-021-14103-1>
- Richards S, Paterson E, Withers PJA, Stutter M (2016) Septic tank discharges as multi-pollutant hotspots in catchments. *Science of the Total Environment*. <https://doi.org/10.1016/j.scitotenv.2015.10.160>
- Richards S, Withers PJA, Paterson E, McRoberts CW, Stutter M (2017) Potential tracers for tracking septic tank effluent discharge in watercourses. *Environ. Pollut*, 228, 245-255. <https://doi.org/10.1016/j.envpol.2017.05.044>.
- Shamsudin SN, Rahman MHF, Taib MN, et al (2016) Analysis between *Escherichia Coli* growth and physical parameters in water using Pearson correlation. Pp 131–136
- Somarelli JA, Makarewicz JC, Sia R, Simon R (2007) Wildlife identified as major source of *Escherichia coli* in agriculturally dominated watersheds by BOX A1R-derived genetic fingerprints. *Journal of Environmental Management* 82:60–65. <https://doi.org/10.1016/j.jenvman.2005.12.013>
- Staley ZR, Grabuski J, Sverko E, et al (2016) Comparison of Microbial and Chemical Source Tracking Markers To Identify Fecal Contamination Sources in the Humber River (Toronto, Ontario, Canada) and Associated Storm Water Outfalls. *Applied and Environmental Microbiology* 82:6357–6366. <https://doi.org/10.1128/AEM.01675-16>

- Stepenuck KF, Wolfson LG, Liukkonen BW, et al (2011) Volunteer monitoring of E. coli in streams of the upper Midwestern United States: a comparison of methods. *Environ Monit Assess* 174:625–633. <https://doi.org/10.1007/s10661-010-1483-7>
- Sui Q, Cao X, Lu S, Zhao W, Qiu Z, Yu G (2015) Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review. *Emerging Contaminants* 1, 14-24. <https://doi.org/10.1016/j.emcon.2015.07.001>
- Tabachnick, BG, Fidell, LS (2007) *Using Multivariate Statistics*. 5th Edition, Allyn & Bacon/Pearson Education, New York.
- Togola A, Budzinski H (2007) Analytical development for analysis of pharmaceuticals in water samples by SPE and GC-MS. *Analytical and Bioanalytical Chemistry*. <https://doi.org/10.1007/s00216-007-1251-x>
- Tran NH, Reinhard M, Yew-Hoong Gin K (2018) Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. *Water Research*, 133, 182-207. <https://doi.org/10.1016/j.watres.2017.12.029>
- Tran NH, Li J, Hu J (2013) Occurrence and suitability of pharmaceuticals and personal care products as molecular markers for raw wastewater contamination in surface water and groundwater. *Environ. Sci. and Pollut. Research*. 21, 4727–4740. <https://doi.org/10.1007/s11356-013-2428-9>
- U.S. Census of Agriculture, County Profile: Calhoun County Alabama. 2017. <https://www.nass.usda.gov/Publications/AgCensus/2017>.
- U.S. Census of Agriculture, County Profile: Talladega County Alabama. 2017.
- U.S. Environmental Protection Agency (2016) *Quick Guide To Drinking Water Sample Collection* 2ND EDITION. 6–20
- USDA NASS Southeastern Region. Alabama County Estimates: Cattle 2019-2020. 2020, No. 706, 1–4.
- U.S. Geological Survey, 2021, National Water Information System data available on the World Wide Web 18 (USGS Water Data for the Nation): <https://waterdata.usgs.gov/nw>.
- Valsecchi S, Polesello S, Mazzoni M, Rusconi M, Petrovic M (2015) On-line sample extraction and purification for the LC–MS determination of emerging contaminants in environmental samples. *Trends in Environmental Analytical Chemistry*, 8, 27–37. <https://doi.org/https://doi.org/10.1016/j.teac.2015.08.001>
- Verhougstraete MP, Martin SL, Hynman DW, et al (2015) Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. *Proceedings of the National Academy of Sciences* 112:10419–10424. <https://doi.org/10.1073/pnas.1415836112>
- Vialle C, Sablayrolles C, Lovera M, et al (2011) Monitoring of water quality from roof runoff: Interpretation using multivariate analysis. *Water Research* 45:3765–3775. <https://doi.org/10.1016/j.watres.2011.04.029>
- Watkinson AJ, Micalizzi GB, Graham GM, et al (2007) Antibiotic-Resistant *Escherichia coli* in Wastewaters, Surface Waters, and Oysters from an Urban Riverine System. *Applied and Environmental Microbiology* 73:5667–5670. <https://doi.org/10.1128/AEM.00763-07>
- Whitman RL, Przybyla-Kelly K, Shively DA, et al (2008) Sunlight, season, snowmelt, storm, and source affect E. coli populations in an artificially ponded stream. *Science of The Total Environment* 390:448–455. <https://doi.org/10.1016/j.scitotenv.2007.10.014>
- Wilkinson JL, Boxall ABA, Kolpin DW, et al (2022) Pharmaceutical pollution of the world’s rivers. *Proceedings of the National Academy of Sciences* 119:e2113947119–e2113947119. <https://doi.org/10.1073/pnas.2113947119>

Zhang Y, Rashid A, Guo S, et al (2022) Spatial autocorrelation and temporal variation of contaminants of emerging concern in a typical urbanizing river. *Water Research* 212:118120–118120. <https://doi.org/10.1016/j.watres.2022.118120>

Appendix A

Table of Contents	
I. Calibration Figures.....	54
II. Field and Lab Data	56
III. Pour Point Maps.....	70
IV. Hydrograph.....	72

Calibration Figures

The data presented in Figures A1 and A2 represent our calibration curves for gas-chromatography mass spectrometry analysis of pharmaceuticals in the Ojeda Lab. This calibration was used to determine the concentration of pharmaceutical compounds in our water samples.

Figure A1. The calibration curve for caffeine using six standards on the Ojeda Lab GC-MS. Calibration levels included 0.5, 1, 5, 10, 20, and 30 ng/mL of pure caffeine. The x-axis is concentration in ng/mL and the y-axis is the response. The linear equation to describe the calibration is $y = 256551x - 452149$. The $R^2 = 0.9709$.

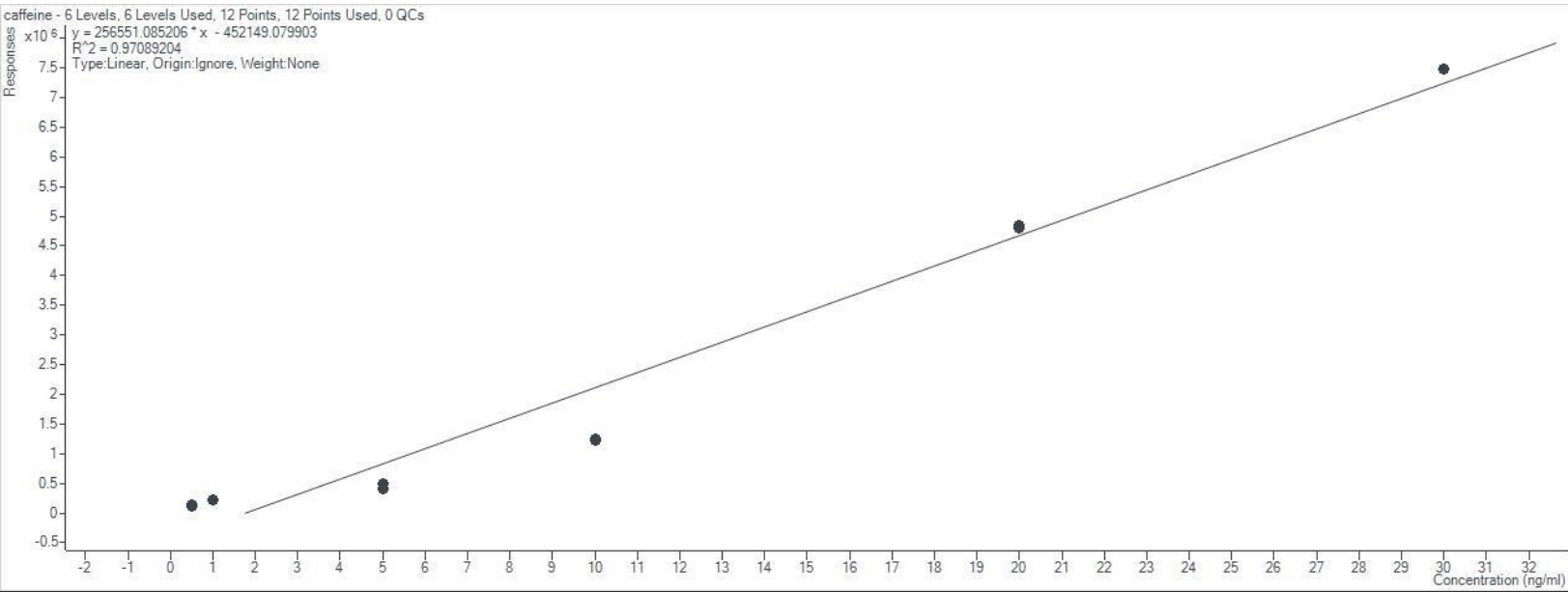
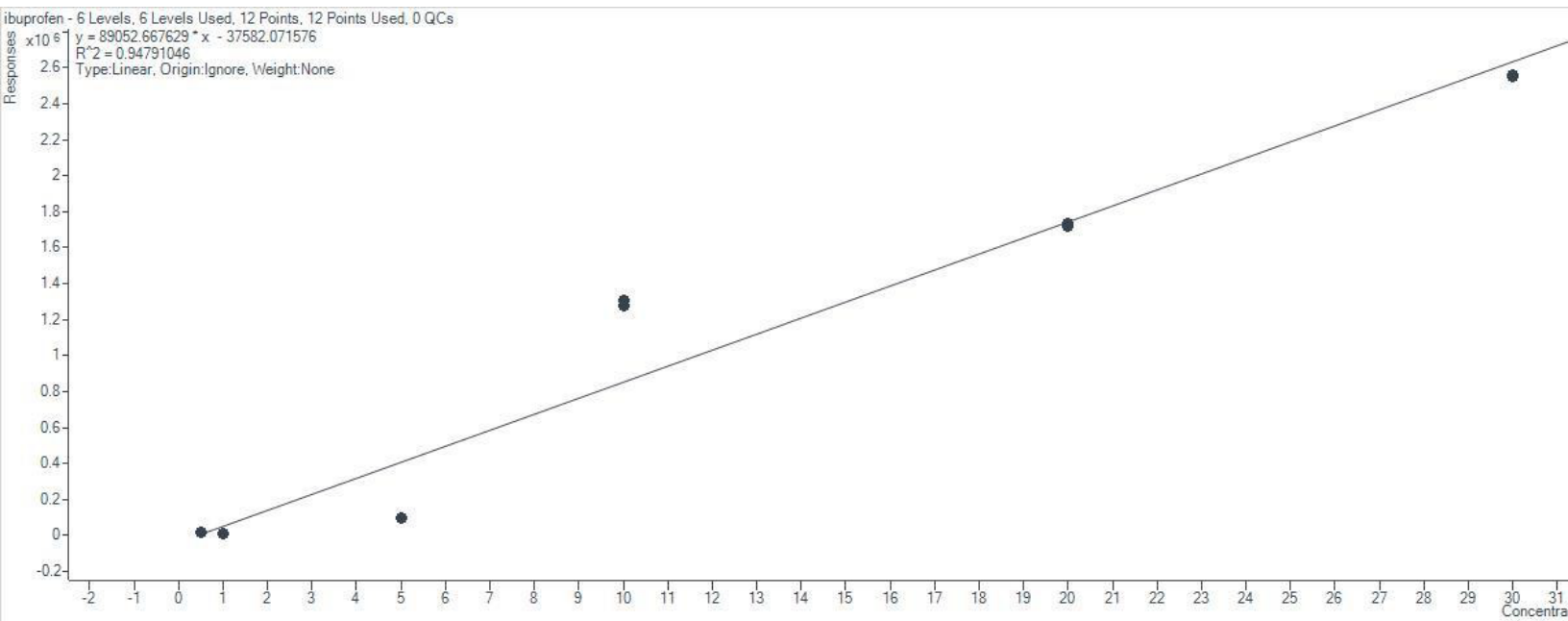


Figure A2. The calibration curve for ibuprofen using six standards on the Ojeda Lab GC-MS. Calibration levels included 0.5, 1, 5, 10, 20, and 30 ng/mL of pure ibuprofen. The x-axis is concentration in ng/mL and the y-axis is the response. The linear equation to describe the calibration is $y = 89053x - 37582$. The $R^2 = 0.9479$.



Field and Lab Data

This section includes all pertinent data collected in the field, in the lab, and from samples sent off for analysis.

Table A1. Pharmaceutical Data: Eurofins

Data reported by Eurofins Analytical Lab is organized by site. Sites 3, 4, 7, and 9 were analyzed during the months of May, June, August, and September 2021. In total, 32 EC compounds were detected in our 16 water samples over 4 months. Eurofins laboratories uses LC-MS techniques for their Endocrine Disruptor suite of chemical analysis. The concentrations reported below are in ng/L.

Date	Site #	1,7-Dimethylxanthine	Acetaminophen	Atenolol	Atrazine	BPA	Bromacil	Caffeine	Carbamazepine	DEA	DEET	DIA
5/27/2021	3	-	-	-	100	-	-	20	5.2	9.6	37	5.4
7/29/2021	3	-	-	-	28	-	-	31	-	18	-	-
8/26/2021	3	-	-	-	-	-	-	-	-	-	-	-
9/30/2021	3	-	-	-	-	-	-	-	-	-	-	-
5/27/2021	4	-	-	8.3	80	19	5.5	17	6	-	66	-
7/29/2021	4	-	-	-	22	37	-	-	-	-	-	-
8/26/2021	4	-	14	6.4	-	-	-	53	-	-	-	-
9/30/2021	4	7.9	29	6.6	15	-	6.7	100	6.3	-	-	-
5/27/2021	7	-	-	-	280	-	-	180	-	5.1	10	-
7/29/2021	7	-	-	-	70	-	-	-	-	74	-	-
8/26/2021	7	-	-	-	-	-	-	-	-	-	-	-
9/30/2021	7	-	-	-	-	-	-	-	-	-	-	-
5/27/2021	9	-	-	-	-	-	-	-	-	-	-	-
7/29/2021	9	-	-	-	-	-	-	-	-	220	-	-
8/26/2021	9	-	-	-	-	-	-	26	-	-	-	-
9/30/2021	9	12	-	-	-	-	-	31	-	-	-	-
Date	Site #	Estradiol	Ethylparaben	Flumequinone	Ibuprofen	Iohexol	Lidocaine	Metformin	Metolachlor	Naproxen	OUST	Phenazone
5/27/2021	3	-	-	-	-	340	12	-	10	-	-	-
7/29/2021	3	-	20	21	-	240	5	-	6	-	-	41
8/26/2021	3	-	-	-	-	120	-	-	-	-	-	-

9/30/2021	3	-	-	-	-	98	-	-	-	26	-	-
5/27/2021	4	-	-	-	-	1400	17	-	-	-	-	-
7/29/2021	4	-	-	-	-	590	15	-	12	-	-	-
8/26/2021	4	-	-	-	-	-	12	-	-	-	6.9	-
9/30/2021	4	-	-	-	-	-	26	-	-	-	-	-
5/27/2021	7	14	26	-	28	-	-	-	-	-	-	-
7/29/2021	7	14	-	28	-	-	-	22	72	-	-	52
8/26/2021	7	-	-	-	-	-	-	-	-	-	-	-
9/30/2021	7	-	-	-	-	-	-	-	-	-	-	-
5/27/2021	9	13	24	-	-	-	-	-	-	-	-	-
7/29/2021	9	-	-	26	-	-	-	-	-	-	-	230
8/26/2021	9	-	-	-	-	-	-	-	-	-	-	-
9/30/2021	9	-	-	-	-	-	60	-	-	-	-	14
Date	Site #	Primidone	Salicylic Acid	Sucralose	Sulfamethoxazole	TCEP	TCPP	Theobromine	Theophylline	Thiabendazole	Trimethoprim	
5/27/2021	3	-	300	710	15	-	-	-	12	7	-	
7/29/2021	3	-	-	460	-	11	-	-	-	-	-	
8/26/2021	3	-	-	490	-	-	-	-	-	-	-	
9/30/2021	3	-	-	590	-	-	-	-	-	-	-	
5/27/2021	4	-	-	1100	29	12	-	-	18	-	6.6	
7/29/2021	4	-	300	690	14	-	230	-	-	-	-	
8/26/2021	4	-	-	-	20	-	-	120	-	-	-	
9/30/2021	4	6.8	-	-	49	22	400	130	17	5.2	-	
5/27/2021	7	-	270	-	-	14	380	-	13	-	-	
7/29/2021	7	-	-	-	-	12	-	-	-	-	-	
8/26/2021	7	-	-	-	-	-	-	-	-	-	-	
9/30/2021	7	-	-	-	-	-	-	-	-	-	-	
5/27/2021	9	-	710	-	-	-	-	-	-	-	-	
7/29/2021	9	-	-	-	-	-	-	-	-	-	-	
8/26/2021	9	-	-	-	-	-	-	50	-	-	-	
9/30/2021	9	-	-	-	-	-	260	74	24	-	-	

Table 2A. Pharmaceutical Data: Ojeda Lab

Data collected from SPE and GC-MS analysis in the Ojeda Lab. The detection limit for these techniques is around 10-15ng/L and none of our data meets that limit. All values reported below are in ng/L.

Date	Site #	Ibuprofen	Caffeine
4/29/2021	1	0.566385	0.113277
4/29/2021	2	0.132962	0.026592
4/29/2021	3	0.27401	0.054802
4/29/2021	4	2.695519	0.539104
4/29/2021	5	0.430209	0.086042
4/29/2021	6	0.321569	0.064314
4/29/2021	7	0.125593	0.025119
4/29/2021	8	0.639399	0.12788
4/29/2021	9	0.117555	0.023511
5/27/2021	1	0.185283	0.037057
5/27/2021	2	0.236582	0.047316
5/27/2021	3	0.479278	0.095856
5/27/2021	4	0.11281	0.022562
5/27/2021	5	0.839793	0.167959
5/27/2021	6	0.10831	0.021662
5/27/2021	7	0.168269	0.033654
5/27/2021	8	0.084666	0.016933
5/27/2021	9	0.084415	0.016883
6/24/2021	1	0.226191	0.045238
6/24/2021	2	0.135098	0.02702
6/24/2021	3	0.123449	0.02469
6/24/2021	4	0.087926	0.017585
6/24/2021	5	0.185176	0.037035
6/24/2021	6	0.11941	0.023882
6/24/2021	7	0.704915	0.140983
6/24/2021	8	0.128604	0.025721
6/24/2021	9	0.096776	0.019355
7/29/2021	1	0.298734	0.059747
7/29/2021	2	0.66983	0.133966
7/29/2021	3	0.136434	0.027287
7/29/2021	4	0.133214	0.026643
7/29/2021	5	0.110862	0.022172
7/29/2021	6	0.612446	0.122489
7/29/2021	7	0.232941	0.046588
7/29/2021	8	0.103461	0.020692
7/29/2021	9	0.202816	0.040563
8/26/2021	1	0.131366	0.026273
8/26/2021	2	0.827805	0.165561

8/26/2021	3	0.528495	0.105699
8/26/2021	4	2.166583	0.433317
8/26/2021	5	0.371315	0.074263
8/26/2021	6	1.444935	0.288987
8/26/2021	7	0.193088	0.038618
8/26/2021	8	0.531868	0.106374
8/26/2021	9	0.71287	0.142574
9/30/2021	1	0.136668	0.027334
9/30/2021	2	0.117463	0.023493
9/30/2021	3	0.121004	0.024201
9/30/2021	4	0.14538	0.029076
9/30/2021	5	0.719074	0.143815
9/30/2021	6	0.730197	0.146039
9/30/2021	7	0.14032	0.028064
9/30/2021	8	0.193569	0.038714
9/30/2021	9	0.136536	0.027307

Table A3. Field and Lab Data

These table reports field and lab measurements. Table A3 includes conductivity, pH water temperature, and dissolved oxygen were measure in the field at each site using an EXO Sonde probe. *E. coli* enumeration, nitrate-nitrogen, and ammonium-nitrogen were measured in the Ojeda Lab. Average total nitrogen and phosphorous were measured in Dr. Wilson’s Lab at Auburn Fisheries. EC data was reported by Eurofins Analysis. * indicates no data was collected for the sample point.

Date	Site #	<i>E. coli</i>	pH	Water Temp. (°C)	Conductivity (µS)	Dissolved Oxygen %	Nitrate-Nitrogen (mg/L)	Ammonia-Nitrogen (mg/L)	Avg. Total Nitrogen (µg/L)	Avg. Total Phosphorous (µg/L)	1-Day Precipitation (mm/day)	Total EC concentration (ng/L)	Number of EC compounds
4/29/2021	1	200.0	7.37	24.25	161.0	211.8	0.407	0.0389	527.81	33.46	0.00	*	*
4/29/2021	2	166.7	6.7	21.53	167.0	138.4	0.686	0.0273	788.54	56.73	0.00	*	*
4/29/2021	3	300.0	6.55	21.46	163.0	140.2	0.652	0.0213	750.80	59.81	0.00	*	*
4/29/2021	4	500.0	6.54	20.91	143.0	166.4	0.697	0.0178	833.07	101.79	0.00	*	*
4/29/2021	5	833.3	6.3	20.53	118.0	154.7	0.581	0.0122	765.65	77.05	0.00	*	*
4/29/2021	6	33.3	6.69	20.41	92.0	140.7	0.371	0.0098	285.03	45.28	0.00	*	*
4/29/2021	7	633.3	6.41	19.90	98.0	135.5	0.315	0.0090	422.35	43.80	0.00	*	*
4/29/2021	8	666.7	6.91	19.35	85.0	120.5	0.265	0.0086	391.73	44.79	0.00	*	*
4/29/2021	9	50.0	6.78	17.39	33.0	145.5	0.136	0.0090	39.46	44.66	0.00	*	*
5/27/2021	1	0.0	7.67	28.42	182.0	142.1	0.298	0.1910	495.18	110.40	0.00	*	*
5/27/2021	2	0.0	6.93	27.34	203.0	145.4	0.538	0.0838	606.29	116.20	0.00	*	*
5/27/2021	3	150.0	6.24	23.77	198.0	120.3	0.578	0.0570	750.64	125.00	0.00	1583.2	14
5/27/2021	4	450.0	6.73	23.03	164.0	132.9	0.684	0.0249	812.81	210.20	0.00	2784.4	14
5/27/2021	5	400.0	6.54	23.16	149.0	115.4	0.616	0.0317	918.07	142.20	0.00	*	*
5/27/2021	6	450.0	6.63	22.92	101.2	101.2	0.307	0.0190	430.55	93.80	0.00	*	*
5/27/2021	7	483.3	6.42	21.71	116.0	92.7	0.314	0.0061	411.16	83.20	0.00	1220.1	11
5/27/2021	8	433.3	6.58	21.27	100.0	102.8	0.277	0.0000	473.02	84.50	0.00	*	*
5/27/2021	9	350.0	6.1	18.17	36.0	114.1	0.000	0.0000	84.91	81.70	0.00	747	3
6/24/2021	1	0.0	7.5	27.61	107.4	127.2	0.518	0.0304	529.12	72.93	0.00	*	*
6/24/2021	2	316.7	7.6	23.89	55.7	101.3	0.598	0.0154	504.45	64.47	0.00	*	*
6/24/2021	3	216.7	7.59	23.46	113.4	109.6	0.566	0.0114	529.36	92.97	0.00	*	*
6/24/2021	4	300.0	7.44	22.69	91.2	100.5	0.393	0.0080	381.44	74.17	0.00	*	*

6/24/2021	5	300.0	7.31	22.06	79.1	116.0	0.380	0.0081	355.24	96.56	0.00	*	*
6/24/2021	6	133.3	7.25	22.07	58.3	88.9	0.252	0.0083	223.44	82.54	0.00	*	*
6/24/2021	7	216.7	7.22	21.73	60.8	101.3	0.203	0.0082	232.53	66.34	0.00	*	*
Date	Site #	<i>E. coli</i>	pH	Water Temp. (°C)	Conductivity (µS)	Dissolved Oxygen %	Nitrate-Nitrogen (mg/L)	Ammonia-Nitrogen (mg/L)	Avg. Total Nitrogen (µg/L)	Avg. Total Phosphorous (µg/L)	1-Day Precipitation (mm/day)	Total EC concentration (ng/L)	Number of EC compounds
6/24/2021	8	50.0	7.24	21.16	53.2	101.8	0.180	0.0083	252.57	54.27	0.00	*	*
6/24/2021	9	200.0	6.95	18.00	32.6	113.8	0.752	0.0081	95.65	42.71	0.00	*	*
7/29/2021	1	33.3	9.22	33.55	144.5	162.4	0.445	0.0370	395.57	49.46	0.00	*	*
7/29/2021	2	50.0	7.76	27.67	122.3	99.5	0.523	0.0322	521.30	66.46	0.00	*	*
7/29/2021	3	316.7	7.76	25.78	124.3	103.1	0.508	0.0289	508.82	58.21	0.00	881	11
7/29/2021	4	116.7	7.97	25.89	151.5	102.8	0.593	0.0265	720.65	64.98	0.00	1910	9
7/29/2021	5	50.0	7.99	24.85	140.9	89.9	0.577	0.0266	770.26	69.17	0.00	*	*
7/29/2021	6	100.0	7.7	25.47	114.9	95.6	0.390	0.0251	467.33	45.64	0.00	*	*
7/29/2021	7	183.3	7.6	24.28	130.6	90.5	0.421	0.0240	494.16	61.29	0.00	344	8
7/29/2021	8	250.0	7.6	24.05	118.1	91.6	0.427	0.0226	377.48	42.44	0.00	*	*
7/29/2021	9	133.3	7.41	21.93	45.1	97.9	0.181	0.0220	60.19	39.73	0.00	476	3
8/26/2021	1	579.4	9.18	30.93	142.0	146.7	0.593	0.0365	532.28	42.53	9.72	*	*
8/26/2021	2	0.0	7.98	27.46	143.1	95.2	0.650	0.0355	677.56	37.22	10.27	*	*
8/26/2021	3	0.0	8.24	27.46	143.3	105.5	0.585	0.0353	743.20	50.75	7.51	610	2
8/26/2021	4	178.5	7.96	26.56	125.4	97.2	0.576	0.0369	788.61	80.46	6.14	112.3	7
8/26/2021	5	12.0	7.7	25.51	14.3	87.9	0.615	0.0306	1034.68	69.84	0.00	*	*
8/26/2021	6	0.0	7.68	24.61	84.5	91.5	0.341	0.0310	467.58	16.99	15.20	*	*
8/26/2021	7	1.0	7.69	23.84	98.9	89.0	0.322	0.0252	633.69	24.33	15.63	0	0
8/26/2021	8	2.0	7.54	23.58	88.6	90.3	0.336	0.0247	597.61	16.99	10.51	*	*
8/26/2021	9	0.0	7.36	21.71	37.1	96.3	0.175	0.0257	299.90	28.37	4.78	76	2
9/30/2021	1	33.3	9.17	26.7	145.7	150.8	0.765	0.0364	472.073	63.080	0.00	*	*
9/30/2021	2	16.7	8.76	25.4	195.4	126.3	0.824	0.0672	541.632	48.310	0.00	*	*
9/30/2021	3	150	8.34	22.446	203.9	107.5	0.923	0.0647	676.193	57.420	0.00	714	3
9/30/2021	4	216.7	8.31	21.475	177.8	107.4	0.922	0.0637	848.724	124.390	0.00	827.5	15
9/30/2021	5	83.3	7.88	21.392	157.8	94.3	0.929	0.0623	1079.271	114.410	0.00	*	*
9/30/2021	6	200	7.81	20.773	121.6	94.7	0.493	0.0584	285.873	30.210	0.00	*	*

9/30/2021	7	233.3	7.75	19.993	130.2	91.1	0.523	0.0567	379.732	32.550	0.00	0	0
9/30/2021	8	383.3	7.62	20.059	114.6	92.4	0.433	0.0558	356.951	36.370	0.00	*	*
9/30/2021	9	216.7	7.45	17.598	44.2	95.9	0.365	0.0529	20.091	31.690	0.00	475	7

Table A4. Field and Lab Data

Table A4 reports field measurements including precipitation, fDOM, turbidity, discharge, and gage height.
 * Indicates no data collection at this sample point

Date	Site #	fDOM CFU	fDOM QSU	Turbidity	Pressure	USGS Mean Discharge	Mean Gage Height	3 day precip	7 day precip
4/29/2021	1	*	*	*	*	*	*	0	43.22
4/29/2021	2	*	*	*	*	*	*	0	40.55
4/29/2021	3	*	*	*	*	*	*	0	41.09
4/29/2021	4	*	*	*	*	*	*	0	41.71
4/29/2021	5	*	*	*	*	*	*	0	39.39
4/29/2021	6	*	*	*	*	478	18.53	0	40.99
4/29/2021	7	*	*	*	*	*	*	0	40.95
4/29/2021	8	*	*	*	*	*	*	0	32.82
4/29/2021	9	*	*	*	*	*	*	0	35.39
5/27/2021	1	*	*	*	*	*	*	0	0
5/27/2021	2	*	*	*	*	*	*	0	0
5/27/2021	3	*	*	*	*	*	*	0	0
5/27/2021	4	*	*	*	*	*	*	0.63	0.63
5/27/2021	5	*	*	*	*	*	*	1.16	1.16
5/27/2021	6	*	*	*	*	425	18.41	0.01	0.01
5/27/2021	7	*	*	*	*	*	*	0.01	0.01
5/27/2021	8	*	*	*	*	*	*	0	0
5/27/2021	9	*	*	*	*	*	*	0	0
6/24/2021	1	13.30	39.44	8.07	752.1	*	*	14	124.4
6/24/2021	2	10.92	32.36	14.58	752.3	*	*	12.27	94.07
6/24/2021	3	8.23	24.41	14.74	752.2	*	*	13.19	81.49
6/24/2021	4	10.67	31.62	22.21	750.6	*	*	20.65	88.35
6/24/2021	5	9.58	28.41	27.84	750.7	*	*	25.28	87.65
6/24/2021	6	10.42	30.89	19.44	750.3	1550	20.36	27.06	90.17
6/24/2021	7	10.39	30.79	19.78	749.7	*	*	26.66	90.16
6/24/2021	8	9.53	28.26	15.85	748.8	*	*	27.41	104.86

6/24/2021	9	5.23	15.49	1.67	743.6	*	*	33.58	106.33
7/29/2021	1	7.04	21.49	3.65	749.4	*	*	4.53	27.61
7/29/2021	2	14.62	44.34	11.49	749.9	*	*	8.2	44.92
7/29/2021	3	14.71	44.63	15.34	750.5	*	*	10.72	52.07
7/29/2021	4	9.74	29.62	9.50	748.8	*	*	21.21	32.42
7/29/2021	5	9.32	28.35	10.98	748.6	*	*	22.64	32.55
7/29/2021	6	8.12	24.73	9.59	747.5	237	4.51	23.52	25.87
7/29/2021	7	7.97	24.27	5.51	746.5	*	*	23.03	25.51
7/29/2021	8	7.02	21.43	5.38	746.1	*	*	7.66	22.2
7/29/2021	9	5.06	15.51	1.98	740.5	*	*	0.91	1.59
8/26/2021	1	8.20	24.80	5.81	752.0	*	*	9.72	27.95
8/26/2021	2	8.84	26.74	4.96	752.2	*	*	10.27	20.47
8/26/2021	3	9.81	29.65	8.95	752.2	*	*	7.51	15.03
8/26/2021	4	12.21	36.85	9.81	750.9	*	*	6.14	18.86
8/26/2021	5	5.20	15.85	332.25	750.6	*	*	5.65	20.11
8/26/2021	6	9.99	30.19	11.97	749.8	267	4.63	15.2	32.39
8/26/2021	7	7.67	23.25	10.05	749.3	*	*	15.63	32.06
8/26/2021	8	9.34	28.23	6.87	748.4	*	*	10.51	39.3
8/26/2021	9	9.57	28.94	0.84	742.5	*	*	4.78	92.84
9/30/2021	1	10.22	30.89	14.25	751.3	*	*	0	0
9/30/2021	2	5.47	16.65	4.76	751.3	*	*	0	0
9/30/2021	3	6.24	18.97	6.51	751.5	*	*	0	0
9/30/2021	4	9.83	29.71	2.45	749.7	*	*	0	0
9/30/2021	5	9.14	27.65	4.35	749.4	*	*	0	0
9/30/2021	6	7.37	22.36	2.29	748.8	180	3.92	0	0
9/30/2021	7	7.23	21.92	4	748.1	*	*	0	0
9/30/2021	8	6.93	21.02	2.55	747.2	*	*	0	0
9/30/2021	9	5.83	17.72	6.25	741.3	*	*	0	0

Table A5. Metals Data

These table reports trace metal analysis data. This data was collected by Auburn Geoscience Department's ICP-MS Lab. All metals measured in ppb. Headers display atomic numbers and type of gas used in analysis

Date	Site #	63 Cu He	9 Be	23 Na He	24 Mg He	27 Al He	39 K He	40 Ca H2	51 V He	52 Cr He	55 Mn He	56 Fe He	59 Co He	60 Ni He
4/29/2021	1	0.7	0.0	2579.9	8385.6	16.9	1333.8	16648.8	0.4	0.0	5.4	28.2	0.3	0.0
4/29/2021	2	0.5	0.0	2935.0	8872.1	20.1	1236.3	16992.1	0.3	0.0	1.8	43.0	1.4	0.2
4/29/2021	3	0.4	0.0	3019.1	8739.4	22.6	1222.1	16544.7	0.3	0.0	2.2	66.1	0.3	0.0
4/29/2021	4	0.5	0.0	4329.7	6741.9	31.9	1516.6	13456.1	0.3	0.0	3.2	121.6	1.0	0.3
4/29/2021	5	0.5	0.0	2673.0	5836.0	42.3	1279.7	11977.3	0.3	0.0	2.3	154.6	0.8	0.2
4/29/2021	6	0.0	0.0	1708.3	4849.7	40.0	989.7	9389.2	0.0	0.0	3.5	197.2	0.2	0.0
4/29/2021	7	0.0	0.0	1625.8	5314.9	47.3	1063.7	10222.0	0.2	0.0	2.2	175.0	0.0	0.0
4/29/2021	8	0.0	0.0	1596.3	4602.1	45.3	1022.2	8903.3	0.0	0.0	2.3	159.5	0.8	0.0
4/29/2021	9	0.0	0.0	2099.8	1251.2	23.6	565.0	3181.9	0.0	0.0	1.2	82.1	0.0	0.0
5/27/2021	1	1.3	0.0	2833.4	9386.5	23.9	1225.1	18674.9	0.5	0.1	42.5	27.8	0.7	0.3
5/27/2021	2	0.6	0.0	2921.5	11062.1	7.1	1144.0	20919.3	0.4	0.1	3.0	18.0	0.3	0.1
5/27/2021	3	1.9	0.0	2987.5	10902.6	32.0	1165.8	20711.3	0.6	0.2	43.1	113.8	0.1	0.2
5/27/2021	4	1.3	0.0	4075.4	8793.0	24.9	1683.4	16756.7	0.3	0.2	34.5	136.2	0.8	0.3
5/27/2021	5	1.1	0.0	3263.2	7737.7	24.9	1519.5	14768.0	0.3	0.2	42.2	174.5	0.5	0.2
5/27/2021	6	0.8	0.0	1817.7	6131.0	17.4	1103.1	11803.0	0.2	0.0	43.2	176.2	0.0	0.2
5/27/2021	7	0.8	0.0	1740.7	6330.3	24.4	1163.0	12120.6	0.2	0.1	56.4	207.9	0.2	0.1
5/27/2021	8	0.5	0.0	1697.9	5528.7	18.0	1086.2	10630.4	0.2	0.0	47.0	176.3	0.6	0.1
5/27/2021	9	0.3	0.0	2304.2	1362.5	6.6	606.2	3431.9	0.1	0.0	4.4	61.5	0.2	0.1
6/24/2021	1	0.8	0.0	3527.9	4831.9	66.0	11424.7	9949.2	0.4	0.2	7.2	119.2	0.8	0.0
6/24/2021	2	0.8	0.0	1915.8	5283.2	104.2	724.4	10685.1	0.5	0.2	30.7	202.8	0.2	0.1
6/24/2021	3	0.6	0.0	1995.3	5130.2	89.0	-675.7	10444.3	0.4	0.3	10.8	174.3	0.0	0.1
6/24/2021	4	0.6	0.1	2072.2	3892.6	90.3	-2052.2	7893.9	0.4	0.2	4.7	193.5	0.1	0.2
6/24/2021	5	0.6	0.0	1668.5	3417.8	68.9	-1166.9	7148.8	0.3	0.2	6.3	181.0	0.0	0.2
6/24/2021	6	0.3	0.0	1519.6	2490.9	79.3	-1604.3	4810.4	0.3	0.1	6.1	195.8	0.1	0.1
6/24/2021	7	0.2	0.0	1329.7	2592.6	72.4	-1412.8	5064.3	0.3	0.1	8.5	180.5	0.0	0.1

6/24/2021	8	0.1	0.0	1291.2	2182.5	48.1	-2554.1	4236.1	0.2	0.1	8.4	148.9	0.7	0.0
6/24/2021	9	0.1	0.0	1743.1	963.3	29.2	-2060.4	2280.3	0.1	0.1	3.2	69.9	0.2	0.0
7/29/2021	1	0.6	0.0	2181.6	6860.6	8.3	1748.6	14091.1	0.5	0.0	0.5	11.1	0.1	0.1
7/29/2021	2	1.2	0.0	2189.7	5645.0	151.2	2447.1	11092.6	0.5	0.1	24.4	276.1	-0.3	0.3
7/29/2021	3	0.6	0.0	2440.7	5920.8	142.5	1337.7	11518.0	0.5	0.1	18.0	264.6	-0.4	0.3
7/29/2021	4	0.5	0.0	3533.9	6878.4	46.6	966.6	13556.5	0.3	0.2	11.1	214.7	0.1	0.3
7/29/2021	5	0.5	0.0	2496.4	6561.0	39.7	-1110.9	13348.4	0.3	0.1	15.2	240.9	-0.1	0.2
7/29/2021	6	0.2	0.0	2003.0	5436.8	29.8	-3102.7	10521.2	0.2	0.0	20.0	272.8	0.6	0.2
7/29/2021	7	0.1	0.0	1870.3	6451.2	30.6	-817.4	12375.9	0.2	-0.1	40.1	276.8	-0.1	0.1
7/29/2021	8	0.4	0.0	1894.4	5633.3	7.1	6056.5	10769.3	0.2	0.0	32.3	202.9	0.2	0.2
7/29/2021	9	0.0	0.0	2666.6	1402.0	6.4	-2914.4	3654.8	0.1	-0.1	2.8	69.2	-0.3	0.1
8/26/2021	1	0.6	0.0	3241.5	7589.5	15.0	1550.6	16906.6	0.7	0.1	0.8	4.1	0.9	0.1
8/26/2021	2	0.5	0.0	2956.8	8098.6	38.0	1300.3	17021.9	0.4	0.2	24.2	116.5	0.2	0.2
8/26/2021	3	0.6	0.0	2917.7	8213.3	34.8	1263.0	16512.8	0.4	0.1	20.3	107.4	0.9	0.1
8/26/2021	4	0.6	0.0	4176.6	6623.4	46.2	1628.2	13307.9	0.4	0.3	11.0	174.9	0.1	0.2
8/26/2021	5	3.4	0.0	2974.7	5723.2	48.2	1597.2	11197.8	0.4	0.2	10.6	198.5	0.2	0.3
8/26/2021	6	0.5	0.0	2065.3	4530.1	39.2	1109.1	9152.2	0.2	0.1	22.2	243.6	0.1	0.1
8/26/2021	7	0.2	0.0	1876.8	5526.8	36.4	1225.7	11639.2	0.3	0.1	27.9	227.0	0.0	0.1
8/26/2021	8	0.5	0.0	1842.3	4838.7	29.7	1234.5	9752.8	0.2	0.1	29.2	207.8	0.4	0.1
8/26/2021	9	1.3	0.0	2332.1	1289.5	14.0	647.9	3642.1	0.1	0.1	3.7	93.7	0.5	0.1
9/30/2021	1	0.5	0.0	3001.2	6628.6	11.8	2008.3	15831.1	0.7	0.1	1.0	11.9	0.1	0.1
9/30/2021	2	0.5	0.0	3175.7	10629.6	25.8	2147.3	20233.1	0.4	0.1	0.8	53.3	0.1	0.1
9/30/2021	3	0.3	0.0	3344.2	11205.7	29.2	1370.8	21403.3	0.4	0.2	19.9	91.4	0.1	0.1
9/30/2021	4	0.5	0.0	5286.9	8646.5	30.2	1995.4	17031.0	0.3	0.2	10.8	148.5	0.1	0.3
9/30/2021	5	0.7	0.0	675.2	8042.5	24.6	1998.1	15093.1	0.3	0.2	16.5	178.3	0.1	0.2
9/30/2021	6	0.6	0.0	2016.4	6579.9	27.1	1883.5	12318.3	0.2	0.1	21.1	223.5	0.0	0.1
9/30/2021	7	0.6	0.0	1854.0	6451.3	27.6	1835.9	12073.2	0.2	0.1	21.0	222.8	0.0	0.1
9/30/2021	8	0.4	0.0	1854.3	7028.1	29.5	1247.5	13467.1	0.2	0.1	29.0	223.3	0.1	0.0
9/30/2021	9	0.6	0.0	2554.5	7057.8	31.2	1196.9	14024.8	0.2	0.1	28.6	222.5	0.1	0.0

Table A6. Metals Data

This table reports trace metal analysis data. This data was collected by Auburn Geoscience Department's ICP-MS Lab. All metals measured in ppb. Headers display atomic numbers and type of gas used in analysis. Values reported were adjusted using the blanks in each sample set. This resulted in some negative values displayed in red text. * Indicates no data collected for that sample point.

Date	Site #	66 Zn He	75 As He	78 Se He	95 Mo He	107 Ag He	111 Cd He	121 Sb He	137 Ba He	205 Tl	208 Pb	232 Th	238 U	56 Fe H2	88 Sr	88 Sr He
4/29/2021	1	0.3	0.4	0.0	0.6	0.0	0.0	0.3	16.1	0.0	0.0	0.0	0.0	29.8	32.5	31.7
4/29/2021	2	0.5	0.4	0.0	0.6	0.0	0.0	0.3	16.8	0.0	0.0	0.0	0.0	43.0	33.5	33.3
4/29/2021	3	0.4	0.4	0.0	0.5	0.0	0.0	0.3	16.4	0.0	0.0	0.0	0.0	64.6	33.3	33.2
4/29/2021	4	0.9	0.4	0.0	0.6	0.0	0.0	0.6	16.8	0.0	0.0	0.0	0.0	127.9	35.0	34.3
4/29/2021	5	0.5	0.3	0.0	0.4	0.0	0.0	0.0	18.7	0.0	0.0	0.0	0.0	160.6	36.5	35.3
4/29/2021	6	0.4	0.3	0.0	0.0	0.0	0.0	0.0	15.2	0.0	0.0	0.0	0.0	197.4	32.0	31.9
4/29/2021	7	0.2	0.3	0.0	0.0	0.0	0.0	0.0	16.7	0.0	0.0	0.0	0.0	178.6	34.3	34.2
4/29/2021	8	0.0	0.2	0.0	0.0	0.0	0.0	0.0	16.0	0.0	0.0	0.0	0.0	157.6	33.0	33.1
4/29/2021	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.8	0.0	0.0	0.0	0.0	80.8	26.2	25.9
5/27/2021	1	4.3	0.6	0.1	0.3	0.0	0.0	0.3	22.0	0.0	0.1	0.0	0.1	27.3	36.3	36.5
5/27/2021	2	0.0	0.4	0.1	0.4	0.0	0.0	0.3	18.6	0.0	0.0	0.0	0.2	17.7	38.2	37.9
5/27/2021	3	3.5	0.4	0.1	0.4	0.0	0.0	0.3	20.4	0.0	0.4	0.1	0.2	116.6	38.6	37.7
5/27/2021	4	7.0	0.4	0.1	0.5	0.0	0.0	0.3	22.0	0.0	0.2	0.0	0.1	136.9	40.5	40.5
5/27/2021	5	6.8	0.4	0.1	0.3	0.0	0.0	0.0	22.5	0.0	0.3	0.0	0.1	180.2	42.0	41.3
5/27/2021	6	3.6	0.3	0.1	0.0	0.0	0.0	0.0	19.5	0.0	0.1	0.0	0.1	178.8	36.4	35.9
5/27/2021	7	4.8	0.2	0.1	0.0	0.0	0.0	0.0	22.0	0.0	0.2	0.0	0.1	212.0	38.3	37.9
5/27/2021	8	3.1	0.2	0.1	0.0	0.0	0.0	0.0	19.8	0.0	0.1	0.0	0.1	181.1	37.2	36.6
5/27/2021	9	4.2	0.2	0.1	0.0	0.0	0.0	0.0	8.9	0.0	0.0	0.0	0.0	63.4	27.0	26.6
6/24/2021	1	0.2	0.6	0.1	0.3	3.1	0.0	0.1	17.0	0.0	0.1	0.0	0.0	124.9	22.4	22.0
6/24/2021	2	1.2	0.5	0.1	0.3	-3.4	0.0	0.1	15.6	0.0	0.2	0.0	0.1	214.0	23.2	22.9
6/24/2021	3	0.2	0.4	0.1	0.2	-4.1	0.0	0.2	14.6	0.0	0.2	0.0	0.1	202.2	22.6	21.5
6/24/2021	4	0.1	0.4	0.1	0.2	-6.3	0.0	0.1	14.4	0.1	0.2	0.1	0.1	219.3	27.8	23.1
6/24/2021	5	2.7	0.4	0.1	0.1	-5.8	0.0	0.0	14.0	0.0	0.2	0.0	0.0	198.3	21.3	20.6
6/24/2021	6	0.9	0.3	0.0	0.1	-4.2	0.0	0.0	11.9	0.0	0.1	0.0	0.0	208.0	18.1	17.6
6/24/2021	7	0.0	0.3	0.0	0.1	-4.7	0.0	0.0	12.1	0.0	0.1	0.0	0.0	204.8	19.1	18.2

6/24/2021	8	0.0	0.2	0.0	0.0	-5.6	0.0	0.0	11.0	0.0	0.1	0.0	0.0	161.1	17.9	16.9
6/24/2021	9	1.0	0.2	0.1	0.1	-5.5	0.0	0.0	6.9	0.0	0.0	0.0	0.0	73.7	19.1	18.1
7/29/2021	1	-2.6	0.6	0.1	0.4	-3.8	0.0	0.2	18.1	0.0	0.0	0.0	0.1	*	*	*
7/29/2021	2	0.4	0.5	0.1	0.2	-1.3	0.0	0.2	16.3	0.0	0.2	0.0	0.1	*	*	*
7/29/2021	3	0.0	0.5	0.1	0.2	-2.6	0.0	0.1	15.9	0.0	0.2	0.1	0.1	*	*	*
7/29/2021	4	-1.1	0.5	0.1	0.3	-3.2	0.0	0.2	19.7	0.0	0.1	0.0	0.1	*	*	*
7/29/2021	5	-0.6	0.5	0.1	0.2	-3.3	0.0	0.0	19.8	0.0	0.4	0.0	0.1	*	*	*
7/29/2021	6	-1.5	0.4	0.0	0.0	-4.5	0.0	0.0	17.6	0.0	0.1	0.0	0.1	*	*	*
7/29/2021	7	-2.0	0.3	0.0	0.0	-3.1	0.0	0.0	20.8	0.0	0.0	0.0	0.1	*	*	*
7/29/2021	8	-0.3	0.3	0.0	0.0	-3.2	0.0	0.0	17.8	0.0	0.1	0.0	0.0	*	*	*
7/29/2021	9	-2.2	0.2	0.0	0.0	-3.3	0.0	0.0	8.1	0.0	0.0	0.0	0.0	*	*	*
8/26/2021	1	-0.3	0.8	0.1	0.5	0.0	0.0	0.3	18.8	0.0	0.0	0.0	0.1	*	*	*
8/26/2021	2	0.2	0.5	0.1	0.4	0.0	0.0	0.3	16.7	0.0	0.1	0.0	0.1	*	*	*
8/26/2021	3	0.3	0.5	0.1	0.5	0.0	0.0	0.3	15.5	0.0	0.2	0.0	0.1	*	*	*
8/26/2021	4	0.4	0.5	0.1	0.6	0.0	0.0	0.4	17.9	0.0	0.1	0.0	0.1	*	*	*
8/26/2021	5	4.8	0.5	0.1	0.4	0.0	0.0	0.0	17.1	0.0	0.1	0.0	0.1	*	*	*
8/26/2021	6	0.8	0.4	0.0	0.1	0.0	0.0	0.0	14.3	0.0	0.1	0.0	0.0	*	*	*
8/26/2021	7	0.0	0.4	0.1	0.1	0.1	0.0	0.0	17.6	0.0	0.1	0.0	0.1	*	*	*
8/26/2021	8	-0.1	0.3	0.0	0.1	0.0	0.0	0.0	16.7	0.0	0.1	0.0	0.0	*	*	*
8/26/2021	9	2.2	0.3	0.1	0.1	0.0	0.0	0.0	8.2	0.0	0.0	0.0	0.0	*	*	*
9/30/2021	1	0.0	0.7	0.1	0.4	0.2	0.0	0.2	24.0	0.0	0.0	0.0	0.1	*	*	*
9/30/2021	2	0.0	0.4	0.1	0.5	0.2	0.0	0.3	17.4	0.0	0.1	0.0	0.2	*	*	*
9/30/2021	3	0.0	0.4	0.1	0.5	0.1	0.0	0.3	17.9	0.0	0.1	0.0	0.2	*	*	*
9/30/2021	4	0.0	0.4	0.0	0.4	0.3	0.0	0.9	-5.3	0.0	0.1	0.0	0.1	*	*	*
9/30/2021	5	0.7	0.4	0.0	0.0	0.1	0.0	0.0	1.7	0.0	0.1	0.0	0.1	*	*	*
9/30/2021	6	2.2	0.3	0.0	-0.3	0.2	0.0	0.0	-0.5	0.0	0.1	0.0	0.1	*	*	*
9/30/2021	7	2.4	0.3	0.1	-0.3	0.2	0.0	0.0	22.5	0.0	0.1	0.0	0.1	*	*	*
9/30/2021	8	0.0	0.3	0.1	0.1	0.1	0.0	0.0	17.5	0.0	0.1	0.0	0.1	*	*	*
9/30/2021	9	0.0	0.3	0.1	0.4	0.1	0.0	0.0	18.9	0.0	0.0	0.0	0.1	*	*	*

Table A7. Land Use Land Cover Data

This table reports the cell count for each site by type of land use. These cell counts were used to calculate percent land use for categories including developed, forested, and agricultural for each sites pour-point watersheds. This data was collected in ArcPro using the Summarize Within Raster tool.

Site #	Open Water	Developed Open Space	Developed Low Intensity	Developed Medium Intensity	Developed High Intensity	Deciduous Forest	Evergreen Forest	Mixed Forest
1	0	179	0	0	0	9729	4209	1635
2	2021	7676	438	199	81	157111	56676	25785
3	257	6357	990	102	4	47013	13462	5939
4	1047	4435	1111	106	0	69916	23345	7141
5	637	35313	22448	10332	4567	61050	14026	7766
6	368	22659	5511	2280	978	72313	32008	7916
7	2645	34454	7927	4267	1691	172654	102024	23522
8	298	3205	1077	371	151	11875	8784	3727
9	15	47	24	5	0	59	82	11
Site #	Barren Land	Shrub Scrub	Herbaceous	Hay Pasture	Cultivated Crops	Woody Wetlands	Emergent Wetlands	Herbaceous Wetlands
1	0	141	0	0	0	0	0	0
2	111	7834	5724	24325	4521	471	113	
3	104	1600	2456	18500	1925	498	67	
4	137	453	1735	8821	1021	456	355	
5	594	2777	4576	18258	273	1087	112	
6	615	6147	4879	12611	2003	465	127	
7	795	16465	18608	78628	25077	5129	997	
8	101	1984	2259	8258	1812	961	68	
9	0	4	16	161	0	0	0	

Pour Point Maps

This section includes the watershed maps of the study area created in ArcPro. Each sample point has its own watershed boundaries created based on the pour points of the region. The Choccolocco Creek flows from the northeast to the southwest (site 9 is near the headwaters and site 1 is near the confluence with major drainage basin).

Figure A3. Land use cover pour point map. Land use data collected from the National Land Cover Database. The sample points and wastewater treatment plants are illustrated. The black lines define the pour point watersheds for each sample point.

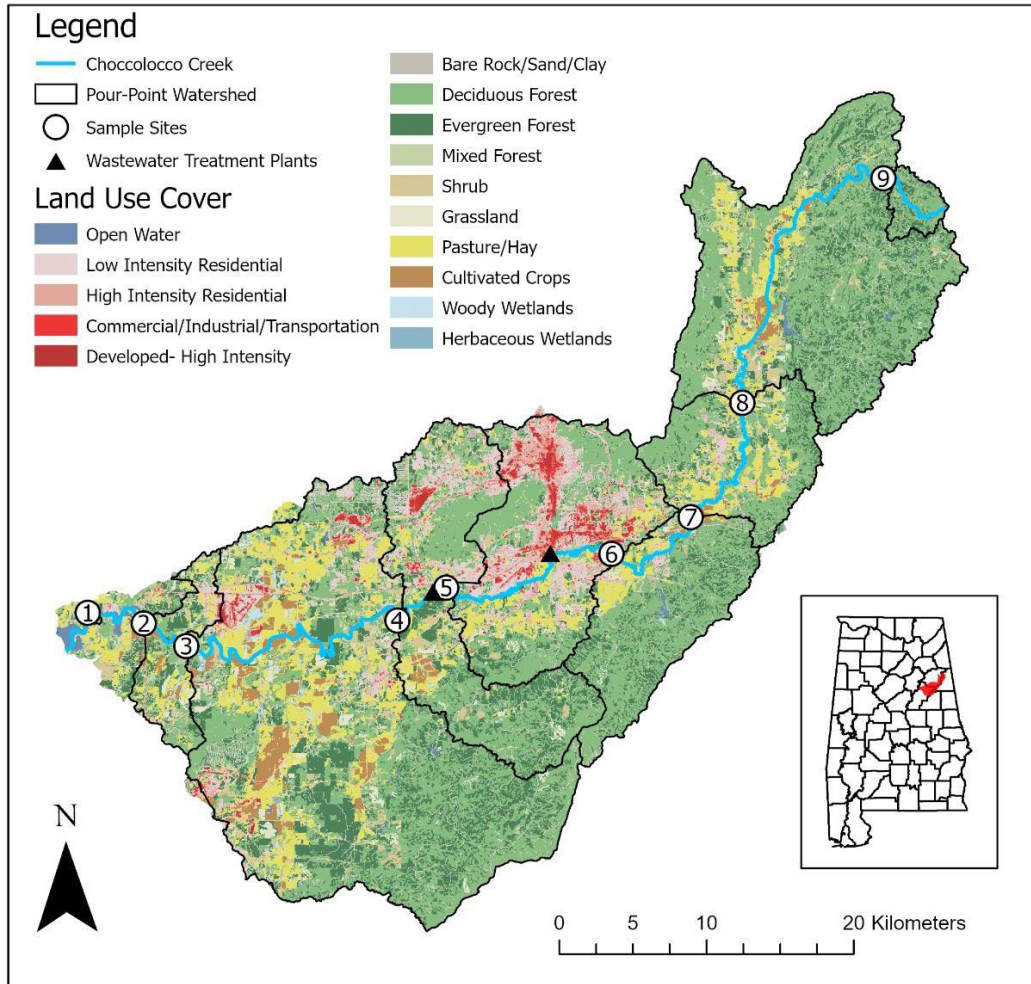
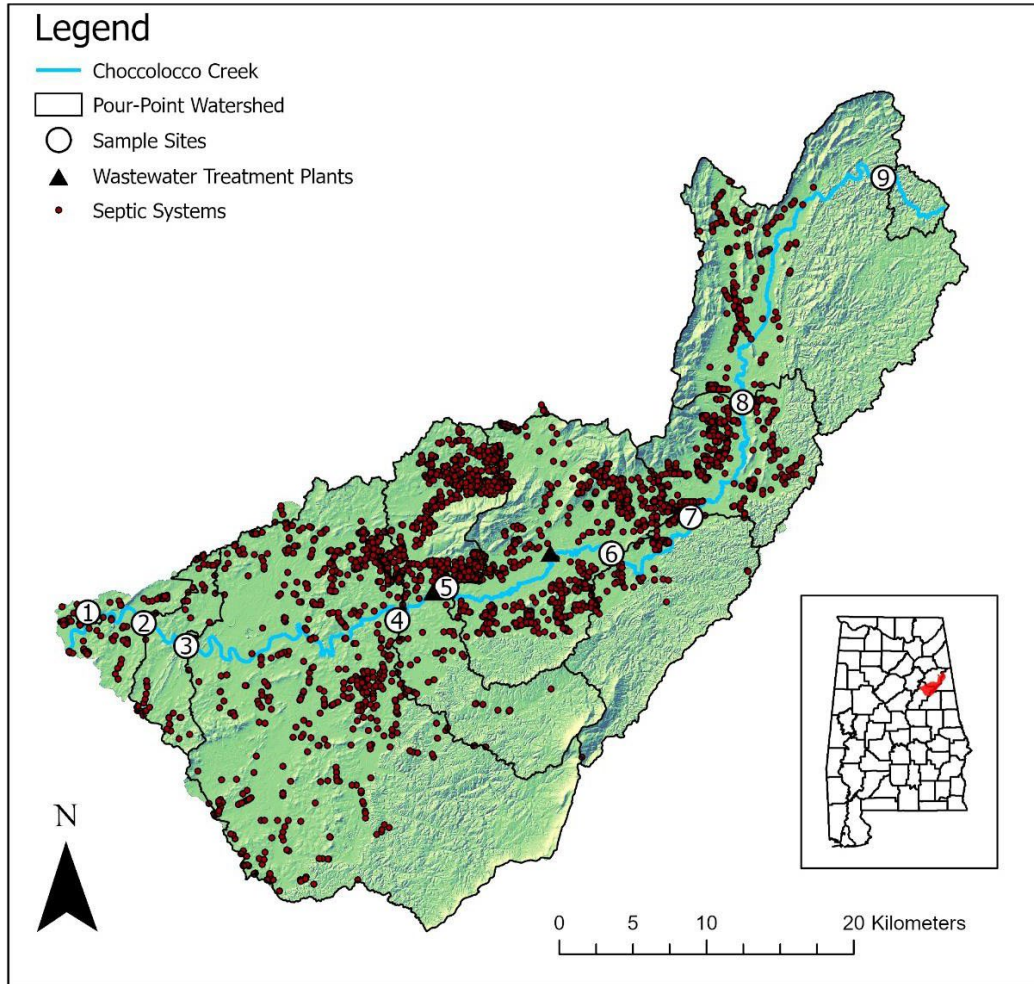


Figure A4. Septic system pour point map. The sample points, septic tank systems, and wastewater treatment plants are illustrated by circles, triangles, and dots, respectively. The black lines define the pour point watersheds for each sample point.



Hydrograph

This section contains a hydrograph for Choccolocco Creek at site 6.

Figure A5. This graph shows the USGS discharge data at site 6. The red arrows indicate when sampling took place.

