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Water-quality assessment:
Bacterial and viral-fecal indicators in an impaired urban watershed, Turkey Creek

Office of Research and Development
Gulf Ecosystem Measurement and Modeling Division

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Water-quality assessment: Bacterial and viral-fecal indicators in an impaired urban watershed, Turkey Creek

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The Center for Environmental Measurement & Modeling (CEMM) is within the Office of Research and Development (ORD). CEMM conducts research to advance EPA's ability to measure and model contaminants in the environment, including research to provide fundamental methods and models needed to implement environmental statutes. The methods and models developed by CEMM are typically applied at the airshed, watershed and ecosystem level.

CEMM's Gulf Ecosystem Measurement and Modeling Division (GEMMD) is located in Gulf Breeze, Florida. GEMMD conducts innovative research and modeling to assess and forecast future risk to ecological integrity from pollutants and other stressors, to develop tools and criteria for supporting resilient watersheds and water resources, to predict the adverse outcomes of chemicals at molecular levels through population scales, and to link environmental condition to the health and well-being of people and society. GEMMD collaborates with other EPA Divisions and Regional Offices to provide solutions to environmental priorities by providing technical support and information transfer to support regulatory criteria.

This report applies bacterial and viral markers of fecal pollution to elucidate impairment issues along an urban watershed, Turkey Creek, in Gulfport, MS.

Dr. Elizabeth George, Director
Gulf Ecosystem Measurement & Modeling Division
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Acronyms and Abbreviations

ANOVA	Analysis of Variance
C	Particulate Carbon
CFU	Colony Forming Unit
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EDTA	Ethylenediaminetetraacetic acid
EPA	U.S. Environmental Protection Agency
FDNA	Male-Specific DNA coliphages
FRNA	Male-Specific RNA coliphages
GIS	Geographic Information System
GEMMD	Gulf Ecosystem Measurement and Modeling Division
GM	Geometric Mean
GMD	Gulf of Mexico Division
GPS	Global Positioning System
hr	Hour
IAC	Internal Amplification Control
ID	Identification
L	Liter
LBNL	Lawrence Berkley National Laboratory
MDEQ	Mississippi Department of Environmental Quality
MED	Method Extraction Blanks
µg	Micrograms
µl	Microliters
µM	MicroMole
mg	Milligram
$MgCl_2$	Magnesium Chloride
ml	Milliliter
MPN	Most Probable Number
N	Particulate Nitrogen
NEEAR	National Epidemiological and Environmental Assessment of Recreational Waters
NGI	NEEAR-GI Illness
NPDES	National Pollutant Discharge Elimination System
NTC	No-Template Controls
ORD	Office of Research and Development
ORISE	Oak Ridge Institute for Science and Education
PCN	Particulate Carbon and Nitrogen
PFU	Plaque Forming Unit
POTW	Publicly Owned Treatment Works
ppt	Parts per Thousand
QAPP	Quality Assurance Project Plans
qPCR	Real Time-Polymerase Chain Reaction
RARE	Regional Applied Research Effort
RNA	Ribonucleic Acid
rRNA	Ribosomal
RT-PCR	Reverse Transcription-Polymerase Chain Reaction

SOP	Standard Operating Procedure
STV	Statistical Threshold Value
TDN	Total Dissolved Nitrogen
TDP	Total Dissolved Phosphorus
TMDL	Total Maximum Daily Load
TN	Total Nitrogen
TNTC	Too Numerous to Count
TSB	Tryptic Soy Broth
TSS	Total Suspended Solids

Acknowledgments

We would like to acknowledge the following individuals for assistance with data collection: Flowers White (Turkey Creek Community), Richard Grady (The University of Southern Mississippi), Lael Butler (EPA – GMD), Gerard Boos (EPA – GMD), Katelyn Houghton (EPA – GEMMD), and Keletia Gordon (The University of Southern Mississippi); and Deb Vivian (EPA-GEMMD) for graphics assistance. We would also like to acknowledge Coen Perrott (Mississippi Department of Environmental Quality) and Daryl Williams (EPA – Region IV) for reviewing this report.

Executive Summary

This final report summarizes the activities and research conducted under RARE project number 1771 for the urban impaired watershed, Turkey Creek, with a cooperative study between the ORD Gulf Ecosystem Measurement and Modeling Division (GEMMD), Gulf Breeze, FL, and the Gulf of Mexico Division (GMD) in Gulfport, MS. The study encompasses the period from October 1, 2016 to September 29, 2018, with subsequent project data analyses occurring from October 1, 2018 to September 2019. The project encompassed educational training and outreach within the local Gulfport community as project staff mentored and trained citizen scientists that included college students and local community volunteers in water-quality collection, fecal-bacteria enumeration and processing environmental waters for nutrient analyses. A major component of this study was identification of fecal-viruses, particularly male-specific RNA viruses (FRNA coliphages) as an indicator of pollution source (human and/or animal). During the final year, water samples were tested for specific bacterial human-markers of fecal contamination. Landscape/land-cover analysis was applied using GIS, sewage-line maps, manholes, wastewater-treatment plant locations and lift-station locations to assess any potential point-source into Turkey Creek. Various nutrients were analyzed to evaluate linkages between nutrient concentrations and *E. coli* enumeration to assess any potential drivers as contributing sources. Two stations out of four along the Turkey Creek watershed appear to be impacted by human sewage by point-source contamination, whereas the remaining stations were impacted most likely due to run-off. Recommendations for applying dye tracers to pinpoint the contamination issues were addressed in this report.

1.0 Introduction

1.1 Background

Contamination of surface waters often leads to drinking water contamination, degradation of aquatic biota and their habitat and the ultimately decline of the quality of life for both humans and animals. Pathogen contamination, as determined by bacterial fecal indicators (e.g., *E. coli* and enterococci), is a leading cause of Total Maximum Daily Load (TMDL) exceedance in watersheds and has been shown to be associated with nutrient enrichment. According to the TMDL, Turkey Creek is listed as impaired for fecal coliform loading consistent with the Clean Water Act (Fecal Coliform TMDL for Turkey Creek Revised Report 2016). Measures taken to reduce, eliminate or remediate pollution sources are only effective if the source is identified. EPA recommends the use of bacteria as indicators of fecal pollution although fecal indicators of bacterial origin are not always an accurate predictor of fecal viral loads and the waters may be deemed "clean" when in fact they are polluted. In addition, viral indicators of pollution often are indicative of fecal source (human vs animal), and the presence of fecal-indicator viruses have been associated with human-health risks. This RARE funding leveraged the current successful community bacterial monitoring program in Turkey Creek and built upon the already established Federal-State-Community partnership that is currently and successfully monitoring in the Turkey Creek community and Citizen Science activities in Gulfport, MS, and directly supports the *Watershed Implementation Plan for Turkey Creek* (http://ltmcp.org/wp-content/uploads/2015/01/final.TurkeyCreek.WIP_.pdf). In addition, this project applied fecal-source identification using viruses (Friedman et al., 2009), identified bacterial communities in the water and sediment, and evaluated the landscape and urban areas. The Community's Plan for the Turkey Creek and North Gulfport Neighborhoods (http://www.leahmahan.com/comehellorhighwater/wp-content/uploads/docs/F11_08-26_CommunityPlan.pdf) had a strategy to "identify and mitigate all pollution sources for both Turkey Creek and Bayou Bernard and establish regular monitoring to ensure water quality." This project aids to inform the Turkey Creek Steering Committee's decisions on seeking solutions to the contamination issues in Turkey Creek.

The Mississippi Department of Environmental Quality (MDEQ) has monitored specific stations along the Turkey Creek watershed for fecal contamination for several years. The Gulf of Mexico Division partnered with MDEQ and GEMMD to monitor Turkey Creek for this RARE project to ultimately help determine sources of bacterial contamination along with related parameters. The stations 02481240 (Canal Rd), CS221 (Arkansas Rd) and 02481252 (Creosote Rd/Rippy Rd), were initially selected by MDEQ as fecal coliform data from these sites were included in the MDEQ January TMDL 2015 report. The GMD added Airport as it was located between the Rippy Rd and Arkansas sites, the Ohio station (wooded area) was upstream from Arkansas and eventually Hutter Rd was added by the GMD because it was the most upstream location. Therefore, these sample stations, Ohio, Airport, Canal, Rippy, Arkansas and Hutter, were selected for this project.

The research approach was to assess water-quality and other indices at various locations/stations in Turkey Creek, including one station upstream (Hutter) from the urbanized Turkey Creek region. Based on the historical MDEQ monitoring, six stations (Rippy, Airport, Arkansas, Ohio, Canal and Hutter) located along the Turkey Creek watershed were selected. Analyses occurred monthly at a minimum and included measurements of nutrient concentrations, viral genotypes (addresses source of fecal pollution), microbial community in the water column, fecal indicator (*E. coli*) abundance in the water column, and microbial community in the sediment (an indication if the fecal bacteria are residing and multiplying in

the sediment versus discharge or run-off). In addition, the landscape, land-use, stream flow rates, and/or wastewater treatment locations were evaluated to provide information regarding point and possible non-point sources of fecal contamination.

This task is Research Action Plan (RAP) SSWR 4.02A: Indicator development.

2.0 Material and Methods

2.1 QAPP and SOPs

The QAPP and SOPs are on file (QAPP-GED/EDEB/SF/2016-01-001, October 16, 2016).

2.2 Sample Collection

Water samples were collected at stations Rippy Rd, Ohio, Canal and Hutter Rd along the Turkey Creek watershed to evaluate water for fecal-viral impacts, nutrients, PhyloChip (microbial community in the water column) and sediment (microbial community in the sediments) and for fecal-indicator bacteria (Table 1). These stations were selected based on historical monitoring by MDEQ. An additional two stations, Airport Road and Arkansas Road, were and assayed for fecal-indicator bacteria, but not for nutrients or viral loads (Table 1; Fig. 1).

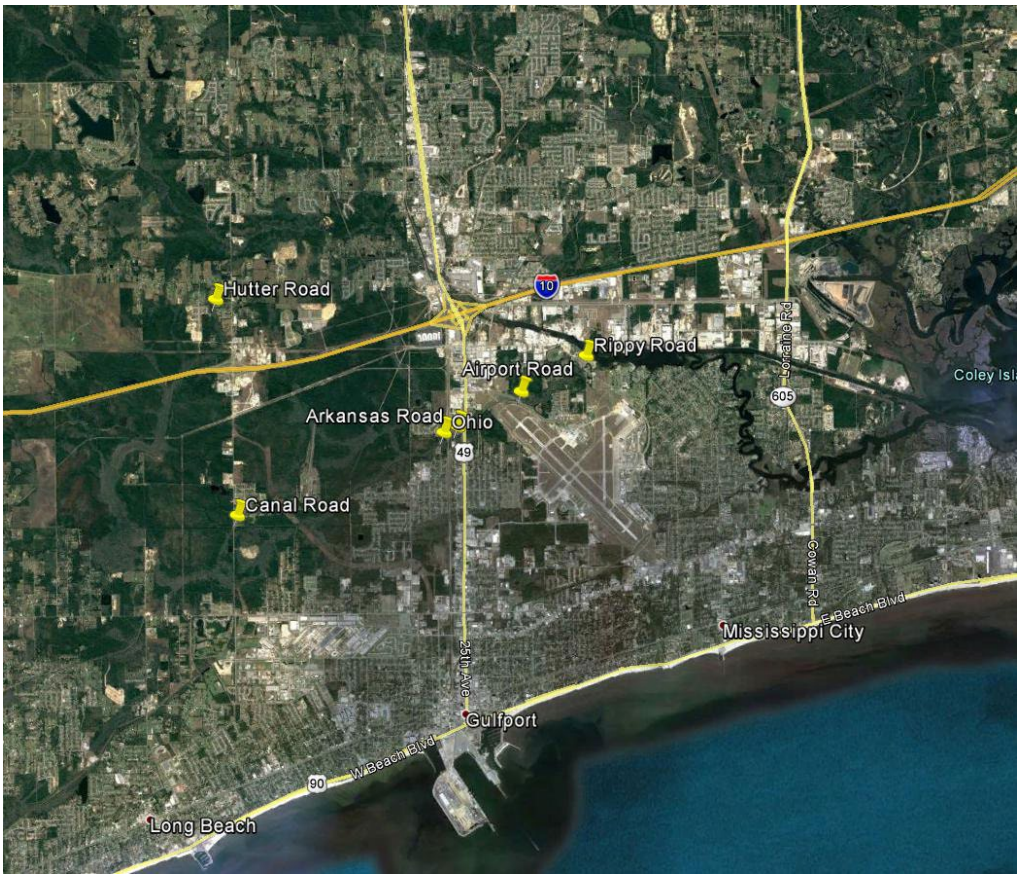


Figure 1. Map of sample stations along the Turkey Creek watershed, Gulfport, MS.

Table 1. Monitoring stations within the Turkey Creek watershed.

Waterbody	Station ID	Location	Latitude	Longitude
*Turkey Creek Most downstream	02481252 Rippy Rd	Airport Rd and Creosote Rd	30.42380556	-89.07027778
**Turkey Creek, Upstream from Rippy	Airport Rd	Airport Road on the bridge just west of the Airport	30.418	-89.08265
**Turkey Creek Upstream from Airport	CS221 Arkansas Rd	Arkansas Road bridge	30.41238889	-89.09477778
*Turkey Creek Upstream from Arkansas	Ohio Ave	Ohio Ave, North Gulfport, wooded	30.4114	-89.09735
*Turkey Creek Upstream from Ohio	02481240 Canal Rd	2.5 mi N of Long Beach at Canal Rd Bridge	30.39805556	-89.1366667
*Turkey Creek Upstream from Canal	Hutter Road 18082 Hutter Rd	At Hutter Road and Magnolia Springs sub-division (by lift station)	30.433370	-89.140552

*Stations sampled/water collected for water-quality parameters, nutrients, viral load, fecal bacteria, PhyloChip and sediments/GEMMD

** Stations sampled/water collected for fecal bacteria and water-quality parameters/GMD

2.3 PhyloChip®

The PhyloChip® is a microarray with oligonucleotides that represent microbes indicative of fecal signatures (human and/or various animals). This information lends evidence that fecal impact originated from either human or animal or both within the water column. PhyloChip® samples were collected once/month by aseptically collecting 100 ml creek water and storing on ice in the dark. The water was filtered through a manifold onto a Polycarbonate membrane filter, 47mm, 0.45µm within 8 hrs of collection and frozen at – 80 º C. Filtered PhyloChip® samples were shipped frozen to the contractor (Lawrence Berkeley National Laboratory; LBNL) for DNA extraction and bioinformatic processing. Initial data processing was performed by LBNL with subsequent bioinformatic analyses performed by EPA Region 7 Phylochip® Community Analysis Automated Sample Source Tracker. Preliminary data was analyzed using default settings for the Fecal Source Prediction Report.

2.4 Citizen Science

Local volunteers were recruited for this study through outreach activities in the Gulfport community. Volunteers included community members, college students and/or ORISE interns.

2.5 *E. coli* Enumeration

Fecal-bacteria indicators, *E. coli*, were determined for Most Probable Number (MPN) per 100 ml of creek water using the IDEXX Colilert assay (IDEXX, Westbrook, ME) at each station (Table 1).

2.6 Water-quality Parameters and Nutrients

Water-quality parameters, including flow and creek-stage height, were obtained twice/month as outlined in Table 2.

Table 2. Optical, Physical, and Chemical Measurements in Sample Medium Water

Parameter	Expected Range	Method Detection Limit (MDL); Accuracy; Precision	Method or Instrument; (SOP)
Dissolved Organic Carbon (DOC)	100 to >1000 µM	C: 0.008 µM; 10%;30%	High Temperature Catalytic Oxidation; *SOP-GED/EDEB/JRA/2017-01-001, May 3, 2017*
Total Dissolved Nitrogen (TDN)	n.d. to >100 µM	N: 0.002 µM; 10%; 30%	High Temperature Combustion; *SOP-GED/EDEB/JRA/2014-01-001, April3, 2014*
Total Suspended Solids (TSS)	0 to 100 mg L^{-1}	10%; 10%; 30%	Analysis of total suspended solids in freshwater SOP-GED/EDEB/JRA/2017-04-002
Total Phosphorus TDP	0 to 15 µM	2.19 µg P/L; 10%; 30%	Persulfate digestion, manual; *SOP-GED/EDEB/BJ/2017-01-001 July 26, 2017*
Particulate Carbon and Nitrogen (PCN)	PC: 0.4 to 2.0 mg L-1 PN: 0.04 to 0.4 mg L-1	PC: 0.053 mg L-1; 10%; 30% PN: 0.002 mg L-1; 10%; 30%	Combustion, TCD detector (CE Elantech); SOP-GED/EDEB/JRA/2017-1-002, April 5, 2017
Water Temperature	EXO2 -5 to 35 °C: ±0.01 °C EXO2 35 to 50 °C: ±0.05 °C	ProDSS ±0.2°C full range HL4 ±0.10°C full range	Thermistor
Pressure		±1.5 mmHg from 0 to 50°C	Integral Barometer
Conductivity	0-100 mS cm-1: ±0.5% or 0.001 mS cm-1 w.i.g.	100-200 mS cm-1: ±1% of reading	Four Nickel Electrode Cell
pH	4.0 to 8.0	EXO2 ±0.1 pH units within ±10°C of calibration temperature; otherwise, ±0.2 pH units ProDSS and HL4 ±0.2 pH units	Glass Bulb Combination Electrode
Specific Conductance	Calculated from Conductivity and Temperature	±0.5% or 0.001 mS cm-1 w.i.g.	EXO2, ProDSS or HL4 Sonde

Depth		±0.04 m	Pressure Transducer
Total Dissolved Solids (TDS)	Calculated from Conductivity and Temperature	Not specific – Calculated from conductivity and temperature	EXO2 and ProDSS
Turbidity	Nephelometric	0 to 999 FNU: 0.3 FNU or ±2% of reading, w.i.g.; 1000 to 4000: ±5% HL4	EXO2 and ProDSS
Dissolved Oxygen	HL4 0–8 mg/L: ±0.1 mg/L; HL4 >8mg/L: ±0.2 mg/L	EXO2 and ProDSS 0 to 20 mg/L: ±0.1 mg/L or 1% of reading, w.i.g.; 20 to 50 mg/L: EXO 2 ±5%, ProDSS ±8%; HL4 >20 mg/L: ±10%	Optical Luminescence
Salinity	Calculated from Conductivity and Temperature	±1% of reading or 0.1 ppt, whichever is greater (w.i.g.)	EXO2, ProDSS or HL4 Sonde
100 ml water	N/A	DNA will be extracted and processed by the contractor	PhyloChip
100 ml water	0-2419 MPN/100 ml	2419 MPN/100 ml IDEXX Colilert 2000	Standard Operating Procedure for the IDEXX Fecal Indicator Assay SOP-GED/EDEB/SF/2017-02-001
sediment	N/A	DNA was extracted and sequenced by the contractor	16S rRNA sequencing
1L of water	N/A	Male-specific coliphage presence/absence	Culture and molecular genotyping; SOP-GED/EDEB/SF/2017-01-001

Water was collected and analyzed for Dissolved Organic Carbon (DOC mg/L), Total Nitrogen (TN mg/L), Particulate Carbon and Nitrogen (N mg/L, C mg/L), Total Dissolved Phosphorus (TDP µM) and Total Suspended Solids (TSS mg/L) (Table 2). A duplicate sample was taken at any one of the four stations (Ohio, Rippy, Canal and Hutter) where nutrient analyses occurred. Field samples were collected and processed according to Standard Operating Procedures and stored at – 20 º C and/or – 80 º C until enough samples were collected for analyses.

2.7 Sediments

Sediments were collected from i) waters edge and ii) middle of creek/bridge area. Sediments were sampled using cores along the shoreline and using a PONAR grab from the middle of the bridges. Collected sediment samples were stored at – 80 º C throughout the project. In November, 2018, all samples were sent to Argonne National Labs for DNA extraction and 16S rRNA sequencing.

2.8 Bacteria Human-Specific Markers

Samples for qPCR were obtained monthly by aseptically collecting 100 ml creek water and storing on ice in the dark. Water was filtered through a manifold onto polycarbonate membrane filter, 47mm, 0.45 µm within 8 hours of collection and frozen at – 80 º C. DNA extracted from the filtered samples were analyzed following EPA methods 1696 (EPA 821-R-19-002; 2019) and 1697 (EPA 821-R-19-

003;2019). Plasmid constructs for calibration standards (both targets on a single construct) and internal amplification controls (IAC) were obtained from (Integrated DNA Technologies, Coralville, IA). Tris 0.1 mM EDTA (pH 8.0) was used to generate calibration standards (10, 10^2, 10^3, 10^4, 10^5 copies /2µl) and IAC reference material (10^2 copies/2µl). Water sample extractions were completed using DNA-EZ kit (GeneRite, North Brunswick, NJ). Modifications highlighted in the EPA methods for the sample extractions were implemented. In addition to 24 Turkey Creek samples, three method extraction blanks (MEB) with purified water substituted for test samples, were completed with each processing batch (8 samples/batch). Turkey creek DNA extraction yields were determined with a NanoDrop UV spectrophotometer (NanoDrop Technologies, Wilmington, DE) and stored and -20 º C. Two qPCR assays were used to examine human related contamination (HF 183/BacR287 and HumM2). Reaction mixtures contained 1X TaqMan Environmental Master Mix (version 2.0; Thermo Fisher Scientific, Grand Island, NY), 0.2 mg/mL bovine serum albumin (Sigma-Aldrich, St. Louis, MO), 1 µM each primer (final concentration), and 2 µL of DNA sample extract. Total reaction volume was 25 µL, and reactions were run in triplicate in MicroAmp optical 96-well reaction plates with MicroAmp 96-well optical adhesive film (Thermo Fisher Scientific, Grand Island, NY). The thermal cycling profile for all assays was 2 min at 95 º C followed by 40 cycles of 5 s at 95 º C, and 30 s at 60 º C. The threshold was manually set to 0.03 (HF183/BacR287) and 0.08 (HumM2) and quantification cycle (Cq) values were exported to Microsoft Excel for analysis. No-template controls (NTC) were used with purified water subsitituted for template DNA to monitor for potential extraneous DNA contamination.

2.9 Coliphage Viruses

One liter samples of water for viral analysis were collected at each station as noted in Table 1, concentrated into 2-4 ml with the InnovaPrep concentrator (Drexel, MO), stored overnight in the dark at 4 º C and plated the following day onto MS2 FRNA phage plates (Scientific Methods, Inc., Granger, IN) to enumerate male-specific coliphage viral plaques. Initially, 1L of creek water was concentrated for viral isolation. Eventually, this was modified to filter/concentrate in 100 ml in triplicates (to determine if we could improve the coliphage enumeration in triplicate/repeatable numbers) and then the remaining 700 ml is concentrated to capture all viruses in the sample for the 1L collected volume. The plates were then incubated overnight (for approximately 24 hours) at 35 º C. The plaques that formed overnight were counted and recorded. The MS2 plates containing the male-specific coliphage plaques were wrapped in parafilm, placed in sealed baggies and stored at 4 º C for up to 4-6 weeks. Phages were isolated from the individual plates by removing the plaque plugs with a sterile pipette, enriched overnight (log phase *E. coli*, 0.01M $MgCl_2$ in 1X TSB at 37 º C), centrifuged and the supernatant was frozen minus 80 º C in 20% glycerol for future genotyping. Phages were screened to determine if the plaques were RNA phages or DNA phages. This is important to know when genotyping them for fecal-source. Phages that were identified as FRNA (RNase-sensitive) were genotyped for genogroups I, II, III and IV using RT-PCR assays (Friedman et al., 2009).

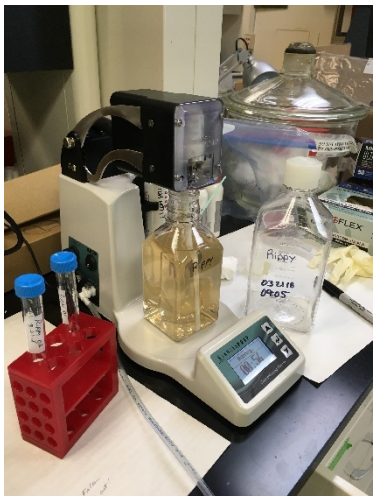


Figure 2. Concentrating water samples using the InnovaPrep instrument.

2.10 GIS and Landscape Analyses

Gulfport City and County offices provided information regarding sewer mains, manholes, and lift stations. Using the area of interest in Gulfport around the Turkey Creek watershed, a map was prepared, including the sample stations.

Maps of location sites and necessary attributes for each site for this report were developed using ArcMap Pro 10.1.6 program. Bridge location, which are sample sites, sewer lines, lift stations, Publicly-Owned Treatment Works plants (POTW), satellite imagery, watershed outline, and roads were added to the map as layers. These layers were adjusted using program capabilities of editing and labeling. Flow direction maps were developed by using 1ft contour data from 2018 remote sensed mapping developed by Harrison County, MS. The contour data was spliced to be more manageable when developing the topography raster. The topographic raster was input for the flow direction tool in ArcMap. Additional steps of flow accumulation tool, and steam flow tool were used to determine accuracy of data. Based on this information a flow model was developed to determine that flow accumulation matched up with current imagery of location of Turkey Creek (Appendix C).

2.11 Communications

GMD staff regularly participated in Turkey Creek Steering Committee Meetings along with local stakeholders, residents, state and federal partners. Throughout this project, staff consulted the committee on project design and reported the study's progress. Results and recommendations will be presented to the Turkey Creek Steering Committee meeting and to MDEQ. The Gulf of Mexico Division will work closely with state and local partners along with the committee by aiding with these contamination issues. A poster titled "Male-specific coliphage: fecal-source identification in an urban watershed" was presented at the Water Microbiology Meeting, Chapel Hill, NC, May, 2019.

3.0 Results

3.1 Field Monitoring, Water-Quality Parameters and Laboratory Analyses

To determine quality objectives and criteria, peer reviewed published field methodologies, the precision and degree of bias acceptable for successful implementation of the project measurements including the analytical capabilities of the instrumentation were accepted as described in Table 2.

3.2 PhyloChip®

Microbial population in the water column analyzed with the PhyloChip® indicated a strong signal for human sewage signature at Ohio and Hutter on September 20, 2017 and a marginal signal for human sewage signature at Hutter on March 30, 2018.

3.3 Citizen Science and Outreach

Outreach activities included public schools in the Gulfport community, students from the University of Southern Mississippi, Mississippi Gulf Coast Community College Jefferson Davis Campus, ORISE interns and local citizens. Volunteers were trained to collect water from the Turkey Creek watershed and assisted in filtering nutrients and *E. coli* (IDEXX) assays. Federal staff were sometimes visitors from other EPA regions and/or ORD. The number of participants per sample date were counted for each respective sample event; hence the same person/staff could be counted multiple times over each quarter. In addition to hands-on training, a few of the volunteer students received college credits for their activities and time with this project.

Table 3. Citizen Science participants per quarter.

DATE	PARTICIPANTS
September to December 2016	7 volunteers/students and 14 federal staff
January to March 2017	4 volunteers/students and 25 federal staff
April to June 2017	14 volunteers/students and 11 federal staff
July to September 2017	14 volunteers/students and 14 federal staff
September to December 2017	20 volunteers/students and 13 federal staff

January to March 2018	20 volunteers/students and 13 federal staff
April to June 2018	25 volunteers/students and 16 federal staff
July to September 2018	21 volunteers/students and 13 federal staff

3.4 Fecal-Indicator Monitoring

According to MDEQ (Regulations for Water Quality Criteria for Intrastate, Interstate and Coastal Waters, approved by EPA February 27, 2017), Turkey Creek is classified as "recreational" waters. The EPA ambient water-quality criteria for freshwaters is an MPN of less than 410 *E. coli* per 100 ml based on a Single-Sample Maximum Allowable Density Statistical Threshold Value (STV) for recreational waters (US EPA Water Quality Criteria, 2012). The Single-Sample value was selected as it was not feasible to assay these watersheds at least five times/month which would be required to determine a geometric mean (GM) for fecal-indicator bacteria. In addition, fecal indicator criteria often vary from state-to-state. Although EPA criteria were utilized as a guide the MDEQ's requirements are consistent with the EPA criteria.

Table 4. US EPA Water Quality Criteria, 2012.

Criteria Elements	**Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators**			**Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators**	
	Magnitude			**Magnitude**	
Indicator	GM (cfu/100mL)[a]	STV (cfu/100 mL)[a]	OR	GM (cfu/100 mL)[a]	STV (cfu/100 mL)[a]
Enterococci – marine and fresh	35	130		30	110
OR					
E. coli - fresh	126	410		100	320
Duration and Frequency: The waterbody GM should not be greater than the selected GM magnitude in any 30-day interval. There should not be greater than a ten percent excursion frequency of the selected STV magnitude in the same 30-day interval					

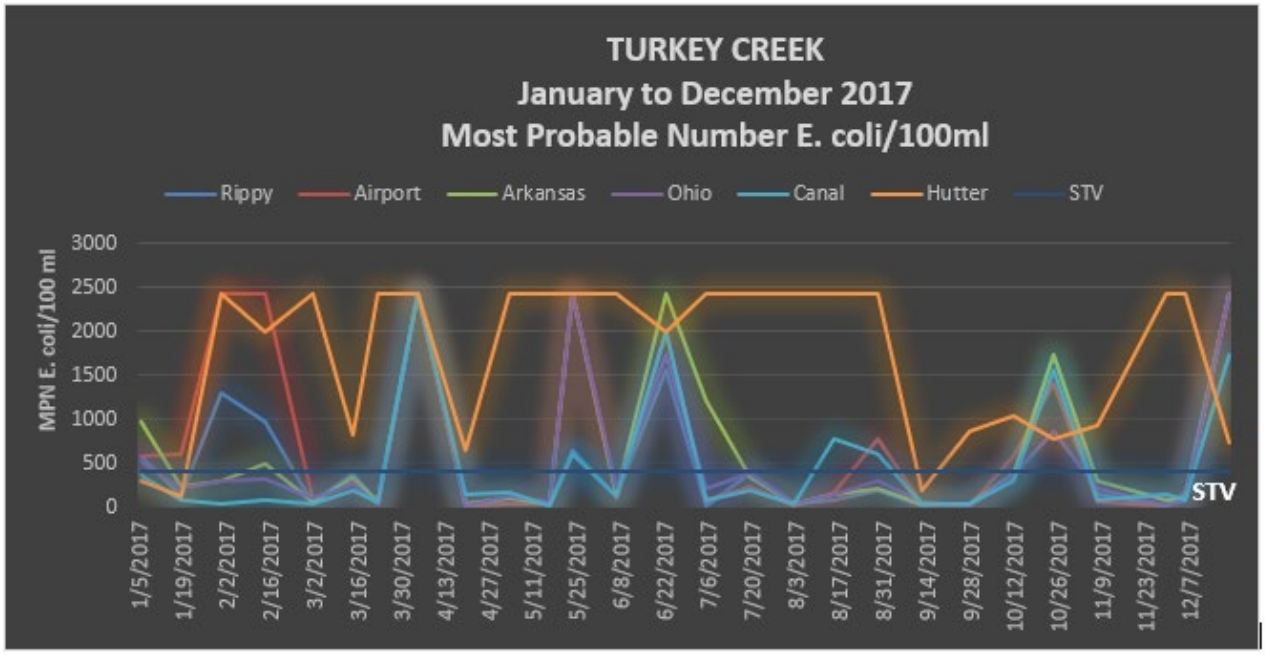
[a] EPA recommends using EPA Method 1600 (U.S. EPA, 2002a) to measure culturable enterococci, or another equivalent method that measures culturable enterococci and using EPA Method 1603 (U.S. EPA, 2002b) to measure culturable *E. coli*, or any other equivalent method that measures culturable *E. coli*.

Following the EPA water-quality recreational freshwaters guidance, the stations Rippy, Airport, Arkansas, Ohio and Canal exceeded the EPA criteria of 410 STV for *E. coli* enumeration for 22-41% of the 2017 sampling season (bi-monthly). The Hutter station, however, exceeded the 410 STV criteria approximately 89% of the twice/month samples (Fig. 3, Table 5).

Similar trends were noted during the bi-monthly *E. coli* analysis as all stations except Hutter had exceedance values ranging from 33-38%, with the Hutter station exceeding the 410 STV 70% of the sample collections during 2018 (Fig 3, Table 5).

Table 5. *E. coli* enumeration exceeded the EPA 410 MPN/100 ml STV standards as follows:

YEAR	STATION	No. of samples collected	No. days exceeded	Percent exceeded
2017	Rippy	27	8	29.6 %
2017	Airport	27	11	40.7 %
2017	Arkansas	27	8	29.6%
2017	Ohio	27	6	22.2%
2017	Canal	27	7	25.9%
2017	Hutter	27	24	88.9%
2018	Rippy	21	7	33.3%
2018	Airport	21	7	33.3%
2018	Arkansas	21	7	33.3%
2018	Ohio	21	8	38.1%
2018	Canal	21	7	33.3%
2018	Hutter	20	14	70%



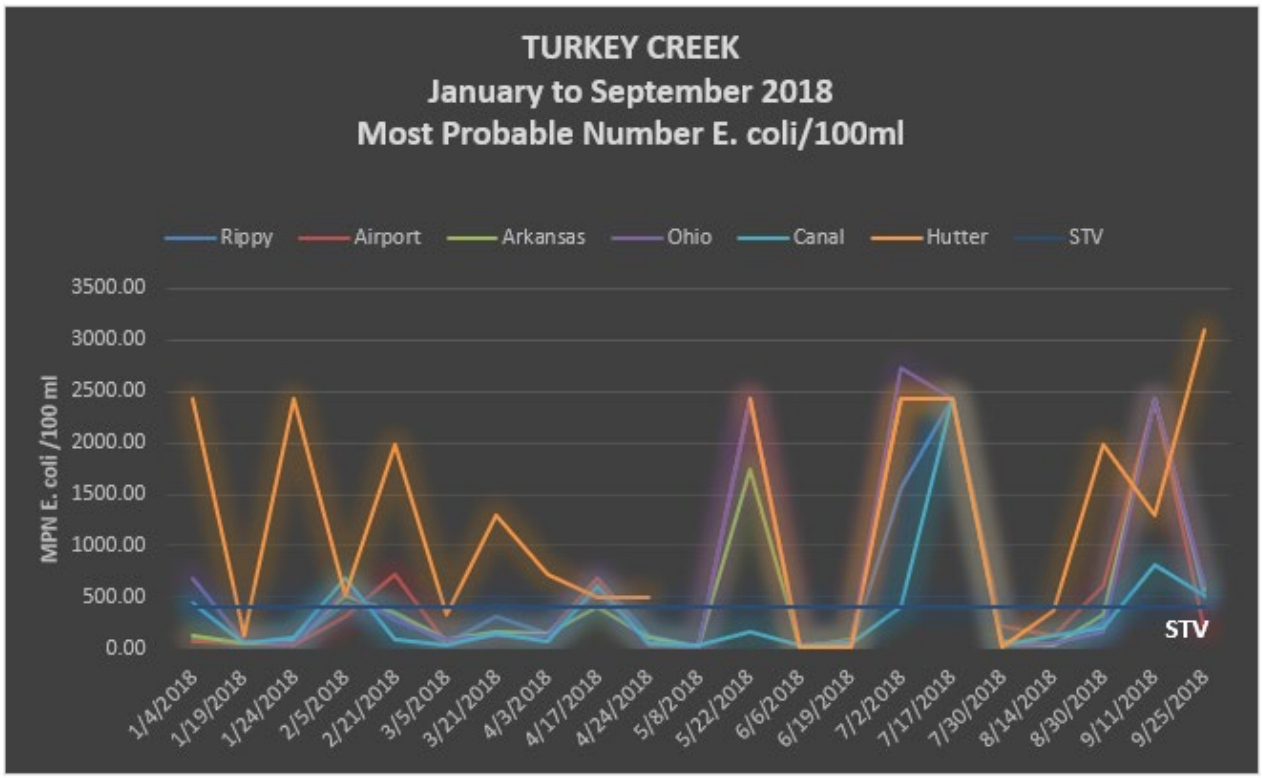


Figure 3. Most probable number (MPN) of E.coli per 100 ml of ambient waters. A Single-Sample Maximum Allowable Density Statistical Threshold Value (STV) for recreational waters is 410 CFU/100 ml.

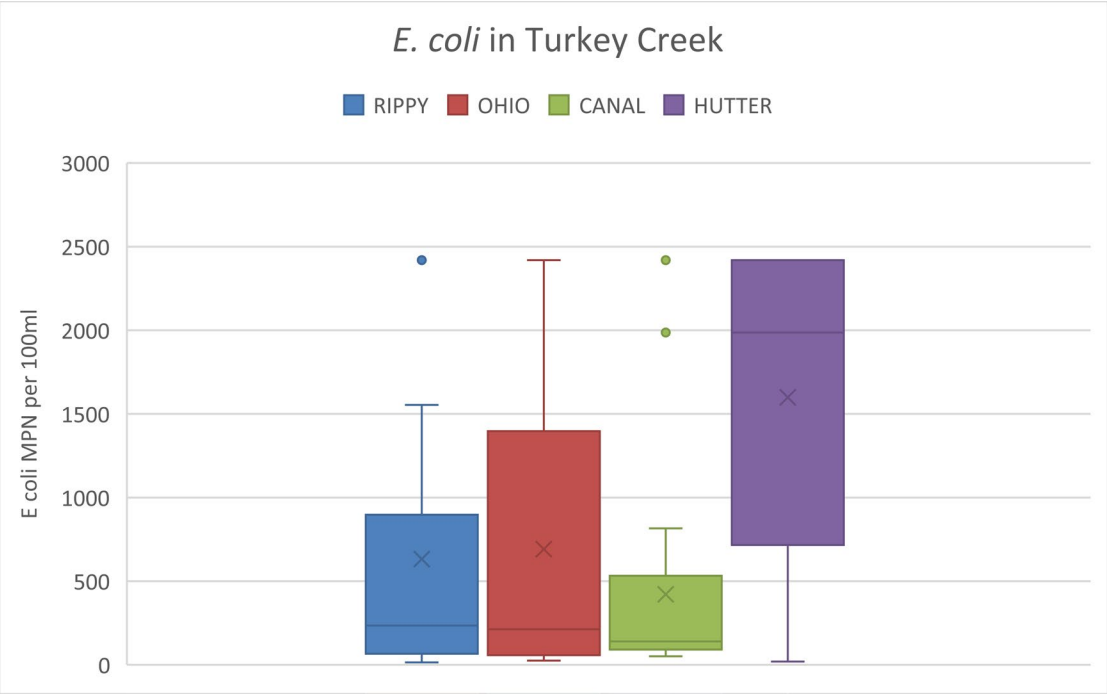


Figure 4. Box and whisker plot of E. coli MPN/100 ml for 2017-2018.

3.5 Water-Quality Parameters and Nutrients

The correlation table (Appendix E: Nutrients) compared all nutrients and water-quality parameters to each other and to *E. coli* MPN values using Excel correlation in the data analysis function. *E. coli* moderately correlated with DOC (0.41). DOC was moderately correlated with TN (0.49) and TSS (0.30). The strongest correlation was between N and C (0.87).

3.6 Sediment

Taxonomy from the "waters edge" from both 2017 and 2018 samples did not harbor *E. coli* or enterococci. The most abundant taxa, approximate top 50 classes in 2017, were *Nitrospira, Deltaproteobacteria, Spirochaetes, Bacteroidia, Verrumicrobia, Actinobacteria, Gammaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Chloroflexi, Pedosphaerae* (*Verrumicrobia*), and *Acidobacteria*. The most abundant taxa, approximate top 50 classes in 2018, were *Betaproteobacteria*, *Chlorobi*, *Nitrospira*, *Sphingobacteria* (*Bacteroidetes*), *Verrucomicrobia*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Bacteroidia*, *Pedosphaerae* (*Verrucomicrobia*), *Alphaproteobacteria*, *Chloroflexi* and *Acidobacteria*. Both 2017 and 2018 had one group of Archaea, the *Crenarchaeota*, as the more abundant taxa.

3.7 Bacterial Human-Specific Markers for Source-Tracking

HF183/BacR287 bacterial human marker was positive on May 22, 2018, with 0.93 log10 copies and on July 17, 2018, 0.806 log10 copies were detected only at the Rippy Station. The bacterial human marker HumM2 was positive on the same dates in 2018, with a value of 0.797 log10 copies and 0.873 log10 copies for May 22 and July 17, respectively, at the Rippy station. No other stations were positive for the two human bacterial markers tested in 2018 and these molecular indicators were not used in 2017.

3.8 Coliphage Viruses

Total male-specific coliphage counts (number of coliphage plaques on the MS2 plates) were determined for 2017 and 2018 per liter. In 2018, counts were also determined per 100 ml. ANOVA analysis for the male-specific coliphage counts per 100 ml (Table 6; Fig 3) resulted in no statistical differences between collection sites (Appendix B4). Male-specific coliphage were further separated from DNA (FDNA) and RNA (FRNA) in order to determine the genotype of the FRNA phages. Rippy had a total of 9 FRNA for groups II and III (suggests a human-viral origin) and a total of 5 FRNA from genogroups I and IV (suggests an animal-viral origin). The Ohio and Canal stations were dominated by animal coliphage signatures whereas Hutter was evenly mixed with animal and human genotypes (Table 7).

Table 6. 2018 Male-specific coliphage plaque-forming unit (PFU) per 100 ml.

2018	RIPPY	OHIO	CANAL	HUTTER
1/24/2018	200	11	1	24
2/21/2018	200	20	5	19
3/21/2019	6	42	18	94
4/17/2018	200	11	1	22
5/22/2018	200	200	6	200
6/19/2018	3	16	1	200
8/14/2018	21	29	11	64
9/11/2018	200	200	200	200
AVERAGE per 100 ml	129	66	30	103

NOTES: Counts were not performed in July, 2018, because the InnovaPrep viral concentrating instrument failed. It was subsequently sent to the manufacturer and repaired. Coliphages were not concentrated per 100 ml in 2017.

Table 7. FRNA coliphages were genotyped (I, II, III and IV) using RT-PCR to determine the fecal source. In most cases, genogroups II and III are indicative of a human signature and genogroups I and IV (mostly IV) are an animal signature. The table depicts the total for each genogroup from January, 2017 to September, 2018.

	Genogroup I	Genogroup II	Genogroup III	Genogroup IV
Rippy	4	5	4	1
Ohio	2	4	0	4
Canal	4	3	2	5
Hutter	3	1	2	2

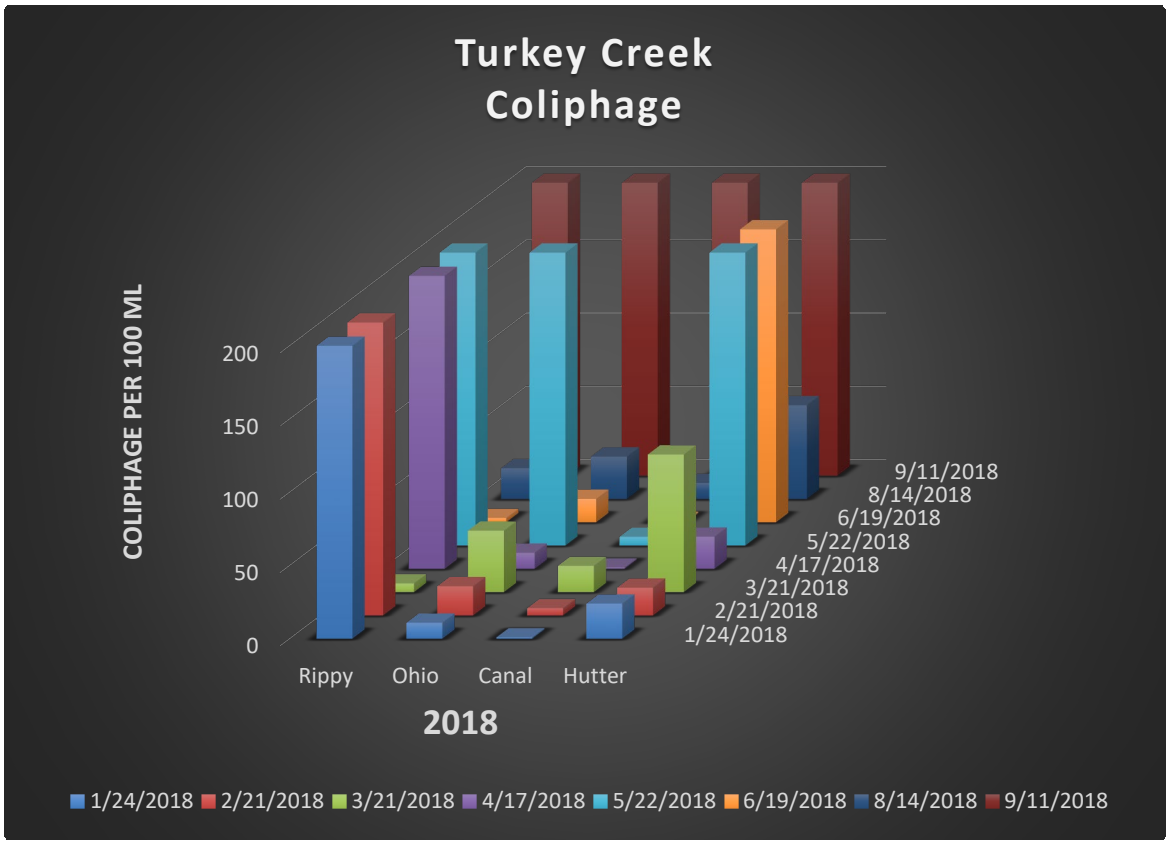


Figure 5. Male-specific coliphage (viral) plaque counts per 100 ml of ambient waters (from Table 5). Ambient waters were concentrated per 100 ml in triplicate. Male-specific coliphage counts are the average of the three-100 ml sample volumes. If the counts were TNTC, an arbitrary value was assigned of 200 MPN/100 ml for plotting purposes. Counts were not performed per 100 ml in 2017, only per liter.

3.9 Rain data

To evaluate the correlation of rain events with *E. coli* enumeration, monthly and daily rain precipitation (in inches) were obtained from the Oregon State PRISM Climate Group (http://prism.oregonstate.edu/explorer/). PRISM assembles datasets by gathering climate observations from a variety of monitoring networks.

Table 8. Dates that all stations exceeded the EPA standard of 410 MPN/100 *E. coli* and the corresponding rain in inches. Note: Canal station did not exceed the standard on May 22, 2018, but the remaining stations were exceptionally high.

DATE EXCEEDED	Rain 48 hr prior (inches)	Rain 24 hr prior (inches)	Rain on collection day (inches)
April 4, 2017	0	2.68	0.79
May 23, 2017	1.3	0.82	0.8
June 22, 2017	0.07	6.52	1.64
October 24, 2017	0.52	5.49	0
December 19, 2017	0	2.58	0.06
April 17, 2018	9.7	0	0
May 22, 2018	0	0	1.29
July 2, 2018	0.31	0.54	0.85
July 17, 2018	0.09	0.93	3.52
September 11, 2018	0	0.9	1.19

When EPA standards were exceeded in 2017 for all stations, heavy rains had occurred within 24- 48 hours of the sample date. Rain data total over a period of 48 hr prior to the collection date of April 4, 2017, was 3.47 inches (April 3, April 4). For the May 23 collection date, rainfall was a total of 2.92 inches within 48 hr; June 22 collection date had a total of 8.16 inches within 48 hr and the October 24 sample date, rain values totaled 6.01 inches over a two-day period. Overall, rainfall was 1-8 inches within 24-48 hr of the actual sample dates in 2017 for the days where all stations exceeded the EPA *E. coli* standards. Although rainfall was 4 inches on January 1, stations were not sampled until January 5 and all of the stations were not in exceedance, suggesting rain that occurs within 24-48 hr prior to sample collection is an important driver in run-off and fecal contamination issues in this watershed and that the contamination decreases within approximately three to five days after a rain event.

During 2018, the dates that *E. coli* levels were exceeded for all six sample stations were as follows: April 17, May 22 (except Canal), July 2, July 17 and September 11, 2018. For the collection date of April 17, there was zero rainfall 48 hr prior but on April 15 (72 hr prior) there were 9.7 inches. On May 22, rainfall was 1.29 inches occurring on the collection date and the July 2 collection date had a total of 1.39 inches within 48 hr. July 17 collection date had a total of 4.45 inches within 48 hr and for September 11, 2018, a total of 2.09 inches of rain occurred within 48 hr of sample collection.

Spearman correlation was used to evaluate all rain (24, 48, 72 and 96 hr prior to collecting the water sample) and IDEXX data for 2017 and 2018. With the exception of the Hutter station, Spearman values

ranged from 0.48-0.60 for the 24 hr rain data, suggesting that 24 hr prior to water collection was driving the IDEXX (*E. coli*) enumeration values. Rain data did not correlate to the Hutter station. On days where rainfall was not a driving factor, is it possible that a sub-surface transport of fecal contamination occurred into the watershed.

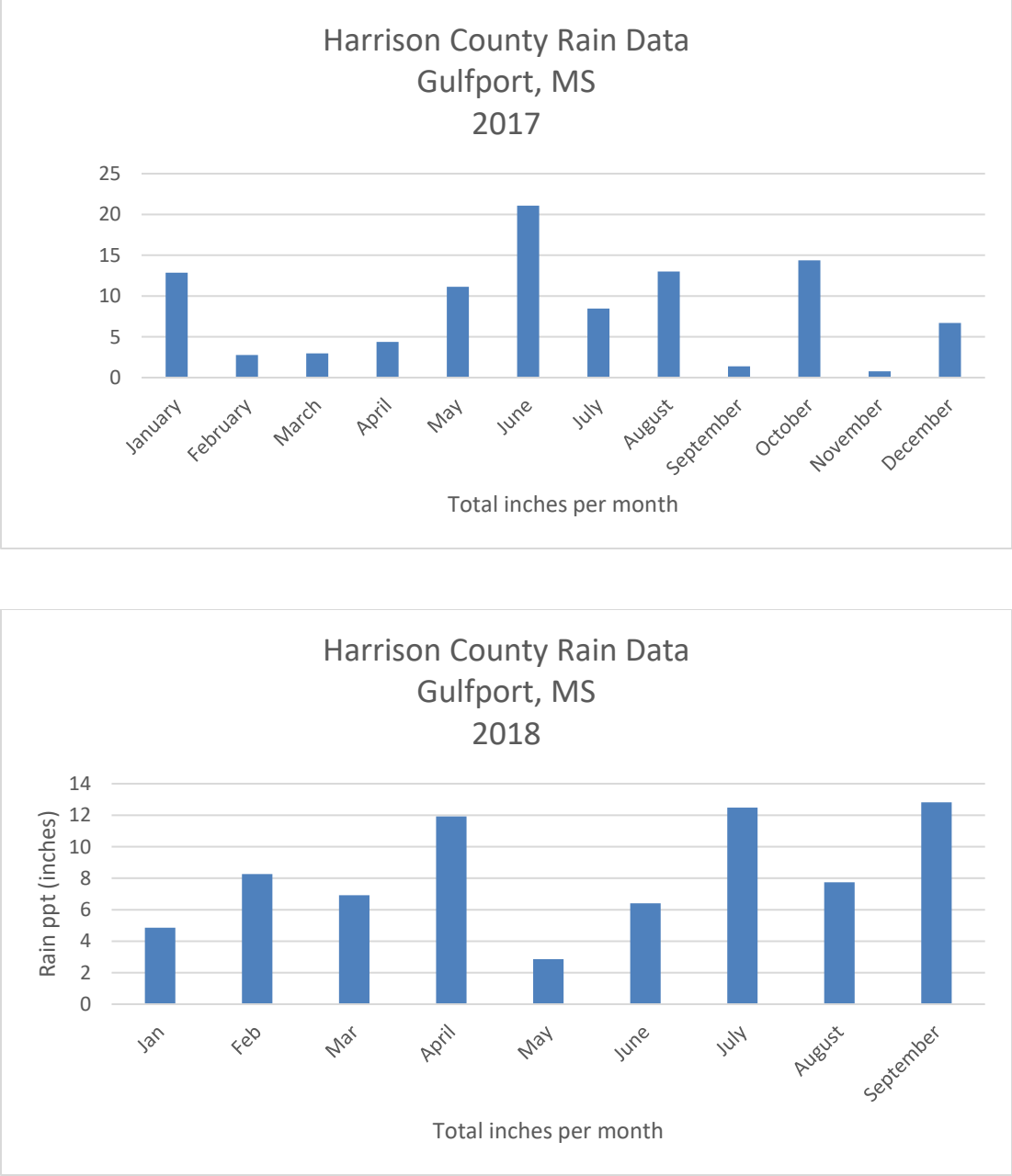


Figure 6. Monthly rain data (inches) for Gulfport, MS, area (Harrison county) for January 2017 to December 2017 and January 2018 to September 2018.

3.10 GIS and Landscape Data

City-sewage lines were located at Hutter, Ohio, Arkansas, Airport and Rippy but not at the Canal station. A lift station was adjacent to Hutter and the Rippy station was closest in proximity to a waste-water treatment plant. Septic tank data was not obtainable. (see Appendix C).

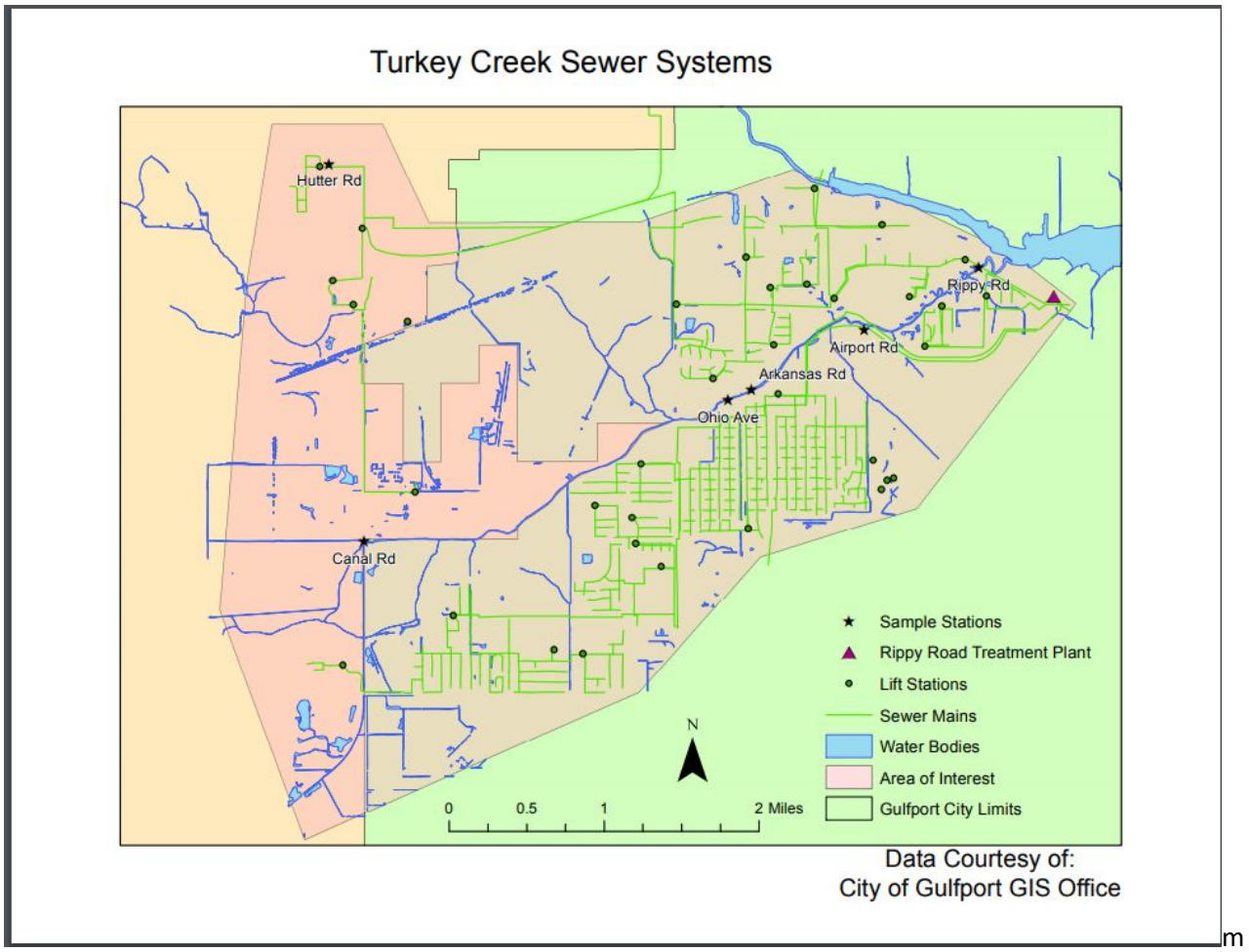


Figure 7. Turkey Creek Sewer Systems

4.0 Discussion

In order to thoroughly evaluate the microbial fecal source, three methods were utilized to distinguish animal from human sewage, i.e., genotyping of FRNA coliphage viruses, PhyloChip of microbial DNA and gene-specific source-tracking markers using qPCR. Viral coliphage genotyping of the Rippy station indicated a human-sewage signature with a total of 9 positive detections for genogroups II and III (human signature trend) and a total of 5 positive detections for animals (genogroups I and IV) throughout this study. The remaining stations, Ohio, Canal and Hutter, were dominated by animal coliphage signatures. Out of two years, the Canal station had a viral human signature on one sample collection day, November 8, 2017; no rain event occurred on or prior to that day. Sometime in the fall of 2017 there was a change-over in a pump station in the Canal area; but it is not certain that this event influenced the human signature on that day. Ohio had mixed animal and human signatures, was animal-dominant by viral genotyping and indicated a human signature with PhyloChip on one sample date in

2017.

The bacterial source-tracking markers HF183 and HumM2 were only positive at Rippy on May 22, 2018 and July 17, 2018, no data were generated or collected for bacteria markers in 2017. These two days also correspond with the times that all stations were above the EPA *E. coli* standards except for Canal (*E. coli* 157 MPN/100 ml on May 22). However, all stations were at the maximum *E. coli* levels of 2419 MPN/100 ml on July 17. Arkansas was 1732 MPN/100 ml and the remaining stations were 2419 MPN/100 ml. Although *E. coli* levels were elevated at the other stations, only Rippy indicated the human contamination for the bacterial molecular markers and, additionally, was dominant for the coliphage FRNA viral genotypes II and III during this study. This evidence adds to the confidence that Rippy was impacted with human sewage.

Rippy could potentially be influenced by the three National Pollutant Discharge Elimination System (NPDES) permitted wastewater effluents if indeed these effluents are mixing with the creek during incoming tides. Three permitted NPDES systems discharge into Bayou Bernard as follows: NPDES 023345 Gulfport POTW South; NPDES 027537 Industrial District; NPDES 051756 Gulfport POTW North. Our observations noted that there was salt-water intrusion at the Rippy sample-collection site, probably due to tidal influences from Bayou Bernard. It may be probable that the treated wastewater effluent could be carried with the tidal flow as the outflow pipe from the plant is approximately 0.5 miles (804.7 m) away from the Rippy sample-collection station. No septic tank data was available but to our knowledge, there are no houses in close proximity along the creek in this area of Rippy Road as there is a forest buffer zone along the Turkey Creek watershed, and, in addition, city sewer lines are also present in this area of Gulfport.

The Canal collection site does have a house that has a few livestock (goats and sheep) along the watershed. However, this station was often the least polluted area when compared to the other stations.

The Airport Road collection site was a more open region, without trees, but with cultivated grass vegetation in the proximity of the Gulfport-Biloxi International Airport. No homes were in this region and more than likely, contamination was due to non-point source run-off as *E. coli* exceedances were similar or identical to the other stations, except Hutter. Human bacterial markers and viral genotyping were not performed at this station.

Hutter Road collection site is adjacent to a lift-station and the nearby subdivision is on city sewer. Google earth shows what appears to be a holding pond just upstream from the Hutter site, and the creek runs through this pond. This area is on private land and is inaccessible but could provide insight as to the potential source of the Hutter contamination issues. The coliphage data suggests the FRNA viral population at Hutter is mixed human and animal. PhyloChip data indicated a human signature and the data was negative for the human bacterial source-tracking markers. If indeed the contamination is from a leaking or groundwater transport from the lift station, the animal contribution could be from domesticated indoor pets and/or nearby wildlife or other upstream sources. Hutter was unique in that there appears to be minimal flow and is somewhat stagnant, especially during dry seasons, whereas the remaining stations were along the open flowing creek waterway. The data trend at Hutter showed *E. coli* levels decreased when the remaining stations increased in fecal contamination during rain events thus suggesting Hutter does not follow the non-point source trend (run-off) but could likely be a point-

source contamination issue.

The State of Mississippi issues "do not swim" advisories based on the Mississippi Beach Task Force recommendations within 24 hr of greater than 1 inch of rainfall (http://www.beachapedia.org/Beach_Water_Quality_Monitoring_Programs_in_Coastal_States#Florida). The Turkey Creek data supports this advisory as elevated values of *E. coli* numbers followed a trend with 24 hr rainfall data. In 2017 when precipitation was 1.5 inches or greater within 48 hr prior to sample collection, all stations exceeded the EPA standards of >410 MPN/100 ml, and often > 1000 or 2000 MPN/100 ml. The exception occurred on October 11, 2017, when 2.7 inches of rain occurred within the 48 hr but only two stations exceeded the criteria. Although rainfall was 4 inches on January 1, we did not sample until January 5 and all of the stations were not in exceedance. This suggests that a rain event that occurs within 1-2 days prior to sample collection is therefore an important driver in run-off and fecal contamination issues in this watershed and that the contamination decreased within approximately three to five days after a rain event. Sample stations were less likely to exceed the criteria when a rain event ended 48 hr before sampling unless the rain event was excessive, such as the 9.7 inches observed in April 2018. What is noteworthy, however, is that the Hutter *E. coli* data trend was lower with rain events when the remaining stations were much higher suggesting the rain had a dilution effect on the fecal contamination at Hutter. This could also be due to increased creek flow as Hutter was often stagnant or very low flow. When precipitation values were averaged for 48, 24 and 0 hours prior to sample collection dates, rain volume data statistically correlated to *E. coli* values for Rippy, Canal and Ohio. Canal had the strongest correlation with $R^2 = 0.84$, followed by Ohio of $R^2 = 0.61$, Rippy with $R^2 = 0.59$ and very weak correlation between rainfall and Hutter ($R^2 = 0.23$) (Table 8; Appendix A2).

5.0 Conclusions

1. *E. coli* fecal indicators (MPN per 100 ml creek water) did not significantly correlate to temperature, pH, nutrients, salinity or total suspended solids in Turkey Creek.

2. Creek stage height had a moderate positive correlation ($R^2 = 0.65$) to *E. coli* MPN at the Canal station but not at Hutter, Ohio or Rippy.

3. Rain data had a moderate to strong positive correlation to *E. coli* MPN values to all stations except Hutter.

4. Landscape and GIS data did not reveal any obvious influences or point-sources that would contribute to contamination. However, Rippy is upstream from a wastewater-treatment plant. It is possible that tidal influxes into Rippy could be influencing the fecal contamination. The maps show the city sewer lines crossing Rippy at the bridge where samples were collected for this study and if there are any breaks in this line, a potential sewage leak could occur near the creek. The Hutter sample site was adjacent to a lift station and could be potentially influenced by leaks, ground-water transport or upstream sources.

5. Septic tank data was not available for Gulfport, however, most of the creek is buffered by a forested riparian zone.

6. FRNA coliphage viral genotyping suggests a human impact at Rippy at different sampling dates in this study. Except for one day at Canal, the remaining sample dates and collection sites were mostly impacted by animal FRNA genotypes.
7. The Hutter collection site is adjacent to a lift station, had both human and animal FRNA genotypes and was positive for human-sewage signatures by PhyloChip analysis.
8. Human-specific source-tracking molecular markers (for bacteria) were positive for Rippy but not for the other stations.
9. Sediments did not harbor *E. coli* or enterococci, therefore, the *E. coli* detected in the IDEXX assays originated from within the water column and not from microbial growth or suspensions from the sediments.
10. Citizens Science outreach program was successful in hands-on training and educating the public and students about water-quality awareness. Some of the local students received college credits for participation.

6.0 Summary

In summary, viral and bacterial signatures support the findings that Rippy has a human-sewage impact. Hutter consistently exceeded the EPA standards for *E. coli,* had a mixed viral signature (human and animal), a human-signature with PhyloChip analysis and no human signature for source-tracking molecular bacteria indicators. Point-source contamination such as leaking pipes, lift station issues or waste-water treatment inflow may possibly be the source of fecal contamination at Hutter and Rippy. The possibility exists that Hutter may be impacted by upstream sources such as the holding pond that appears to intersect the creek. However, it is on private property, therefore not accessible and was not included in this study. Ohio, Canal, Airport and Arkansas were not as contaminated and exceeded the EPA standards for freshwater fecal contamination following rain events, most likely due to run-off.

If the microbes were transported via the groundwaters, the fecal origin could be due to old septic lines/tanks. However, this could not be determined as we were unable to obtain septic data. The watershed is bordered by heavily forested vegetation and is unlikely impacted by the few adjacent homes. There is one home adjacent to the Canal station that has livestock, however, it was often the least contaminated station.

7.0 Recommendations and Suggestions

1. Investigate the Hutter station by the use of tracer dyes (injected into the lift station) to trace the possibility of pipe leaks and/or transport into Hutter via groundwater flows.

2. Investigate what appears to be a holding pond on private property in the middle of Turkey Creek just upstream (236.91 m or 0.184 mile or 974 ft) from the Hutter station (see map in Appendix D)

3. Investigate the Rippy collection site by tracer dyes to determine if the wastewater-treatment effluent is entering the Rippy site during in-coming tides.

4. Investigate the Rippy sewer lines for leaks that, according to the GIS maps, cross Turkey Creek at the sample site near the bridge.

5. Investigate adding green infrastructure such as filtering plants, bioswales, etc, in the areas of the Ohio, Canal, Airport and Arkansas stations to capture and filter runoff and thus minimize contamination into Turkey Creek.

5.0 References

EPA Method 1696. 2019. Characterization of human fecal pollution in water by HF183/BacR287 TaqMan quantitative polymerase chain reaction (qPCR) assay. EPA 821-R-19-002.

EPA Method 1697. 2019. Characterization of human fecal pollution in water by HumM2 TaqMan quantitative polymerase chain reaction (qPCR) assay. EPA 821-R-19-003.

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Watershed implementation plan Turkey Creek. 2006. Prepared in collaboration with Turkey Creek and North Gulfport community partners. Sponsored by Land Trust. Funding assisted by US EPA, Region IV. http://ltmcp.org/wp-content/uploads/2015/01/final.TurkeyCreek.WIP_.pdf

6.0 Appendices

Appendix A. *E. coli* results and enumeration

Table A1. *E. coli* MPN per 100 ml for each station at Turkey Creek for 2017.

Date	Rippy	Airport	Arkansas	Ohio	Canal	Hutter
1/5/2017	579.4	579.4	980.4	517.2	365.4	290.9
1/18/2017	122.3	613.1	240	206.4	93.3	133.3
1/31/2017	1299.70	2419.60	307.60	290.90	40.80	2419.60
2/14/2017	980.40	2419.60	488.40	325.50	86.00	1986.30
3/1/2017	43.50	72.80	63.80	113.70	41.40	2419.60
3/14/2017	387.30	298.70	344.80	248.10	186.00	816.40
3/22/2017	38.80	44.10	60.90	44.60	70.60	2419.60
4/4/2017	2419.60	2419.60	2419.60	2419.60	2419.60	2419.60
4/19/2017	14.60	25.90	32.70	24.60	156.50	648.80
5/3/2017	43.90	36.40	98.40	116.90	161.60	2419.60
5/16/2017	38.30	35.90	34.10	50.40	24.70	2419.60
5/23/2017	648.80	2419.60	2419.60	2419.60	613.10	2419.60
6/6/2017	127.4	113.7	155.3	214.3	118.7	2419.6
6/22/2017	1553.1	1986.3	2419.6	1732.9	1986.3	1986.30
7/5/2017	24.3	88.2	1203.3	209.8	86.5	2419.6
7/19/2017	378.40	240.00	344.80	387.30	198.90	2419.60
8/2/2017	41.40	28.20	31.70	36.80	31.30	2419.60
8/15/2017	83.90	178.50	148.30	148.30	770.10	2419.60
8/29/2017	195.60	770.10	209.80	307.60	613.10	2419.60
9/12/2017	25.90	32.30	37.90	57.30	36.40	184.20
9/27/2017	36.80	27.50	31.50	15.80	45.20	866.40
10/11/2017	387.3	579.4	365.4	365.4	290.9	1046.2
10/24/2017	1732.9	1413.6	1732.9	866.4	1553.1	770.1
11/7/2017	139.6	63.3	298.7	218.7	93.4	920.8
11/29/2017	26.2	26.6	83.9	25	148.3	2419.6
12/5/2017	162.4	104.6	107.6	72.3	90.6	2419.6
12/19/2017	2419.6	2419.6	2419.6	2419.6	1732.9	727

Table A2. *E. coli* MPN per 100 ml for each station at Turkey Creek for 2018.

Date	Rippy	Airport	Arkansas	Ohio	Canal	Hutter
1/4/2018	93.3	76.3	129.6	686.7	435.2	2419.6
1/19/2018	43.1	53.7	58.8	62	44.8	129.1
1/24/2018	59.8	28.5	65	50.4	108.8	2419.6
2/5/2018	435.2	307.6	517.2	579.4	686.7	512.2
2/21/2018	325.5	727	344.8	275.5	90.6	1986.3
3/5/2018	59.1	57.3	88	81.6	35.5	325.5
3/21/2018	307.6	155.3	166.4	129.6	148.3	1299.7
4/3/2018	151.5	116.9	142.1	125.9	72.7	727
4/17/2018	686.7	686.7	410.6	547.5	613.1	488.4
4/24/2018	127.4	41.1	98.8	52.0	50.4	488.4
5/8/20122	11.0	16.0	21.6	19.3	40.2	*
5/22/2018	2419.6	2419.6	1732.9	2419.6	157.6	2419.6
6/6/2018	4.1	8.4	22.3	33.1	34.5	13.4
6/19/2018	19.9	71.2	81.3	78.9	60.9	18.8
7/2/2018	1553.1	2419.6	2419.6	2720.0	410.6	2419.6
7/17/2018	2419.6	2419.6	2419.6	2419.6	2419.6	2419.6
7/30/2018	30.1	224.7	71.2	36.4	25.0	14.5
8/14/2018	32.3	128.1	39.3	43.7	132.0	365.4
8/30/2018	248.9	613.1	325.5	172.3	193.5	1986.3
9/11/2018	2419.6	2419.6	2419.6	2419.6	816.4	1299.7
9/25/2018	488.4	190.4	579.4	613.1	517.2	3090.0

*Could not collect water for IDEXX on 8May2018 Hutter station because a snake was in the path.

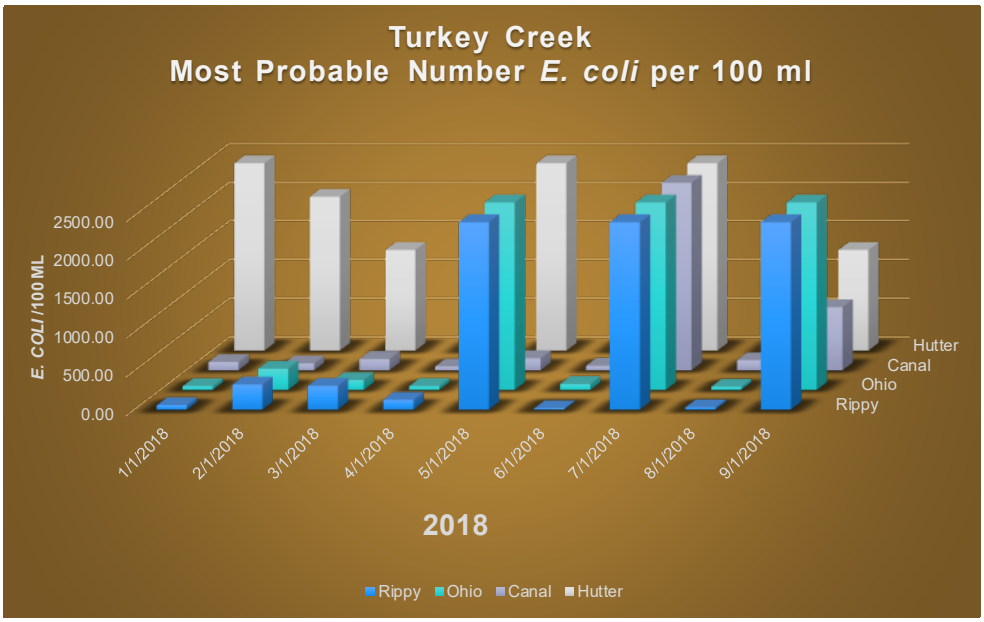


Fig. A1. *E. coli* enumeration for 2018.

Fig A2. Correlation for *E. coli* MPN/100 ml and three-day average rain data for 2017 - 2018.

Hutter correlation with rain

	Column 1	*Column 2*
Column 1	1	
Column 2	0.232696	1

Canal correlation with rain

	Column 1	*Column 2*
Column 1	1	
Column 2	0.845126	1

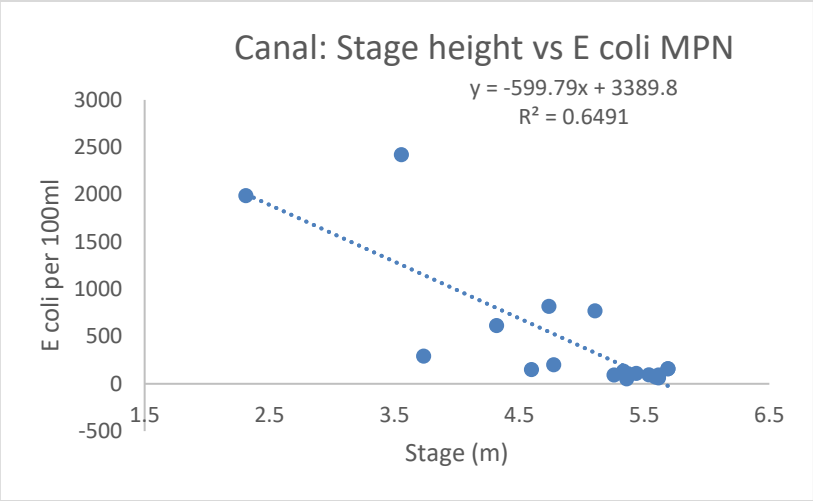
Ohio correlation with rain

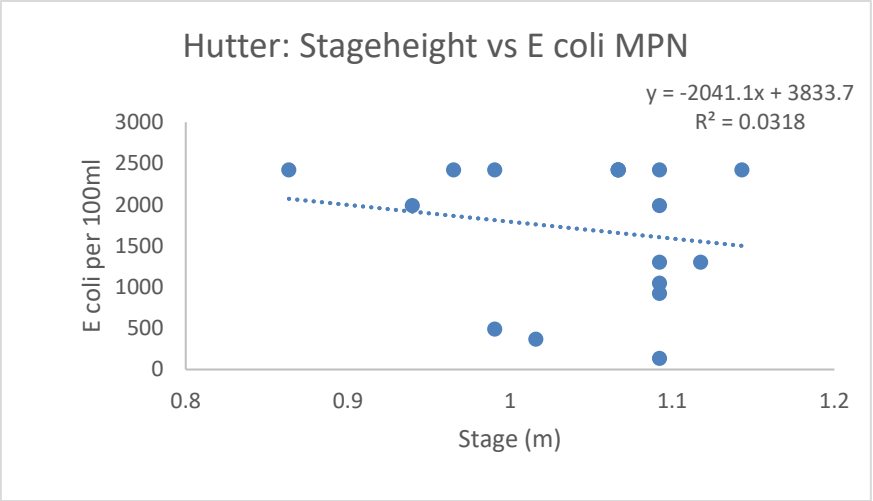
	Column 1	*Column 2*
Column 1	1	

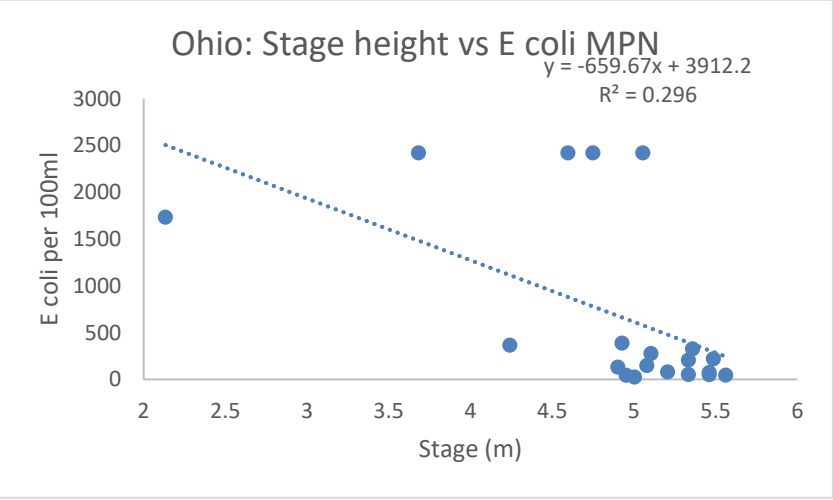
Column 2	0.612158	1

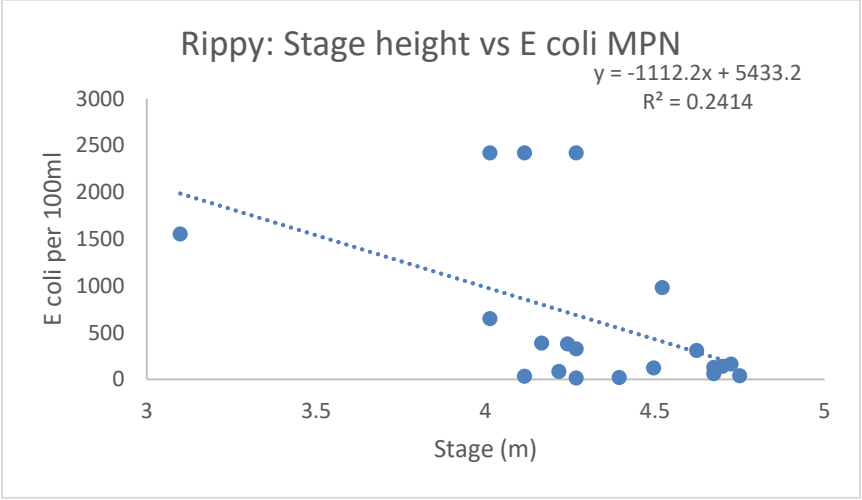
Rippy correlation with rain		
	Column 1	*Column 2*
Column 1	1	
Column 2	0.590604	1

A3. Correlations for *E. coli* MPN and creek-stage height 2017-2018.









Appendix B. Results of male-specific coliphage viral detection.

Table B. One liter of water was collected, concentrated to extract the viruses, and approximately 2-4 ml of concentrated water was plated onto a MS2-phage specific selective-media plate. Counts denote approximate male-specific coliphages (FRNA and FDNA) per Liter. TNTC is "Too Numerous To Count." TNTC was denoted as 200 PFU/L.

Table B1. 2017 Male-specific coliphage plaque-forming unit (PFU) per liter.

DATE 2017	RIPPY	OHIO	CANAL	HUTTER
1/20/2017	3	0	0	0
2/15/2017	6	4	1	56
3/23/2017	1	15	7	200
4/20/2017	38	200	50	44
5/24/2017	135	200	24	200
7/19/2017	200	104	200	200
8/15/2017	200	200	200	200
11/7/2017	36	29	20	56
12/5/2017	130	42	18	200
AVERAGES (no Jan data) per Liter	93	99	65	145

Note: Too Numerous To Count (TNTC) was given a value of 200 PFU.

Table B2. 2018 Male-specific coliphage plaque-forming unit (PFU) per liter.

DATE 2018	RIPPY	OHIO	CANAL	HUTTER
1/24/2018	200	40	11	97
2/21/2018	200	200	60	200
3/21/2019	200	200	200	136
4/17/2018	200	40	11	56
5/22/2018	200	200	70	200
6/19/2018	42	200	45	200
8/14/2018	191	102	200	200
9/11/2018	200	200	200	200
AVERAGES per Liter	179	148	100	161

Table B3. 2018 Male-specific coliphage plaque-forming unit (PFU) per 100 ml.

2018	RIPPY	OHIO	CANAL	HUTTER
1/24/2018	200	11	1	24
2/21/2018	200	20	5	19
3/21/2019	6	42	18	94
4/17/2018	200	11	1	22
5/22/2018	200	200	6	200
6/19/2018	3	16	1	200
8/14/2018	21	29	11	64
9/11/2018	200	200	200	200
AVERAGE per 100 ml	129	66	30	103

Fig. B1. 2018 Male-specific coliphage plaque-forming unit (PFU) per 100 ml.

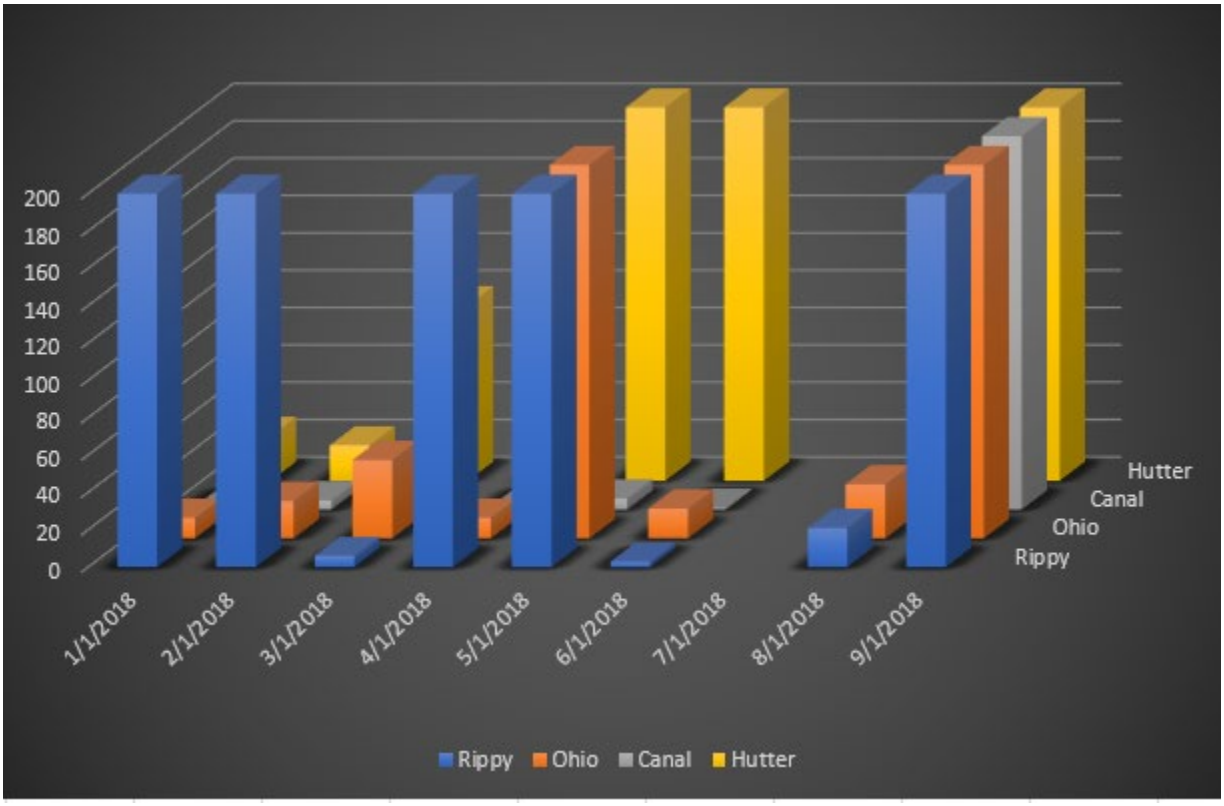


Table B4. Statistical analysis using ANOVA to evaluate differences in viral concentrations for each station.

ANOVA of coliphage MPN/100 ml for 2018.

Anova: Single Factor

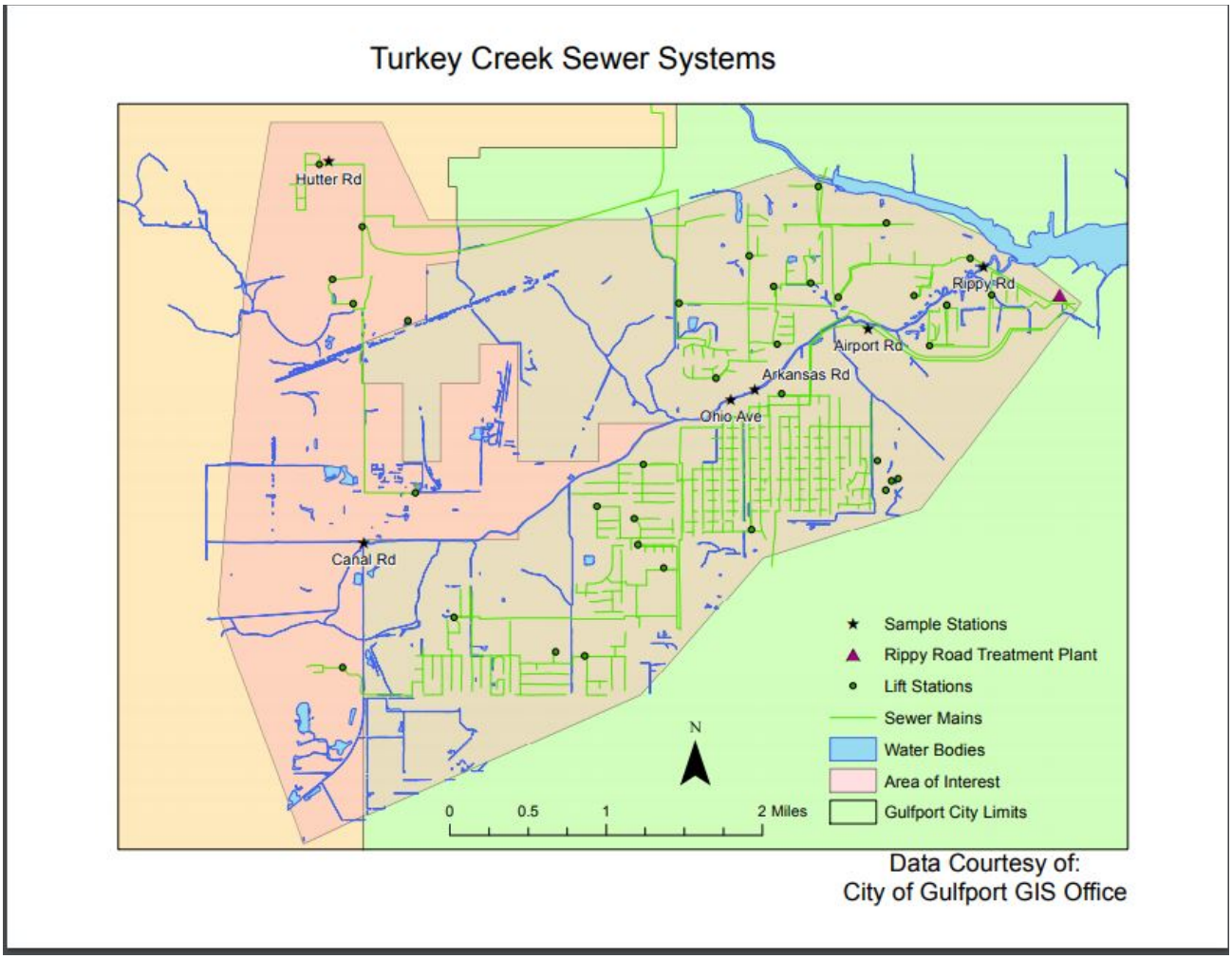
SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1		103		9696.21
Rippy	8	0	128.75	4
Column 2				6931.83
Ohio	8	529	66.125	9
Column 3				4732.55
Canal	8	243	30.375	4
Column 4				7098.12
Hutter	8	823	102.875	5

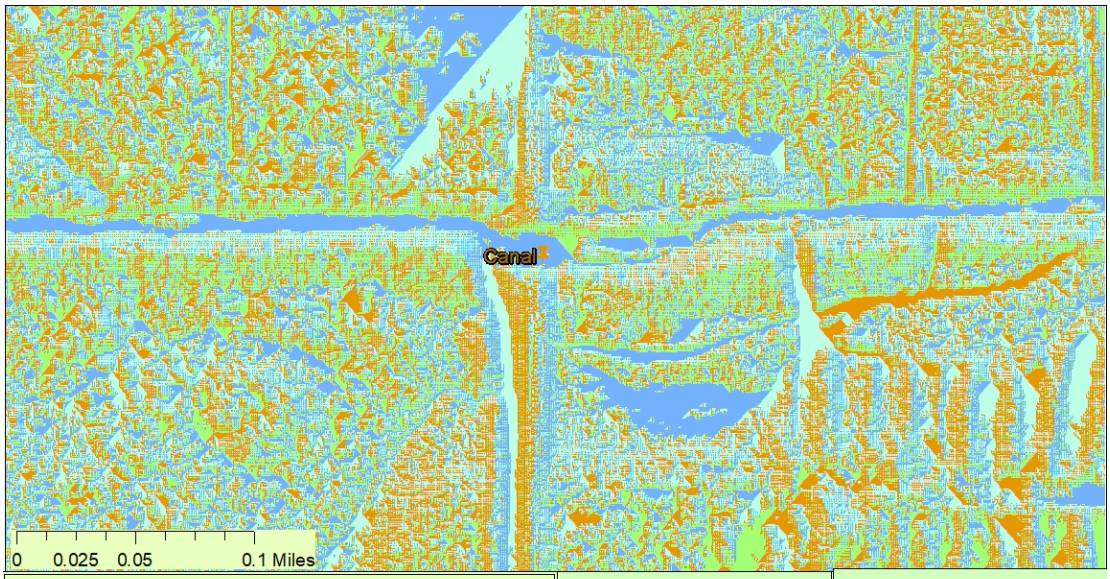
ANOVA

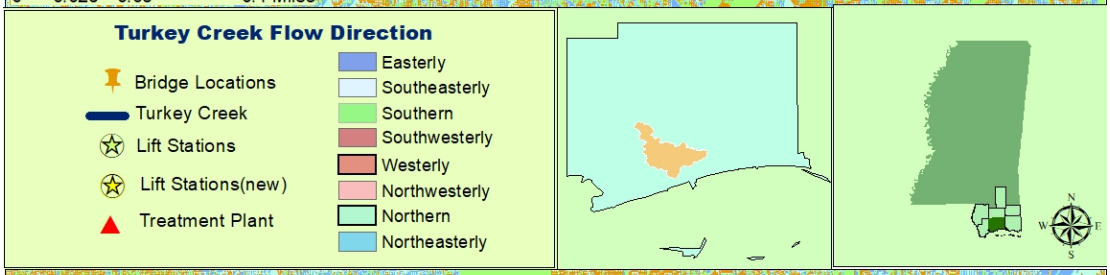
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	44307.84	3	14769.28125	2.075887	0.126029	2.946685
Within Groups	199211.1	28	7114.683036			
Total	243519	31				

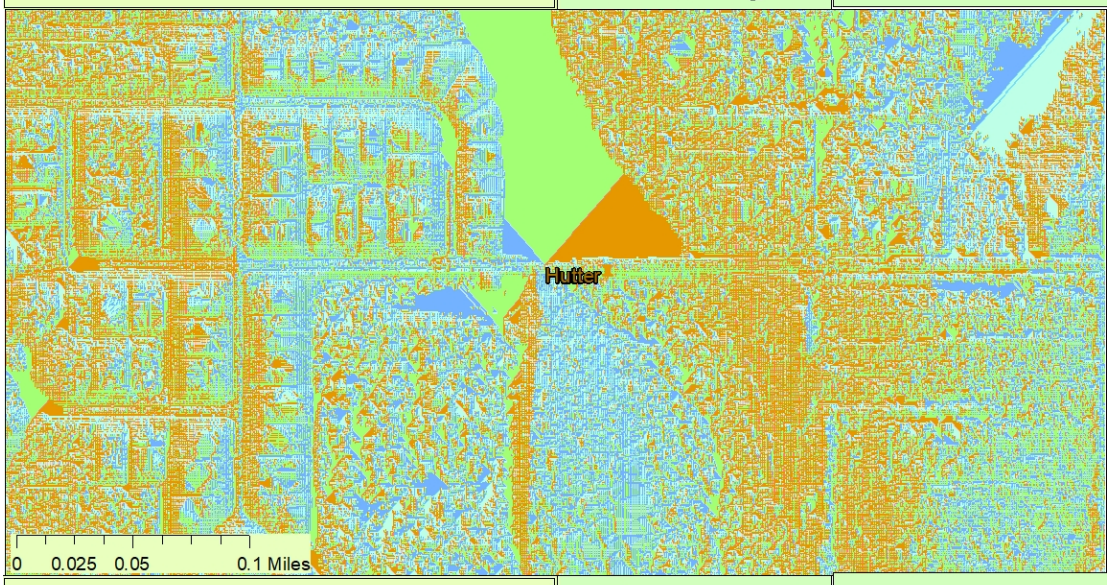
Appendix C. GIS and landscape results for Gulfport, MS.

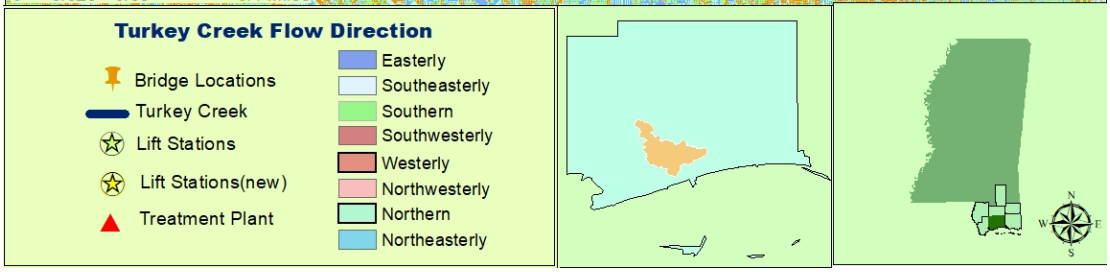


Flow maps: Canal station and Hutter Station**.**

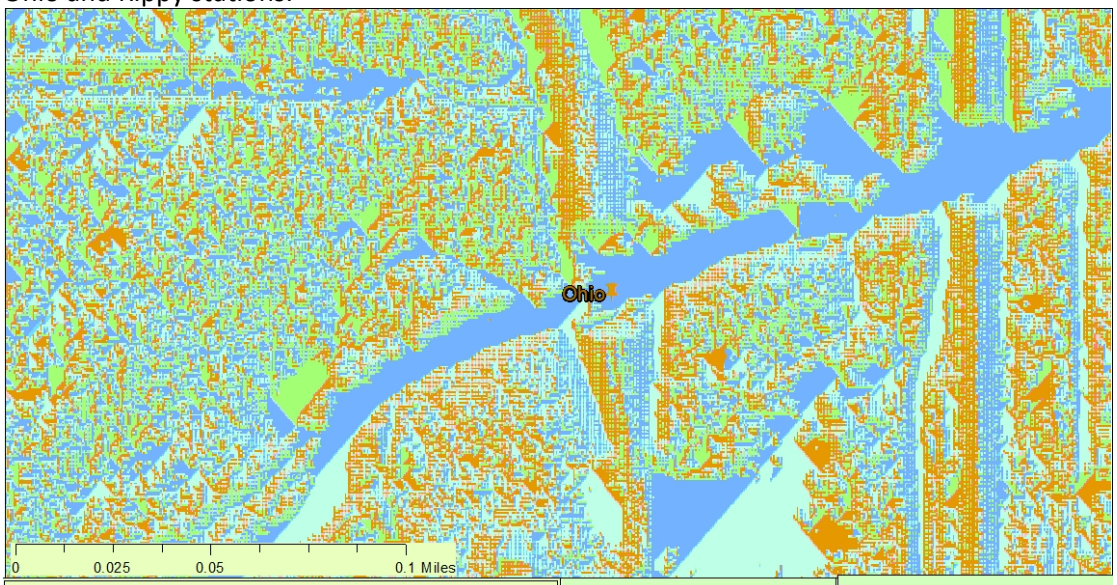


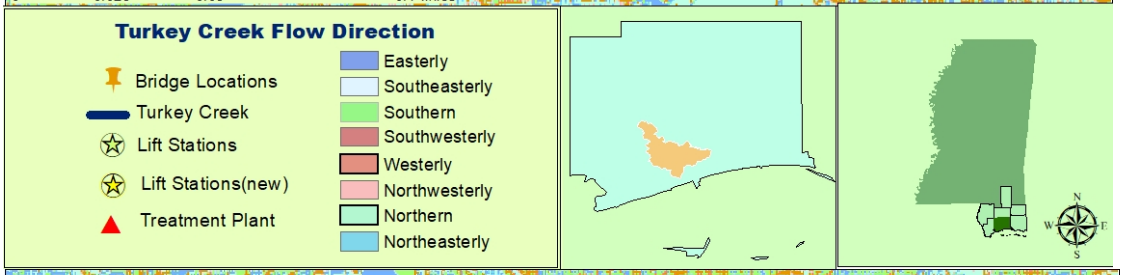


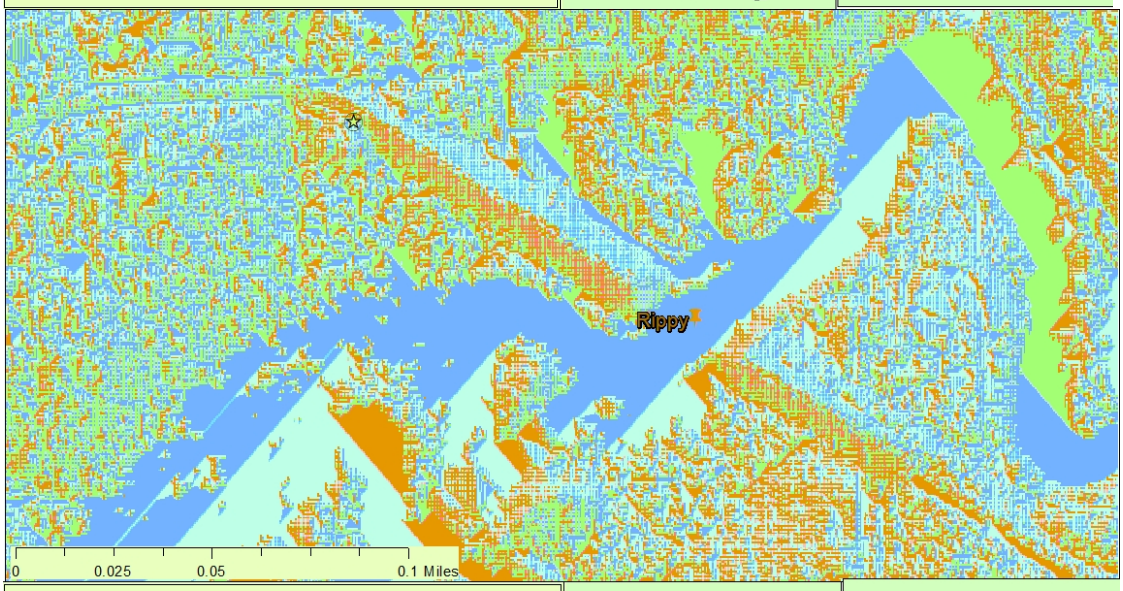


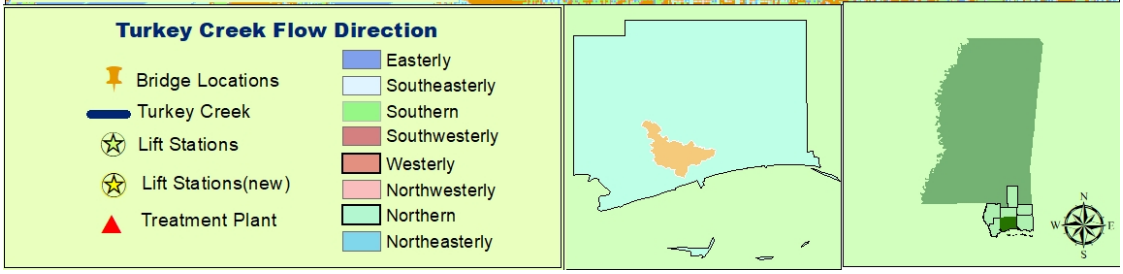


Ohio and Rippy stations.









Appendix D. Google Earth Maps

Fig. D1. Turkey Creek crosses through the Gulfport Airport (black arrow) and flows east into Bayou Bernard.

Fig. D2. Canal station (bridge, orange arrow) with nearby house that has livestock (goats and sheep).

Fig. D3. A holding pond upstream from Hutter. It appears that Turkey Creek runs through this pond; the pond is on private property.

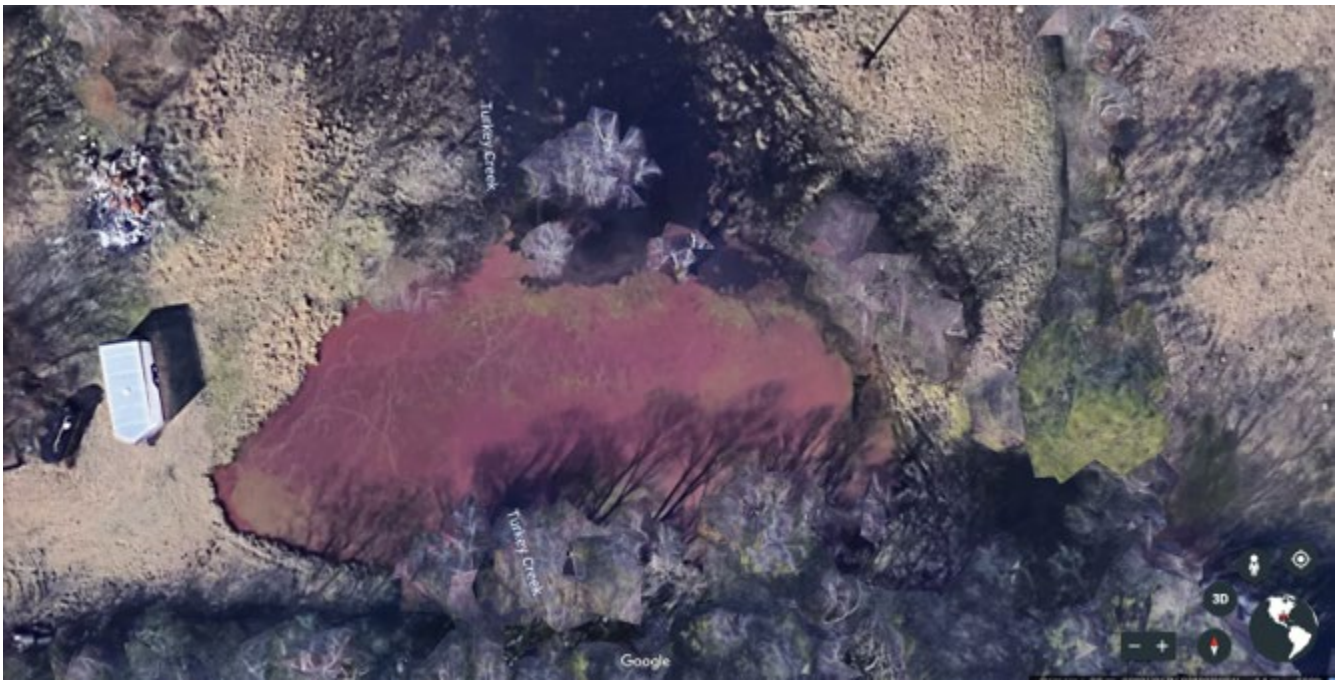


Fig. D4. Hutter sampling station (lower middle, orange arrow) and across the street is the newer subdivision. The lift station is to the left (west) of the Hutter sampling station. This area is on city sewer. The cemetery is to the lower right.

Fig. D5. Hutter station (below, at orange arrow) and view upstream where Turkey Creek flows through what appears to be a holding pond (black arrow)

Fig. D6. Arkansas Ave station at the bridge (orange arrow).

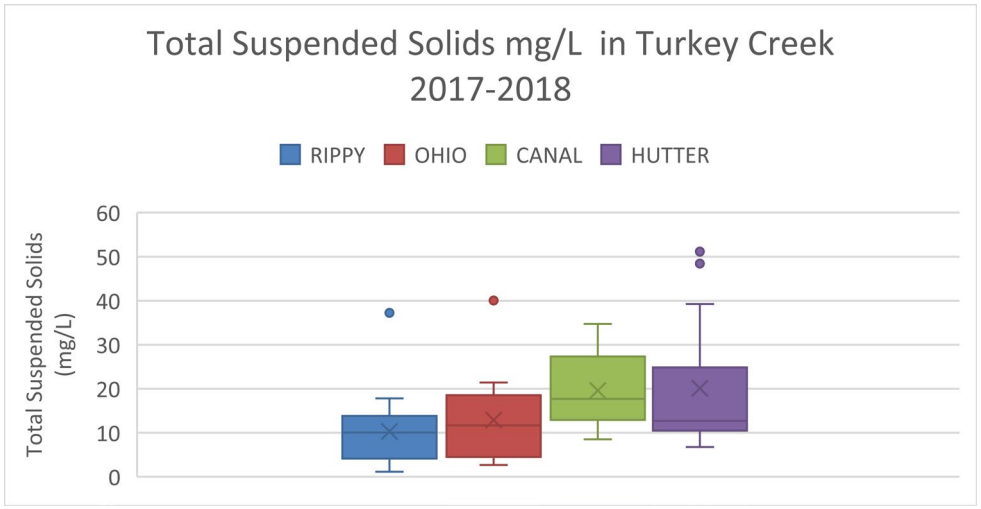
Fig. D7. Ohio Ave station (orange arrow), on the bridge just north of the school.

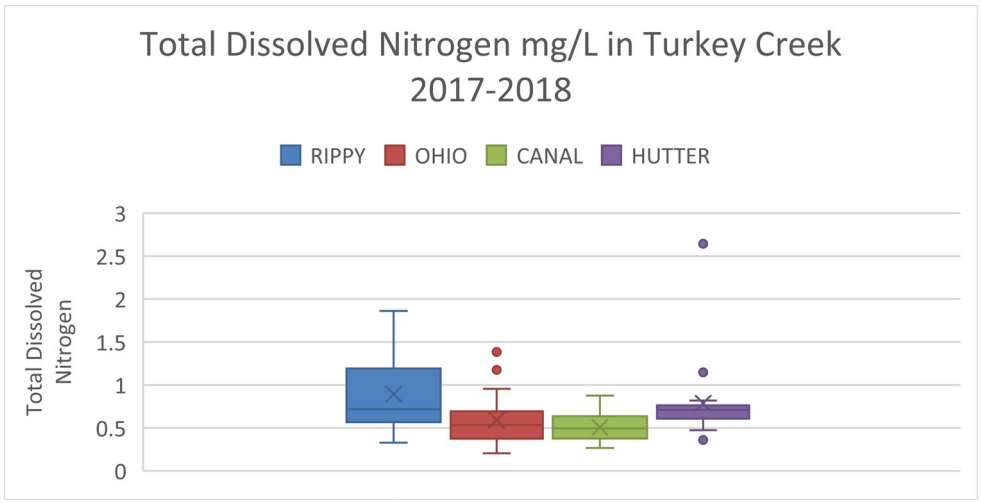
Appendix E: Nutrients

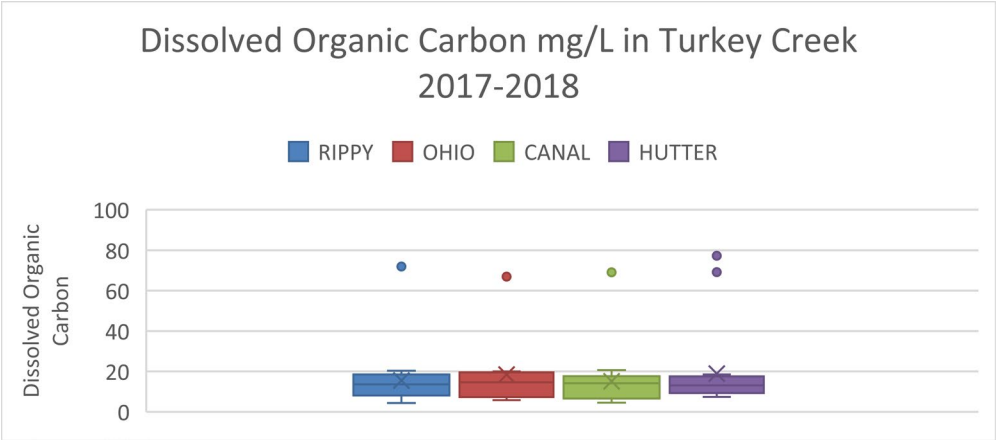
Table E1. Statistical correlation (Microsoft Excel) comparing all nutrients and *E. coli* MPN to each other. Colored boxes depict a statistical correlation; the darker the color the higher the correlation probability.

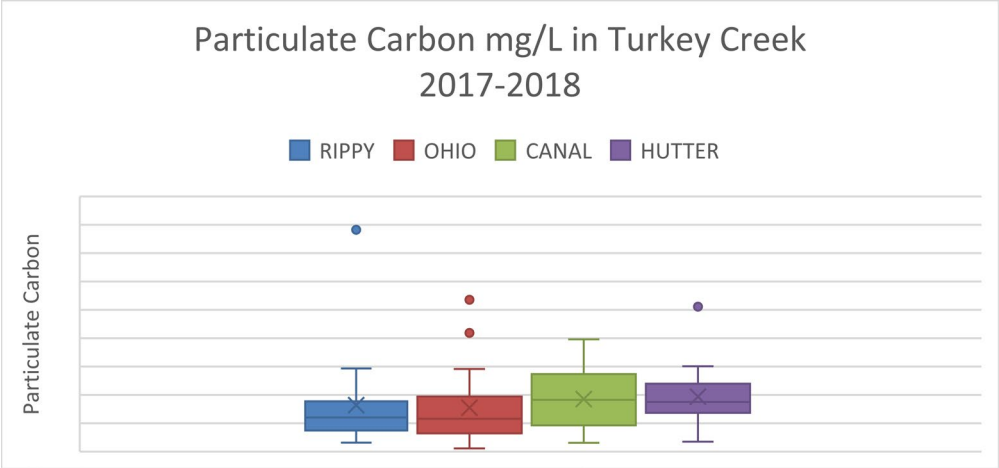
	DOC mg/L	TN mg/L	TDP uM	N mg/L	C mg/L	TSS mg/L	IDEXX E coli per 100 ml	TEMP o C	pH	Salinity ppt
DOC	1									
TN	0.493988	1								
TDP	-0.09612	0.365699	1							
N	0.156988	0.249945	0.281332	1						
C	0.199991	0.247272	0.131536	0.870487	1					
TSS	0.300429	0.118284	0.194972	0.376192	0.407674	1				
IDEXX	0.408026	0.027586	0.217492	0.045838	-0.03775	0.228645	1			
Temp	0.071128	0.004786	0.127905	0.032404	0.066396	0.047329	0.129098522	1		
pH	-0.26422	0.036996	0.12804	0.020507	0.040701	-0.04646	-0.254825553	-0.30172	1	
Sailinity	-0.14631	0.423996	0.368558	-0.07693	-0.14923	-0.26138	-0.100227378	0.006137	0.043996	1

E2. Box and whisker plots of nutrients and total suspended solids (TSS). The box represents the middle half of the data (25th to 75th percentile) and the line represents the 50th percentile.






Dissolved Organic Carbon mg/L in Turkey Creek
2017-2018
RIPPY
OHIO
CANAL
HUTTER
Dissolved Organic Carbon
100
80
60
40
20
0


Particulate Carbon mg/L in Turkey Creek
2017-2018
RIPPY
OHIO
CANAL
HUTTER
Particulate Carbon

EPA
United States
Environmental Protection
Agency

PRESORTED
STANDARD POSTAGE
& FEES PAID EPA
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