BACTERIA ABUNDANCE IN URBAN AND RURAL WATERSHEDS THAT LEAD TO THE NORTHERN GULF OF MEXICO

By

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A NON-THESIS RESEARCH PAPER PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SOIL AND WATER SCIENCES

UNIVERSITY OF FLORIDA

2022

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Introduction

Northwest Florida is known for its ecotourism attractions such as its wide variety of stunning state parks and famous white sandy beaches along the Gulf of Mexico. Around 4 million people visit these pristine beaches every year to enjoy a nice relaxing vacation in the beautiful Gulf waters with their families. What most visitors do not realize is the threats of what they cannot see in these seemingly calm waters. In Escambia and Santa Rosa County, Florida, the local agencies have confirmed bacteria-impaired waterways that are considered unsafe for recreation and commercial use. These impaired water bodies include wetlands, rivers, bayous, bays, and rural and urban stream systems that run throughout each county. All of these lead to the Gulf of Mexico through drainage networks.

Bacteria are common single celled organisms that are, in sparse numbers, no serious threat to humans. According to the CDC, Escherichia coli (E. coli) bacteria live in the intestines of humans and animals; thus, their presence indicates fecal inputs. High concentrations of E. coli bacteria and associated fecal pathogens cause illness such as diarrhea and deterioration of the intestinal tract. In rare cases, high exposure can cause death to both people and animals. E. coli bacteria that cause these illnesses can be transmitted through impaired waterways, contaminated tap water or food, and person-to-person contact (CDC). In urban watersheds, bacteria are transferred by runoff from neighborhoods or development with impervious surfaces. E. coli bacteria are naturally occurring in nature from wild animals and other organisms. Often, in rural and urban settings, bacteria are being contributed by livestock operations or areas with substantial numbers of animals such as dog parks and ponds. Humans can also be a major contributor to high bacteria concentrations. Septic systems are a potentially large contributor to urban bacteria when the systems leach out underground and contaminate the groundwater.

A direct human influence are centralized wastewater treatment systems that dump "filtered wastewater" at lift stations or dilapidated infrastructure that slowly leak wastewater into nearby streams.

Dumping of wastewater by treatment plants is legal in most areas because the water is filtered and are believed to contain low enough levels of E. coli bacteria to be considered unharmful to the nearby environment. When low bacteria concentrations mix with other factors contributing bacteria to a stream, the stream can then become impaired due to high concentrations. Both Escambia and Santa Rosa Counties benefit largely from tourism brought on by the Gulf Coast beaches such as Pensacola beach and Navarre Beach. When the local EPA tests for E. coli bacteria at these beaches they are hoping to have the "Most probable number or MPN" not exceeding 400mpn. When the recreation beach areas are higher than 400mpn they are forced to advise citizens and visitors to stay out of the impaired waters.

State agencies in Escambia County has high interests in levels of E. coli bacteria levels due to potential harm to the health of the community. Through a project coordinated by the University of Florida-Milton and funded by the US EPA, we were able to conduct field assessments of urban and rural bacteria levels. We were able to see, on a small scale, how population density plays a part in the bacteria contamination in the Gulf of Mexico. This paper will outline sample methods, identify sample site locations in Escambia and Santa Rosa County, and discuss and compare the results of rural and urban water sample collection. A connection between septic systems and bacteria results will be an aid in showing how high development density effects these dangerous bacteria levels.

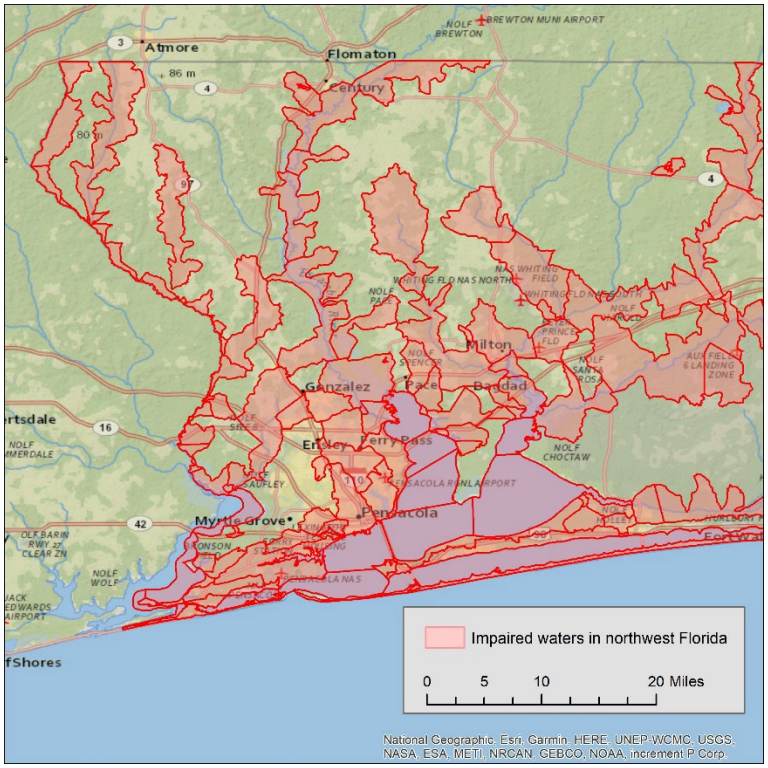
Project Objective

The hypothesis for this bacteria project was that *E. coli* bacteria levels will be higher in densely developed urban areas and lower in less developed rural areas because of less septic systems in rural areas. The main objective was to take water samples in urban and rural stream sites in Escambia County. Another objective was to compare the data of each site to each other by their relative location to other sites and septic systems.

Methods

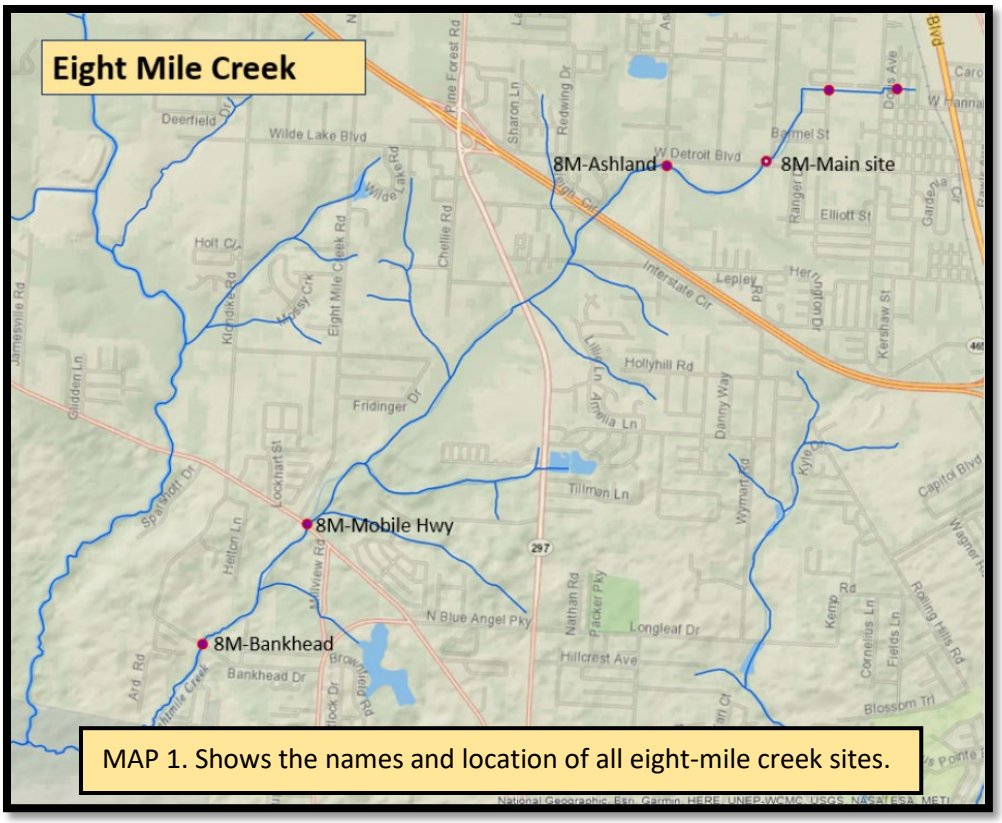
Choosing Sampling Sites

In total, fifty-two sites were sampled for this project. Forty-two urban sites were selected and 10 rural sites were selected. Some of the southernmost sites were chosen based on FDEP-classified impaired stream areas (shown on the map below). These areas have been previously identified as impaired waterways by high bacteria and nitrogen. Sites were also chosen for this project based on their relativity to each other in the watershed. Optimal streams chosen had sites located either upstream or downstream to one another. This placement of site locations allowed for water quality to be evaluated throughout a stream channel in hopes to determine which location was receiving more pollutant input. The sites could also be near wastewater treatment or known septic areas. The following images (Maps 1-6, pages 7-9) show the locations of the chosen sites.

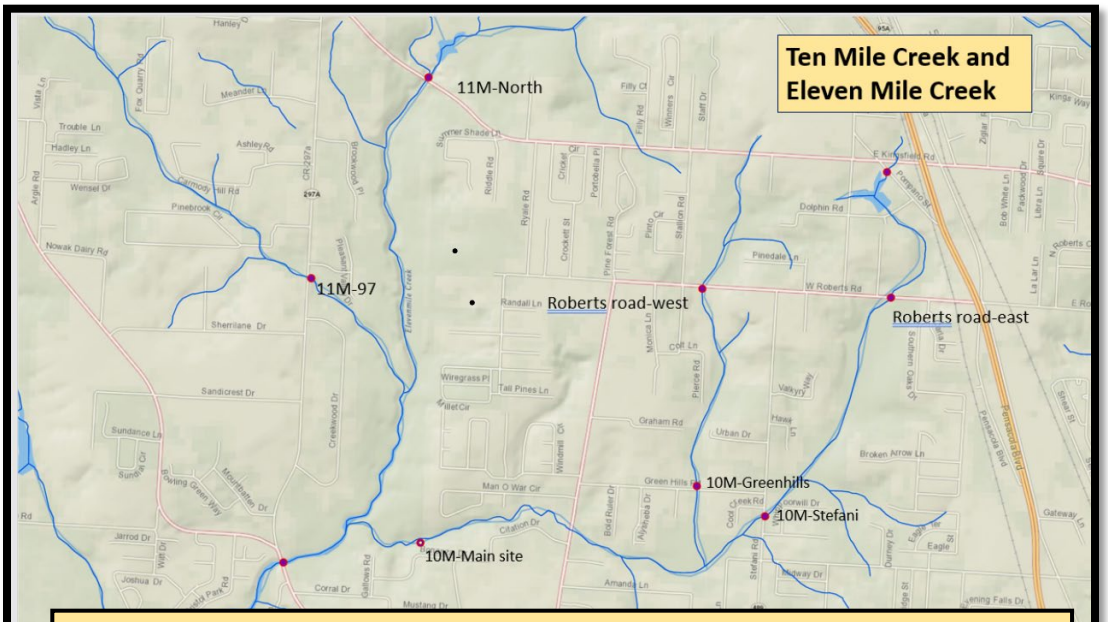


Picture 1 *Shows areas designated as impaired by Escambia EPA. The red areas are waterways that have assessed high for bacteria or nitrogen levels.*

SITE LOCATIONS: ZONE 1-EIGHT-MILE, TEN-MILE, ELEVEN MILE CREEKS

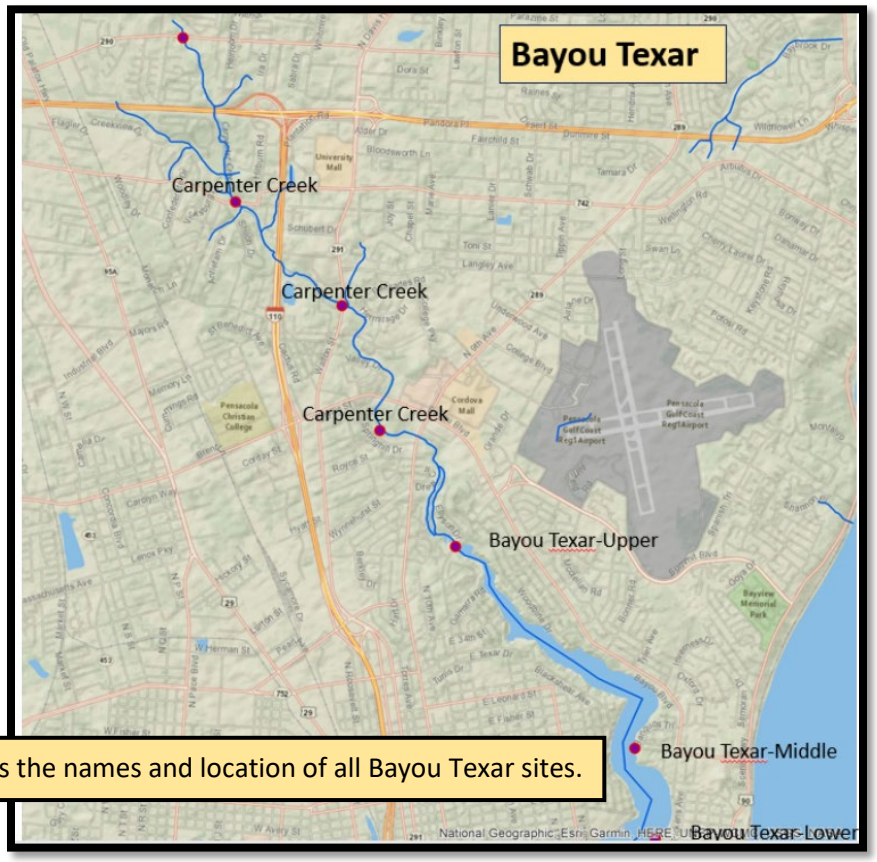


MAP 1. Shows the names and location of all eight-mile creek sites.

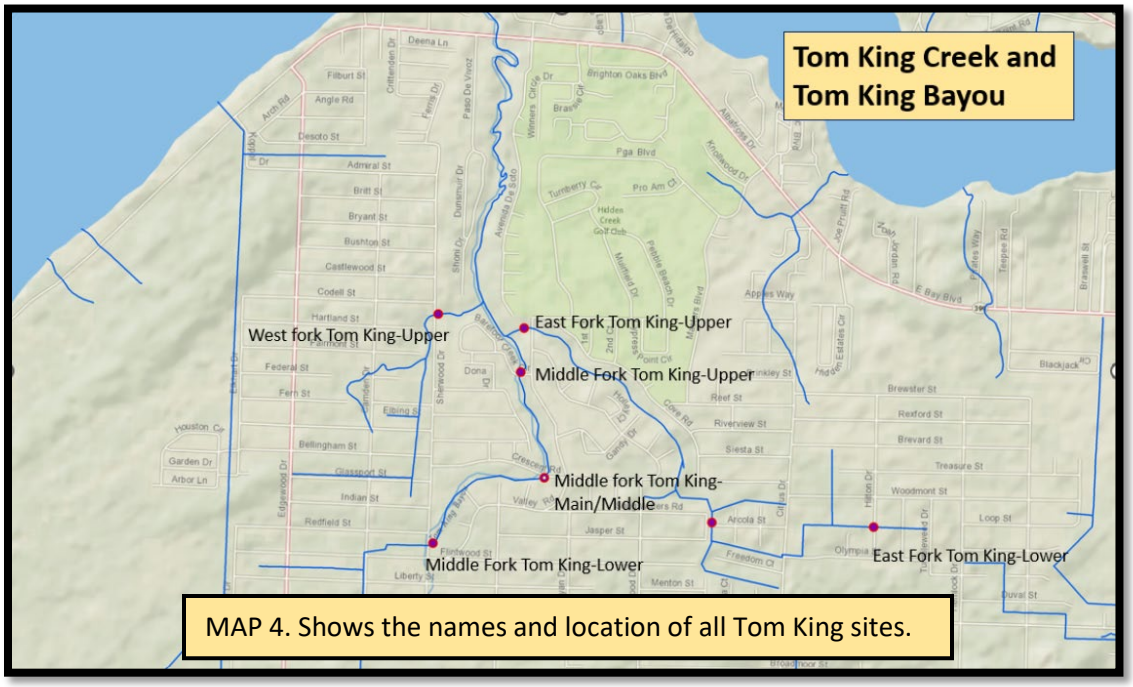


MAP 2. Shows the names and location of all ten mile and eleven-mile creek sites.

SITE LOCATIONS: ZONE 2-BAYOU TEXAR AND TOM KING CREEK/BAYOU

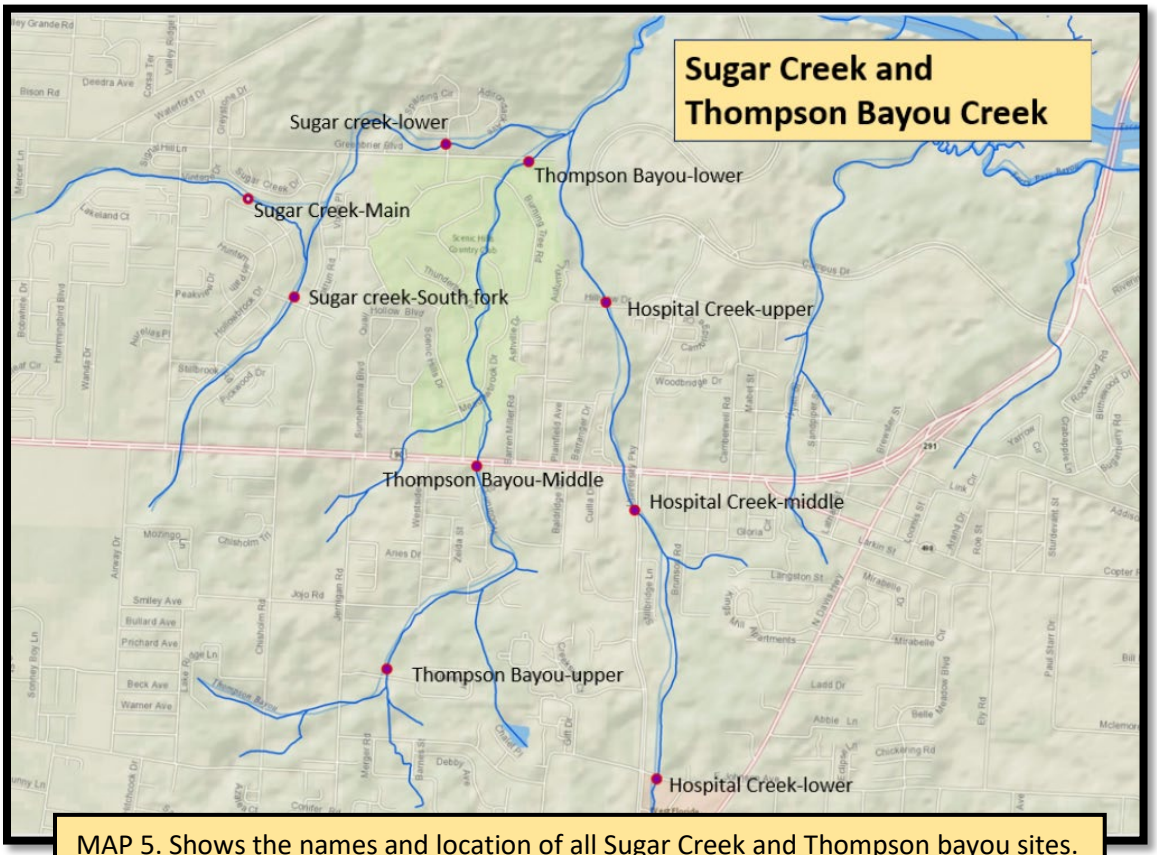


MAP 3. Shows the names and location of all Bayou Texar sites.

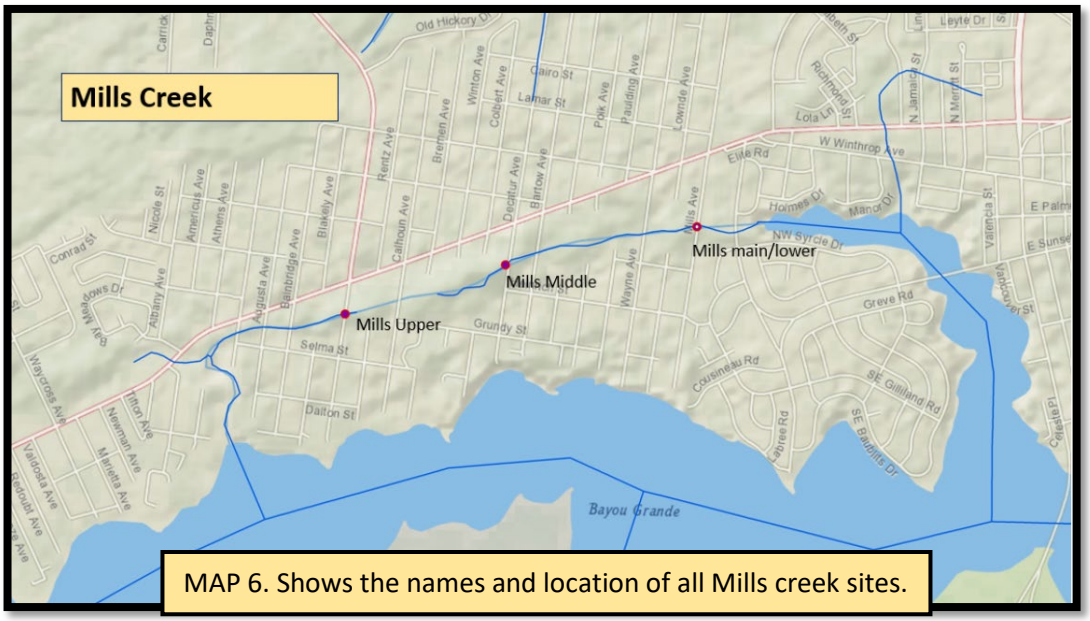


MAP 4. Shows the names and location of all Tom King sites.

SITE LOCATIONS: ZONE 3-SUGAR CREEK, THOMPSON BAYOU, AND MILLS CREEK



MAP 5. Shows the names and location of all Sugar Creek and Thompson bayou sites.



MAP 6. Shows the names and location of all Mills creek sites.

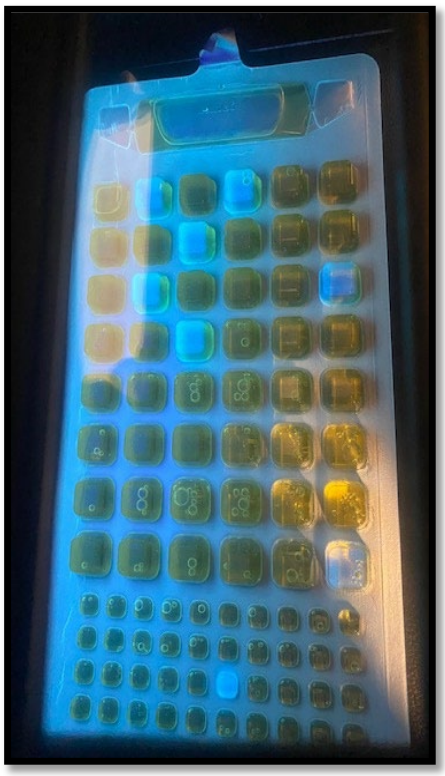
Collecting Nitrogen and Bacteria Data

Water samples were analyzed for nitrate concentrations using an ion chromatograph and for E. coli using IDEXX Colilert enumeration methods. Two 100ml samples were taken at the same time and location. To take each sample, the bottle was lowered into the stream halfway from the surface of the water and the bottom of the stream so that no debris was collected. The bottle was then inverted into the stream and rinsed three times to ensure all unwanted particulate was rinsed out. The sample was then capped off and put on ice immediately. The nitrogen sample was prepped for ion chromatography based on the SOP designated for ion chromatography in drinking water (EPA Method 300.1). Nitrate was not part of the focus of this project, but the results may aid to show pollution impairment of the streams.

Bacteria sampling was conducted using the same procedure as the nitrogen sample. Using a 100ml IDEXX sample bottle, a single water sample was collected and stored in ice for up to 6 hours. Using IDEXX quantification tray, the sample is poured into the divided IDEXX tray and incubated at 35°C for 24 hours. After 24 hours, the tray is revealed to an Ultraviolet light and the cells that glow are counted positive for *E. coli* bacteria. The number of large and small glowing cells are counted and the MPN of bacteria in the sample based on the IDEXX quantification chart is recorded for each tray. It is important to note that a cell must show yellow without a UV light AND glow under a UV light to be counted as positive for *E. coli*. The following images on page 11 show real sample trays from two different sites on the same day.

Picture 2a. Shows real IDEXX bacteria results taken in the lab showing positive glowing cells for *E. coli* bacteria with a score of 1203.3 mpn.

Picture 2b. Shows real IDEXX bacteria results taken in the lab from a different site on the same day showing positive glowing cells for *E. coli* bacteria with a score of only 7.4 mpn.



Collecting GIS Data

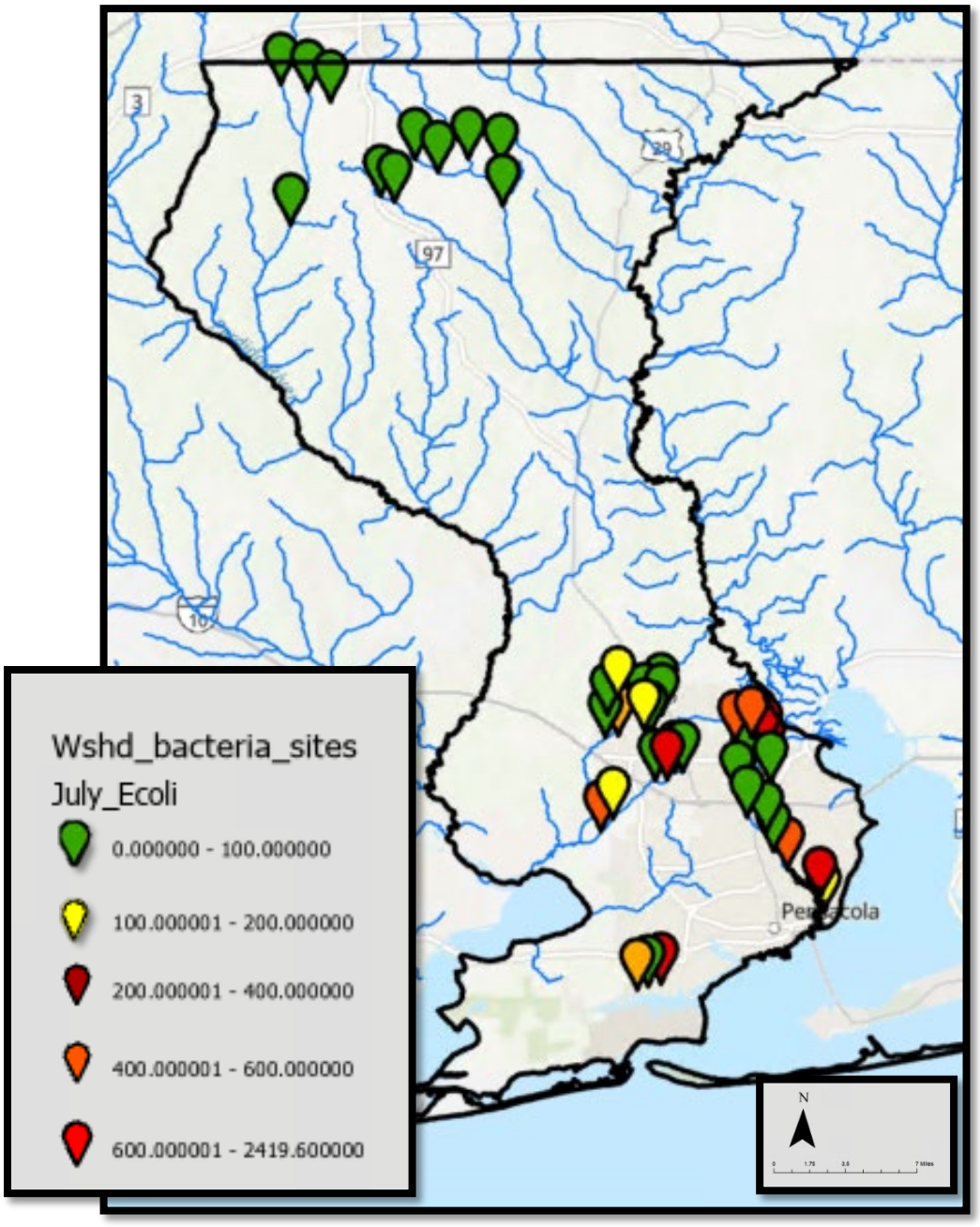
To collect the appropriate components for the GIS maps, I created a hot spot map from a raster layer previously created by the Escambia EPA. Using the hot spot tool, I was able to create a layer showing the bigger clusters of septic systems in Escambia. I used Avenza map app to personally map each site location and created a shapefile in GIS showing these locations. I obtained Escambia County boundary shapefiles from Escambia County GIS service. I created the streams layer by using the flow accumulation tool. I decided not to use a clip tool to limit the streams so that the viewer could see the incoming direction of the streams. It helps give an overall understanding of the watershed distribution.

RESULTS AND DISCUSSION

The hypothesis for this bacteria project was that *E. coli* bacteria levels will be higher in densely developed urban areas and lower in less developed rural areas because of less septic systems in rural areas. Based on the data collected, bacteria levels are higher in urban areas than rural areas.

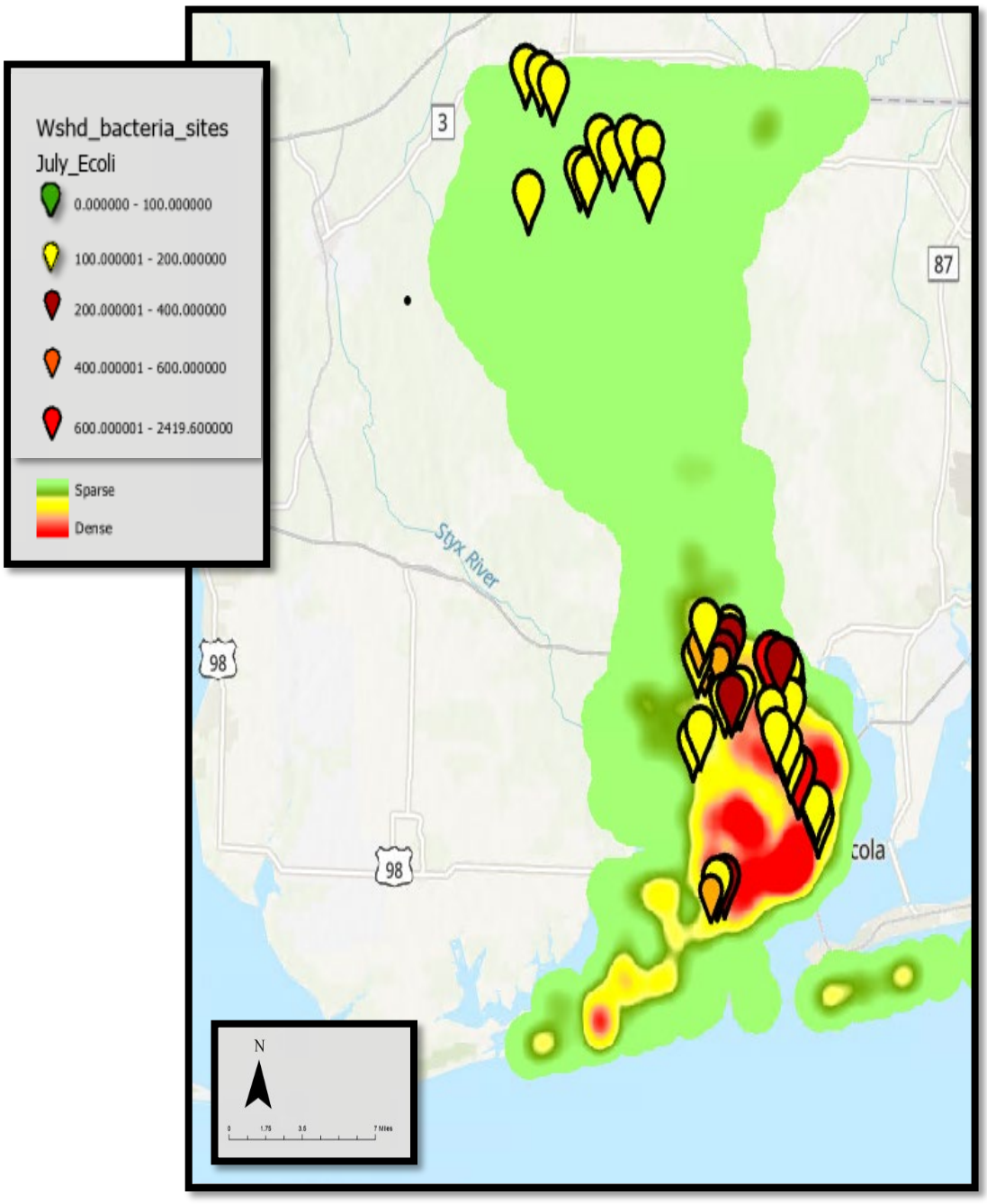
Zone 1 (Ten, Eleven, and Eight Mile) sites had only four samples that were greater than the EPA limit of 400mpn over the 4-month period. Eight mile-main site (Map 1, Pg.7) had the highest recorded level of 1172.95 in September. The other three months were more consistent as they were all within 100mpn of each other. The level for September was much higher at the Eight mile-main site. The other two Eight-mile creek locations also had a spike in bacteria only in September. No other sites had a spike in bacteria like the increase seen at Eight-mile creek. It was inferred that something caused this increase throughout Eight-mile creek but not in other creeks in Zone 1.

Zone 2 (Bayou Texar and Tom King bayou/creek) sites had the highest number of impaired sites with nine samples scoring above 400mpn. Bayou Texar (Map 3, Pg.8) had five samples over 400mpn. Bayou Texar is a bigger water way than the other streams and creeks used for this study. It is a location for several boat launches. The highest level being 1413.6 at the middle site on Bayou Texar. Bayou Texar-upper had three consecutive extreme amounts that were close to consistent. There was a big decrease in the month of November at this location. Bayou Texar-middle and Bayou Texar-upper were both high in the month of June and September. Both were low in November. There was a noticeably significant difference in the July scores for these two sites. Bayou Texar-middle had the highest scores but had a score of 1 in July. This could be due to high rain events recorded on that sampling day. The newly fallen rain may have diluted the stream to have nearly no bacteria. This theory is also seen at the lower site



Urban Bacteria Map. Shows a map representing all forty-two collection sites.

(With their corresponding bacteria levels for July. The colors representing their respective bacteria level can be seen in the legend.)

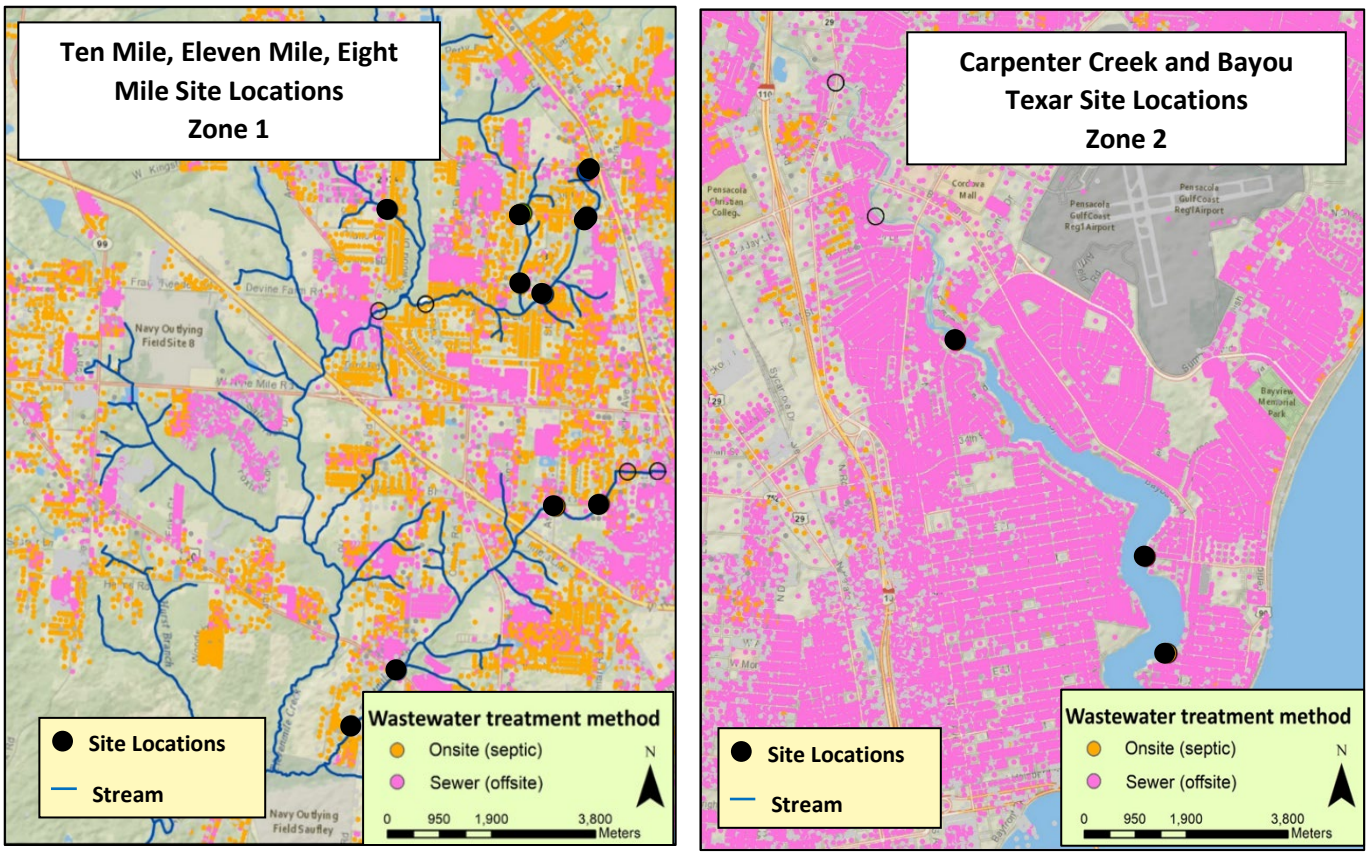


Septic Density Map. Shows a map of septic system density from sparse in light green to dense in bright red. The sites with their bacteria levels for July are also shown in relation to the septic hot spots.

having only 7.5 on that sampling day in July. The upper site, however, had the highest amount for that site with a concentration of 1299.7mpn. This could be due to the collection of runoff after the recorded rain event.

Zone 3 (Sugar Creek, Thompson Creek, and Mills Creek) sites were extremely high in September. Six out of ten sites were over 400mpn. Four of those six sites exceeded the IDEXX detection limit of >2419.6mpn. Hospital Creek (Map 5, Pg.9) is part of Thompson Creek/Bayou system. For the purpose of this project, we named it Hospital creek because West Florida Hospital is located at Hospital Creek-lower site. Hospital Creek reached the max score at the middle and lower site. Thompson Bayou middle site is located near the confluence with Hospital Creek. The two creeks run parallel to each other. It is inferred that there were significant contributions of bacteria to most creeks in zone 3 during September sampling. These high concentrations are likely due to the lack of rain recorded on this sampling day. With less flow of water through the creek, the water is allowed to grow more bacteria in stalled pools and release it slowly. Bacteria also may be entering as runoff from bacteria-laden water into these streams.

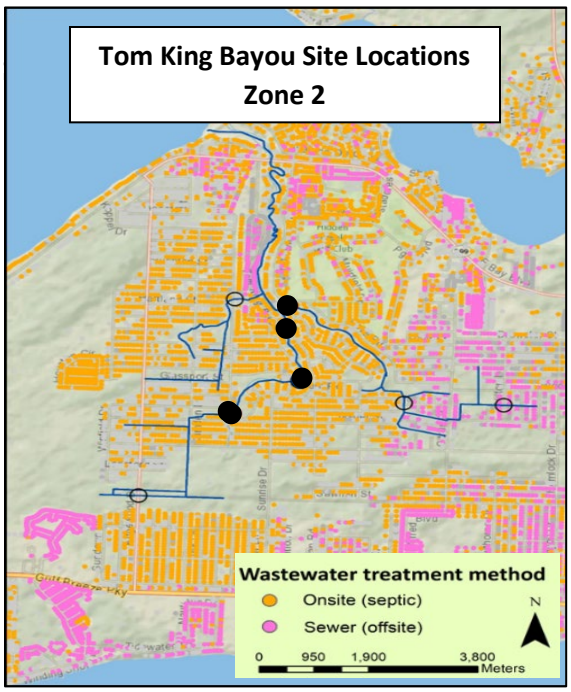
Due to the scope of the project, it is hard to determine exactly the cause of high bacteria levels in the urban sites and their fluctuations in bacteria concentration levels. The data collected for bacteria during the month of September was the highest of all the urban sites. The mpn was considered maxed out for the IDEXX quantification at >2419.6mpn. Table 4 is a comparison of the highest ten sites for the month of September compared to the samples taken at the ten rural sites in September. There were no mpn levels that were over 214mpn found in the rural sites throughout this project. The rural sites shown to have nearly no elevated bacteria concentrations. These rural site levels are a representation of the surrounding livestock fields and naturally found bacteria. This information adds to the belief of a potentially true hypothesis. The hypothesis

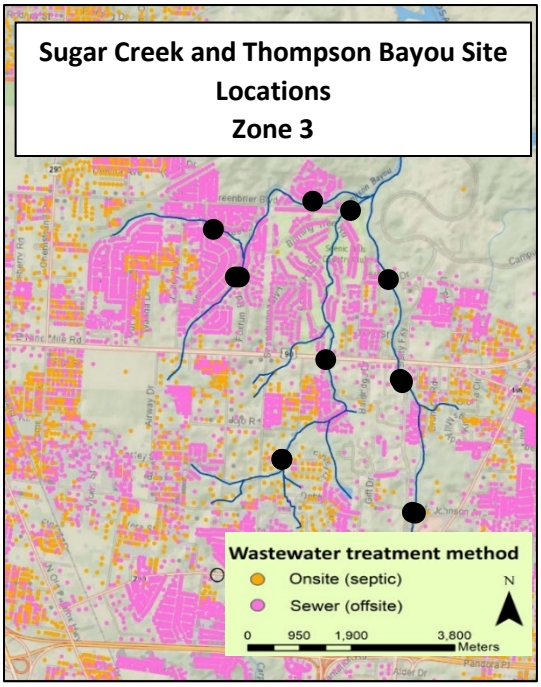


Wastewater Treatment Maps Zone 1 and Zone 2. Shows Ten Mile, Eleven Mile, and Eight Mile Site locations (black dots) on the left map. The right map shows Carpenter Creek and Bayou Texar site locations. The Orange color is a representation of Onsite septic systems within the urban area. The pink color represents offsite sewer areas.

being that the bacteria was higher in the urban sites due to more septic systems and development, which will be seen in the following map graphics.

In Wastewater Treatment Map Zone 1, the onsite septic (orange) is the primary waste management that surrounds the Ten-, Eleven-, and Eight-mile sites. The Bayou Texar sites are almost completely surrounded by offsite sewer. This is probably due to the bayou boundaries being unsuitable for onsite septic. The eleven-, ten-, and eight-mile sites are still surrounded by less dense of septic areas than the Bayou Texar sites. Based on our zone 1 data, the bacteria levels were much less in zone 1 than the other two zones.





Wastewater Treatment Maps Zone 2 and Zone 3. Shows locations of Tom King Bayou sites on the left image. The right image shows Sugar Creek and Thompson Bayou site locations. Both show their designated wastewater treatment methods.

In the Tom King Bayou Wastewater treatment map, there is mostly onsite septic. The sugar creek and Thompson creek areas on the right map have mostly offsite sewer. In the illustration maps containing the most offsite (pink) sewer systems, the sites with the highest bacteria are present.

The difference between onsite and offsite sewer systems has been seen, in this study, to be a potential contributor to the high bacteria levels. Onsite septic are tanks that are in the ground that hold and process human waste at people's homes or businesses. Offsite treatment means that it is running through pipes to a waste facility lift station. According to an anonymous employee for Escambia County's local wastewater treatment plant, the company is allowed to dump the lift stations into the nearby creeks because it is "filtered wastewater". The anonymous source also stated that, "The pipes are old and sometimes leak and there is no way we (the company) are able

to locate and fix every leak." Any sewer pipes that are carrying human waste could potentially be leaking into the groundwater and adding to the bacteria concentrations in the streams.

Conclusion

After analyzing the data, it is acknowledged that there would need to be samples taken over longer periods of time with a more set standard of when a sample should be taken. Factors such as rainfall and temperature also would have an effect on bacteria level concentrations. There are many factors that could affect the bacteria levels in streams. Septic systems that leak or overflow are an everyday occurrence in urban areas such as southern Escambia County and Santa Rosa County. It can be inferred, based on the data seen in this project, that a major cause of bacteria pollution in Escambia County urban streams is largely in part to wastewater. More specifically, delivery to offsite treatment. The density of development in the southern portion of Escambia County is very well seen to make a drastic difference when compared to the rural agriculture lands of northern Escambia County. In table 4, only ten urban sites were chosen to be compared to the ten rural sites. Even in those ten urban sites we can see that the bacteria levels are much worse in the urban area. It is known that runoff is the enabler of pollution in watersheds. It could also be assumed that any bacteria upstream in the rural areas could flow through the river networks and into the impaired streams where it collects and grows in concentration. The data in this report are inconclusive as to determine the direct cause of high bacteria. High temperature, urban contributors, and natural bacteria all play a part, along with other factors. To get better evidence of urban contributors, more water quality tests should be conducted to determine all probable causes including storm events, temperature, dumping of wastewater, and high animal impact areas.

APPENDIX

Table 1. (Results for ZONE 1.) *E. coli* bacteria and nitrogen results at ten-, eleven-, and eight-mile sites for the months of June, July, September, and November 2021. The light red indicates bacteria levels that exceed the EPA limit of 400 mpn. The dark red indicates bacteria levels that were >1,000 mpn

Site	June 2021		July 2021		September 2021		November 2021	
	E. coli (mpn)	NO3 (mg/L)	*E. coli (mpn)*	NO3 (mg/L)	*E. coli* (mpn)	NO3 (mg/L)	*E. coli* (mpn)	NO3 (mg/L)
10 Mile-Greenhill	108.1	No Data	187.2	0.007	147.5	0.00072	65.7	0.0377
10 Mile-Roberts Road East	86	0.0106	201.4	0.0781	38.4	0.1533	29.5	0.0879
10 Mile-Roberts Road West	178.9	0.00001	67.7	No Data	33.6	0.00001	46	0.00001
10 Mile-Stefani Road	58.3	No Data	224.7	0.007	90.8	0.0304	218.7	0.0888
10M-Main site	No Data	No Data	198.9	0.4435	235.9	0.2772	980.4	0.271
10M-Pompano	86	0.00001	No Data	No Data	31.8	0.00001	24.7	0.00001
11 Mile-97A	90.9	1	178.9	0.0478	60.5	0.0397	33.1	No Data
11 Mile-North	No Data	No Data	70.8	0.0288	131.7	No Data	111.2	0.0856
8 Mile-Ashland	200.8	0.018	No Data	No Data	61.7	0.1238	63.1	1
8 mile-Bankhead	13.2	0.023	No Data	No Data	456.5	0.0969	54.6	No Data
8 Mile-Mobile Hwy	18.7	0.171	No Data	No Data	142.1	0.0676	66.3	No Data
8 Mile-Main Site	344.1	0.1448	290.9	No Data	1172.95	0.391	416	No Data

Table 2. (Results for Zone 2) *E. coli* bacteria results comparing Bayou Texar and Tom King Bayou sites for the months of June, July, September, and November 2021. The light red indicates bacteria levels that exceed the EPA limit of 400 mpn. The dark red indicates bacteria levels that were >1,000 mpn.

Site	June 2021		July 2021		September 2021		November 2021	
	E. coli	NO3	*E. coli*	NO3	*E. coli*	NO3	*E. coli*	NO3
Bayou Texar-Lower	235.9	No Data	7.5	0.1218	123.4	No Data	41.4	0.55
Bayou Texar-Middle	1046.2	No Data	1	0.214	1413.6	0.1344	110	0.648
Bayou Texar-Upper	920.8	No Data	1299.7	0.754	920.8	0.777	186	0.943
East Fork Tom King- Middle	No Data	No Data	101.2	0.0425	148.3	0.0236	95.9	0.0162
East Fork Tom King-Upper	410.6	No Data	127.3	0.2775	148.3	0.1929	119.1	0.5734
Mid Fork Tom King-Lower	866.4	0.0725	57.6	0.1528	157.6	0.0157	325.5	0.0462
Mid Fork Tom King-Main Site	73.3	0.0892	69.7	0.058	135.4	0.0522	2400	0.1706
Mid Fork Tom King-Upper	104.6	0.1226	44.1	0.0792	172.5	0	686.7	0.2003
West Fork Tom King-Upper	No Data	No Data	No Data	No Data	53.7	0.0164	48	0.0153

Table 3. (Results for Zone 3) *E. coli* bacteria results comparing Mills Creek, Sugar Creek, and Thompson Bayou sites for the months of June, July, September, and November 2021. The light red indicates bacteria levels that exceed the EPA limit of 400 mpn. The dark red indicates bacteria levels that were >1,000 mpn.

Site	June 2021		July 2021		September 2021		November 2021	
	E. coli	NO3	*E. coli*	NO3	*E. coli*	NO3	*E. coli*	NO3
Mill-Main/Lower	214.3	0.2469	307	0.3717	2419.6	0.1982	No Data	No Data
Mills-Upper	No Data	0.2023	108.6	0.0076	275	0.0097	No Data	No Data
Sugar Creek-Lower	387.3	0.0115	209.8	0.0619	579.4	0.0837	204.6	No Data
Sugar Creek-Main Site	275.5	0.00001	2419.6	0.0473	533.9	0.0362	201.3	No Data
Sugar Creek-South Fork	122.3	0.034	488.4	0.1073	344.8	0.1285	143.9	No Data
Thompson Bayou-Lower	186	0.39	218.7	0.6867	38.6	0.0626	10.8	No Data
Thompson Bayou-Middle	88.4	0.0576	365.4	0.1183	2419.6	0.1082	39.3	No Data
Thompson Bayou-Upper	12.1	0.0016	20.3	0.0401	No Data	No Data	44.3	No Data
Hospital Creek-Lower	186	0.0605	No Data	No Data	2419.6	0.2272	76.3	No Data
Hospital Creek-Middle	275.5	0.0511	325.5	0.0964	2419.6	0.0567	37.3	No Data
Hospital Creek-Upper	20.9	0.00465	No Data	No Data	No Data	No Data	No Data	No Data

Table 4. Shows the urban sites with the highest bacteria levels in September compared to all ten rural sites collected in September. The color ranges from light green being the lowest numbers to dark red being the highest number of bacteria.

Urban Sites	Bacteria Levels	Rural Sites	Bacteria Levels
Sugar Creek-Lower	579.4	Little Pine Barren	9.7
Sugar Creek-Main	533.9	Hwy 99A-Collect	178.5
Hospital Creek-Lower	>2419.6	Big Pine Barren Creek	151.5
Hospital Creek-Middle	>2419.6	Hall Branch	21.2
Eight Mile-Bankhead	456.5	Breastwork Branch	91.1
Eight Mile-Main	1172.95	Hwy 64-Collect	214.3
Bayou Texar-Middle	1413.6	Still road-PBC	111.9
Bayou Texar-Upper	920.8	Rockaway Creek	69.7
Mills-Main Site	>2419.6	Brushy Creek	172.3
Thompson Bayou-Middle	>2419.6	Rocky Creek-Collect	76.8