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A Forensic Assessment of Current Water Quality Using IDEXX Techniques in the Himmarshee Canal and New River in Fort Lauderdale, Florida

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Thesis of
Catherine Margaret Bilodeau

Submitted in Partial Fulfillment of the Requirements for the Degree of

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Marine Science

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NOVA SOUTHEASTERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

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I would like to dedicate this research to my family, especially those not here, to see me finish. My grandmother, Betty, always joked about how I could tell the sex of a fish, and I'm sure if she was here, she would be teasing me about how I could tell if there was fecal contamination in the water. I want to take a moment to thank her and my grandfather, Ben, my great-aunt, Kathy, and then my great-grandmother Margie. I would then like to thank my parents and my brother for the sacrifices they have made over the years to ensure I could achieve my dream. Then I would

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Lastly, I would like to thank the Bank Family Endowment for funding this project through Nova Southeastern University. Without this funding, this project and my dream to complete my master's would not have happened.

ABSTRACT

Fecal contamination has continued to plague local communities around the United States and the world, especially in highly populated areas like Southern Florida. Newer techniques are beginning to be used to track microbes properly as they are found in waterways. Some more recent techniques in Microbial Source Tracking (MST) use IDEXX kits to detect fecal indicator bacteria (FIB). Two IDEXX kits Colilert-18® which detects total coliforms and *Escherichia coli* in water or fecal coliforms in wastewater, and Enterolert®, which targets *enterococci*, were used in the waterways of the Himmarshee Canal and South Fork of the New River in Fort Lauderdale, Florida. These kits yielded high levels of *Enterococcus*, *E. coli*, and thermotolerant total coliforms. The highest levels of *Enterococcus* were measured at over 22,000 MPN/100 mL, while the highest *E. coli* and thermotolerant total coliforms were measured at 5,800 MPN/100 mL and 7,700 MPN/100 mL. All these high levels were well over the recommended levels of 130 CFU/100 mL for *Enterococci* and 410 CFU/100 mL for *E. coli* (including total coliforms). This forensic assessment has shown that fecal contamination is still an ongoing problem and that canals are a potential source of buildup for these bacteria to thrive.

Keywords: IDEXX Enterolert®, *Enterococci*, Colilert-18®, Total Coliforms, *E. coli*, Microbial Source Tracking

INTRODUCTION

Fecal contamination has been a leading cause of pollution for decades and has continued to plague ecological communities, either land-based or in the water (Choudhary et al., 2019; González-Saldía et al., 2019; Shuval, 2003). This contamination has been a source of diseases that put the environment and public health at risk, such as thalassogenic diseases (diseases that are from wastewater) (González-Saldía et al., 2019; Shuval, 2003) and other potentially harmful bacteria found in humans and animal intestines that enter the water through point and non-point sources (Bernhard & Field, 2000; Fewtrell & Kay, 2015). Point sources are sewage outfalls and other direct sources, while non-point sources are storm runoff, septic tank drainage, and agricultural runoff (Fewtrell & Kay, 2015). It is estimated that 300-400 million tons of metals, toxic sludge, and organic waste are leaked into local waters (Choudhary et al., 2019). The 300-400 million tons of pollutants that are leaked into the waters can be attributed to many different sources, such as humans, septic systems, storms, industrial companies, as well as even wildlife and pets (Cao et al., 2015; Choudhary et al., 2019). In 2000, the EPA implemented the Beaches Environmental Assessment and Coastal Health Act, which requires states to develop programs to monitor and notify recreational waterways of any high levels of possible disease-causing bacteria (106th US Congress, 2000). To adequately determine the cause of these diseases, Microbial Source Tracking (MST) is performed by environmental labs to find these Fecal Indicator Bacteria (FIB) (Nshimyimana et al., 2017; Nshimyimana et al., 2019; Paruch & Paruch, 2022; Shanks & Korjkic, 2020; Zhu et al., 2020).

Environmental labs use different techniques to quantify the amounts of fecal indicator bacteria (FIBs) in the water (Cao et al., 2015; Ahmed et al., 2016; Bashir et al., 2020). MST is commonly tracked using different orders of FIBs, specifically Bacteroidales using qPCR (real-time PCR) when looking for HF183 (human-specific Bacteroidales marker), and Enterobacteriales which is commonly used with Defined Substrate Technology (DST) that is involved with kits like IDEXX Enterolert®, and Colilert-18® (IDEXX, 2015). All orders of bacteria and markers are located in the intestinal tract of all vertebrates, including humans (Johnson & Russo, 2002; Teixeria et al., 2022). FIBs are often called enteric, meaning they are related to the intestines. Because of this, researchers use various tests to culture the enteric-specific bacteria to show there is fecal contamination (American Public Health Association et al.,

2017). Some of the bacteria found in these orders are *Bacteroides fragilis* (*B. fragilis*), *Escherichia coli* (*E. coli*), the genus *Enterococcus*, and other total coliforms (Merino-Mascorro et al., 2018; Shahin et al., 2022). Each bacteria genus, family, or order has different morphologies that make them unique, meaning specific tests are needed. The genus *Enterococcus* is the targeted bacteria for the IDEXX Enterolert® test, while *E. coli* and total coliforms are the targeted bacteria for the IDEXX Colilert-18® test (Figure 1). By understanding these different bacteria, especially their taxonomy and human genetic markers, a researcher can adequately plan how to track these FIBs.

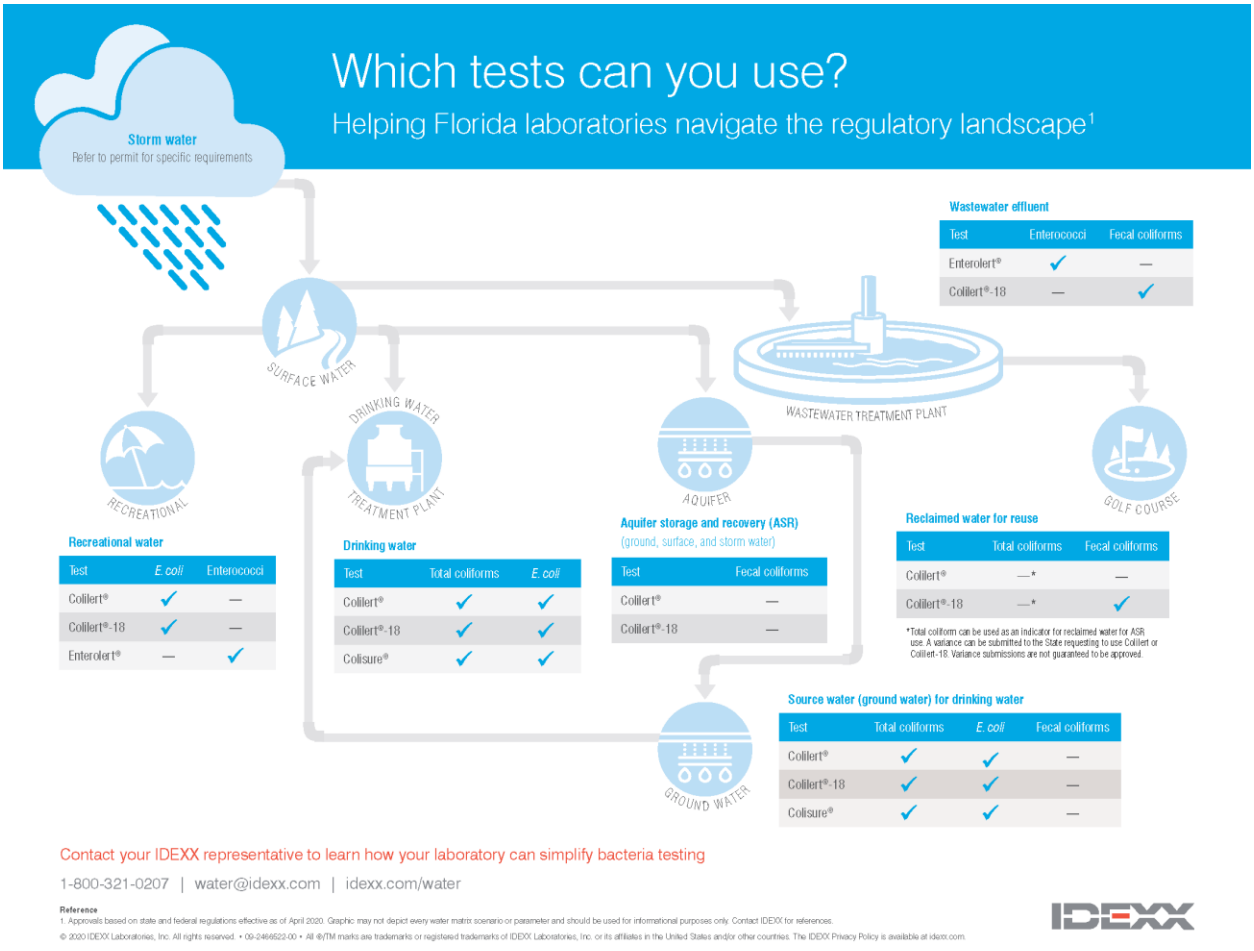

Figure 1: IDEXX Test Chart. This chart shows which IDEXX products can be used depending on the study site and targeted bacteria. IDEXX Colilert® and Coliler-18® test for *E. coli*, while IDEXX Enterolert® tests for *Enterococci* (plural form of *Enterococcus*, which is a genus of bacteria). Source: IDEXX.

The IDEXX Enterolert® test solely tests for bacteria in the genus *Enterococcus*, which have a completely different taxonomic phylogenic tree from *E. coli* and other coliforms. To understand *Enterococcus*, we must discuss its origins and taxonomic tree (Figure 2). The order *Lactobacillales* contains the genus *Enterococcus*, first proposed in 1903 and described as gram-positive, round-shaped bacteria (Thiercelin & Jouland, 1903). A few years later, there was a question about whether or not this genus should be renamed to *Streptococcus* due to the similarities in cell shape and other bacteria like *S. faecalis* being found in fecal material (Andrews & Horder, 1906). However, it was decided to keep the genus separated because not all bacteria in *Streptococcus* are genotypically and morphologically like *Enterococcus*. They are found in various habitats (Byappanahalli et al., 2012; Teixeira et al., 2022). Some of these habitats can be from humans, animals, plants, insects, surface waters, sediment, and sewage (Byappanahalli et al., 2012). Table 1 outlines some species of *Enterococcus* that the Enterolert® test can detect; some individuals are human pathogens. However, these are not the only human pathogenic FIBs found in water.

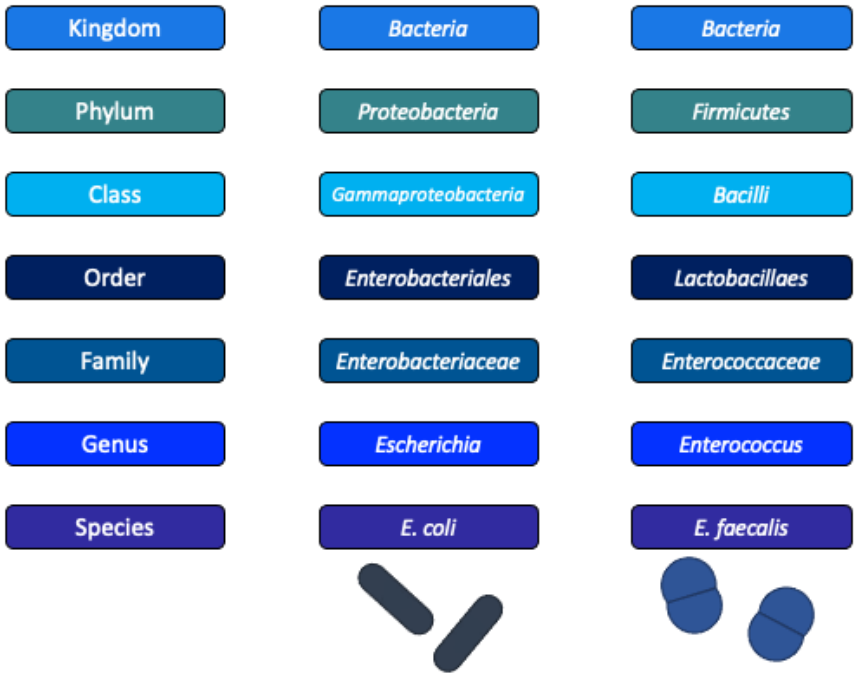

Figure 2: Taxonomic Tree of *E. coli* vs *E. faecalis*. The above figure depicts the taxonomic tree for both *E. coli* and a member of the genus *Enterococcus*, *E. faecalis*. Both bacteria come from a different phylum, order, and family, which will be discussed later on when looking at bacteria found in sediment. The figure was made using the taxonomic levels from ITIS (Integrated Taxonomic Information Report) and Microsoft PowerPoint® (https://www.itis.gov/).

Table 1: Species of *Enterococcus* and known habitat(s). This table shows some of the known species of *Enterococcus*, their groupings, and their known habitats. The table also outlines pathogenic species that can cause diseases in humans. (Source: Byappanahalli et al., 2012).

Group	**Species**	**Known habitat(s)**	**Human pathogen**
E. faecalis	*E. faecalis*	Human, animal (multiple), plant, insect	Yes
	E. haemoperoxidus	Surface water	
	E. moraviensis	Surface water	
	E. silesiacus	Drinking water	
	E. termitis	Animal (termite)	
	E. caccae	Human	
E. faecium	*E. faecium*	Human, animal (multiple), plant, insect	Yes
	E. durans	Human, animal (multiple), insect	Yes
	E. hirae	Animal (multiple), plant	
	E. mundtii	Soil, plant	Yes
	E. villorum	Animal (hog)	
	E. canis	Animal (dog)	
	E. ratti	Animal (rat)	
	E. asini	Animal (donkey)	
	E. phoeniculicola	Animal (bird)	
	E. canintestini	Animal (dog)	
	E. thailandicus	Human, animal (cattle)	
E. avium	*E. avium*	Human, animal (multiple)	Yes
	E. pseudoavium	Human	
	E. malodoratus	Animal (cattle)	
	E. raffinosus	Human	Yes
	E. gilvus	Human	
	E. pallens	Human	
	E. hermanniensis	Animal (dog)	
	E. devriesei	Animal (cattle)	

	E. viikiensis	Animal (broiler plant)	
E. gallinarum	*E. gallinarum*	Human, animal (multiple), insect	Yes
	E. casseliflavus	Plant, soil, human, animal (multiple)	Yes
E. cecorum	*E. cecorum*	Animal (chickens)	
	E. columbae	Animal (pigeon)	
Ungrouped	*E. saccharolyticus*	Animal (cattle), sewage	
	E. aquimarinus	Seawater	
	E. sulfureus	Plant	
	E. dispar	Human	
	E. italicus	Animal (cattle)	
	E. camelliae	Plant	

The order *Enterobacterales* contains the family *Enterobacteriaceae* where 59% of the bacteria in that family are total coliforms (Brenner & Farmer, 2005). Total coliforms have long been used as a FIBs for water quality due to the fact they are enteric bacteria (American Public Health Association et al., 2017). Total coliforms are anaerobic, gram-negative bacteria, and most ferment lactose, which they use to produce gas (American Public Health Association et al., 2017). Some traditional coliforms are *Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella* (American Public Health Association et al., 2017). *Escherichia* is the genus of *E. coli*. *E. coli* is a gram-negative rod-shaped bacterium where one form is a pathogen, O157:H7 (Johnson & Russo, 2002). There is also a form of *E. coli*, *Enterotoxigenic E. coli*, that produces toxins that cause diarrhea, which can cause hemorrhagic colitis and irritable bowel syndrome (Daniels, 2006; Woodman et al., 2019). *E. coli* and *Enterococci* are also common in wound infections, urinary tract infections, and any infections that occur at a surgical site (Dowd et al., 2008; Maki & Tambyah, 2001; Nallapareddy et al., 2006; Nielsen et al., 2013; Keogh et al., 2016). By testing for both of these bacteria using methods like IDEXX, researchers can obtain a better profile of the FIBs in the water.

IDEXX can detect two of the FIBs, *Enterococci*, and *E. coli*, after incubating from 18-24 hours and is commonly used across environmental labs because of its straightforward approach as well as how inexpensive it is (Brand & Barnes, 2014; Kinzelman et al., 2005). Multiple studies have found that when compared to using the membrane filtration method, the IDEXX kits have quicker processing times. Kinzelman, in 2005, stated it saved them 6 hours and reported consistent,

reliable results (Kinzelman et al., 2005; Pisciotta et al., 2001). The two kits used the most are Colilert® and Enterolert® (Peperzak & Bleijswijk, 2021; Ramoutar, 2020;). Each one looks for either *E.* coli, *Enterococcus*, and total coliforms by diluting the samples and using sodium thiosulfate to stop bacterial growth until they can be processed in the lab (American Public Health Association et al., 2017). Another reason the samples are diluted when using marine water is that other bacteria in the water can cause microbial interference, like *Bacillus*, an aerobic endospore bacteria (American Public Health Association et al., 2017). Aerobic endospores are environmentally resistant bacteria that are non-pathogenic. However, because they can germinate on all surfaces and contaminate other bacteria, they can cause that microbial interference and remain there for a very long time (American Public Health Association et al., 2017). IDEXX kits can provide a quantitative number to tell researchers how much FIBs contaminate a water sample. This is done using the Most Probable Number (MPN) table, which estimates the mean density of the bacteria in the sample with the assumption of a Poisson distribution (American Public Health Association et al., 2017; Hurley & Roscoe, 1983). IDEXX calculated the MPN for each combination of positive small and large wells by using the formula outlined below, and the IDEXX procedure calls for the researcher to simply times the MPN number on the table by the dilution factor (Appendix A for MPN Table by IDEXX) (Hurley & Roscoe, 1983).

$$\sum_{i=1}^{k} \frac{v_i d_i p_i}{1 - e^{-v_i d_i mpn}} = \sum_{i=1}^{k} v_i d_i n_i$$

k= Number of dilution levels

n_i= number of wells at level i

p_i= number of positive wells at level i

d_i= dilution factor at level i

v_i= volume of wells at level i

(small wells = 0.16 mL, large wells = 1.9 mL, overflow well = 11 mL)

It is important to note that 1 MPN/100 mL equals 1 CFU/100 mL. The only difference is that CFUs are bacteria grown in a solid medium instead of a liquid medium found in IDEXX (Dubois, 2022). Multiple kits can be incubated simultaneously, allowing different waterways to be sampled on the same day. Shahin et al. (2022) recently did a study on New Orleans canals where they used the

Colilert-18® kit to quantify the concentrations of *E. coli*. They found that *E. coli* concentrations were high around densely populated areas (Shahin et al., 2022). These kits are an accepted EPA technique for water quality.

EPA guidelines recommend using *Enterococcus* for marine and freshwater and *E. coli* for freshwater only (EPA, 2012). In 2012, the EPA released a table outlining the new statistical threshold values (STV) and geometric means (GM), where if samples were to be over these values, they should be flagged (Table 1). Most environmental labs use an estimated illness rate of 36 per 1,000 primary contact rectors and use the STV. If Enterococcus levels are above 130 cfu/100 mL, it is a reason for concern; if *E. coli* levels are above 410 cfu/100 mL, it is also another concern. Environmental labs use these guidelines, and researchers examine if their waterways have high levels of fecal contamination. Some of these places are highly populated cities and states that are plagued with sewage pipes breaking (Barszewski, 2019a; Barszewski, 2019b; O'Neill, 2019; Perez, 2022; Wallman & Maines, 2017). Most recently, Fort Lauderdale has had a share of recent pipe bursts (2016-2022) (Barszewski, 2019a; Barszewski, 2019b; O'Neill, 2019; Perez, 2022; Wallman & Maines, 2017).

Table 2: Recommended 2012 Recreational Water Quality Criteria (RWQC) from EPA. GM stands for geometric mean, while STV stands for statistical threshold values. Most state governments, like Florida, use 36 per 1,000 NGI and follow statistical threshold values. (Source: EPA, 2012).

Criteria Elements	**Estimated Illness Rate (NGI): 36 per 1,000 primary contact recreators**		**Estimated Illness Rate (NGI): 32 per 1,000 primary contact reactors**	
	Magnitude		**Magnitude**	
Indicator	GM (cfu/100 mL)	STV (cfu/100 mL)	GM (cfu/100 mL)	STV (cfu/100 mL)
Enterococci -marine & fresh	35	130	30	110
E. coli -fresh	126	410	100	320

Fort Lauderdale is a highly populated city on the Atlantic Ocean, with intercoastal canals flowing through the city where people reside. For example, at the beginning of 2022, pipes burst in Fort Lauderdale, which caused after-effects where residents had to boil their water before they cooked, drank, and cleaned dishes (Perez, 2022). According to the Florida Department of

Environmental Protection, these rivers and canals are classified as Class III water systems, which are defined as waters being used for fish consumption, recreation (i.e., boating and swimming), and maintenance of a healthy population of fish and wildlife (Rule 62-302.500 F.A.C). Under this rule, most surface waters are considered Class III water systems (Rule 62-302.500 F.A.C). One specific waterway is the New River, which also has a branch called the Himmarshee Canal. These waterways run through residential and industrial areas, which have been subject to fecal contamination in the past (Barszewski, 2019a; Barszewski, 2019b; O'Neill, 2019; Perez, 2022; Wallman & Maines, 2017). New River has been the subject of several other studies examining these waters' contamination.

In 2000 and then again in 2020, two studies examining the water quality in different parts of New River were done. The first study, in 2000, focused on the North Fork of New River and looked at the concentrations of *E. coli* in the area (Solo-Gabriele et al., 2000). This study wanted to look at possible sources for *E. coli*, and they used the Colilert-18® kit and membrane filtration method with water and soil samples. The researchers collected samples either themselves or with two autosamplers over a year. Their results showed high levels of *E. coli* around I-95 and the Argyle Canal (Figure 3) during high tide. Regarding the soil samples, the researchers found they were a plausible source for the high levels of *E. coli*. They also noticed the high levels after a rainfall event, explaining that the runoff from the soil was allowing *E. coli* to seep into the water further. It is important to note that these researchers diluted the water samples for Colilert-18®. For the study done in 2020, the researcher examined the presence of HF183, which is a 16S rRNA sequence marker found in the *Bacteroides* genome in the interstitial tracks of humans which makes it a crucial part of MST because it is host-specific (Green et al., 2011; Haugland et al., 2010; Louis, 2020; Madeja, 2015). Samples were collected over a month and were all water samples. These samples showed the discomforting presence of HF183; however, the researcher could not answer whether there was a pattern between HF183 and weather conditions. Regardless, both studies done in 2000 and, more recently, 2020 bring up more questions about the water quality in New River and its possible sources, as well as if Broward County's efforts to fix the water quality have worked. In 2021, two protein skimmers were installed to help clean up Himmarshee Canal by Clean Waterways LLC®. This new study in 2022 and 2023 has been conducted to answer these plaguing questions.

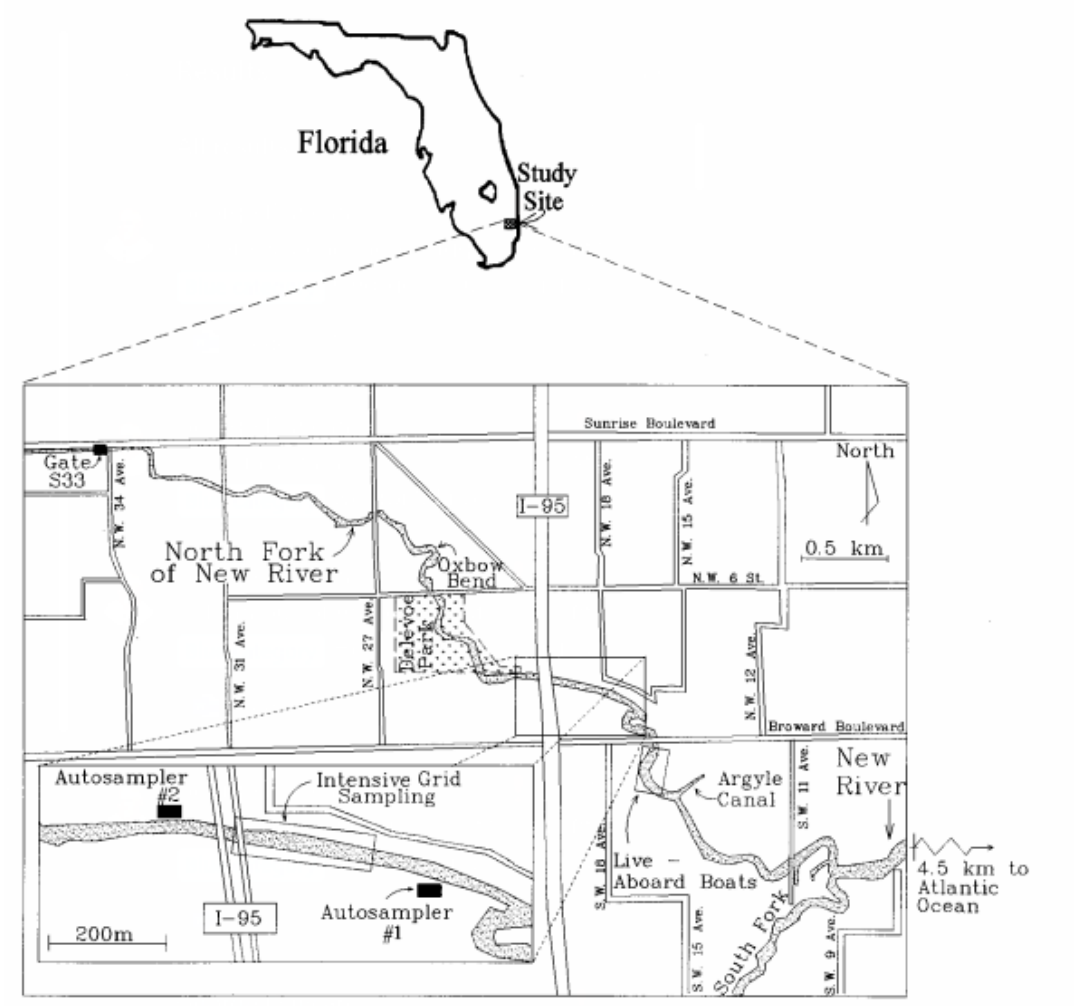

Figure 3. North Fork of New River and Argyle Canal. This figure is taken from Dr. Solo-Gabriele, made for their 2000 study showing the testing sites along New River and Argyle Canal. The Argyle Canal is northwest of the Himmarshee Canal. (Source: Solo-Gabriele et al., 2000).

Ten sites were chosen for this new study based on preliminary data from a homeowner's group. Sixty water samples were collected along the Himmarshee Canal (Figure 4) and one part of the New River itself. Two sets of the samples were subjected to IDEXX kits, the first round being Enterolert® and the second round being Colilert-18®, to cover the fact that the Himmarshee Canal is a body of brackish waters where fresh and marine water mix. The aim of this new current study was to evaluate if *Enterococcus*, *E. coli*, and total coliforms are still running a high risk of fecal contamination and whether or not a particular site is the source.

Preliminary Data

A homeowner's group approached NSU about the water quality in Himmarshee Canal (Figure 4). Sediment samples were collected from some of the sites depicted in Figure 4. The samples were extracted for DNA and then ran through the Illumina® MiSeq to conduct 16s RNA sequencing. The 16S rRNA sequencing data yielded essential insights into the bacteria in the water. Figure 5 illustrates a heatmap from that data showing the highest concentration of the order of bacteria within each sample. The order *Bacteroidales* (which contains bacteria linked to HF183) was the third highest concentration, and *Enterobacteriales* was the fourth, showing that bacteria like total coliforms are in the area. Out of all the sites, site 10 yielded the most *Bacteroidales*. Again, these sites are mapped out in Figure 4 and match the sites used in this study (i.e., Site 10 is the same in both studies). This preliminary data showed how effective 16S rRNA methods were in characterizing Broward County canals. Due to possible fecal contamination and locals voicing concerns about their water quality, an updated forensic assessment was done to determine whether this waterway is still plagued by fecal contamination.

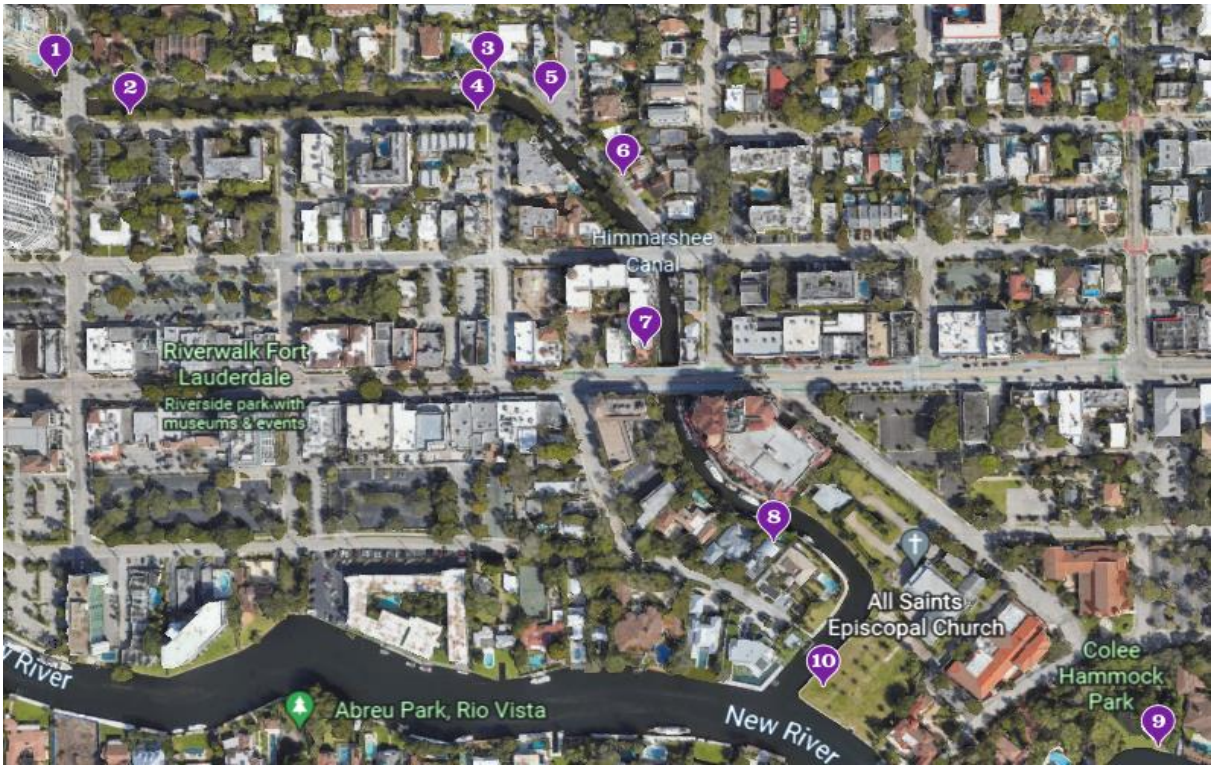

Figure 4. The Ten Sample Sites. Sample sites along the Himmarshee Canal and part of New River.

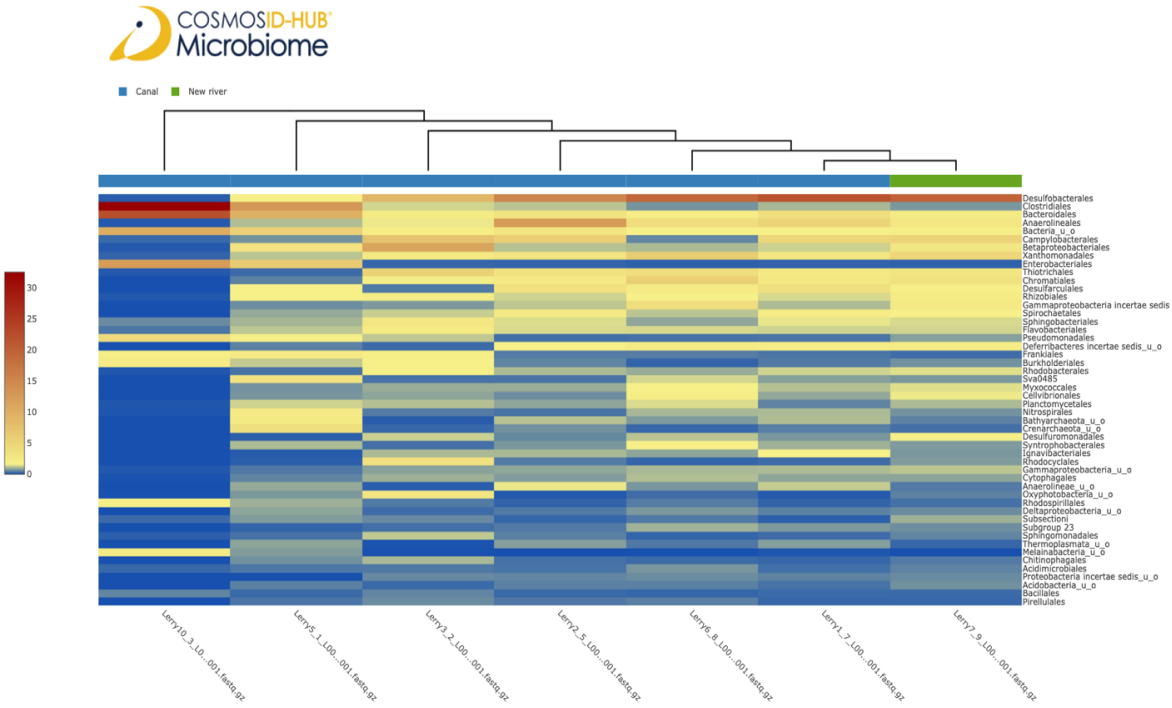

Figure 5. Cosmos ID ® Generated HeatMap of New River Sites. Each column represents a FASTA file from each site. The site is labeled "Larry #(site number)." The side of the figure shows what orders of bacteria are highest in concentration (goes from greatest to least). Site 10 has the highest concentration of *Bacteroidales*. (Source: CosmosID Metagenomics Cloud).

OBJECTIVES AND HYPOTHESES

This study aimed to determine whether or not waterways in New River are contaminated with FIBs (e.g., *Enterococci*, total coliforms, and *E. coli*) and to build upon previous work done by Broward County. Statistical analysis will be performed to find the abundances of *Enterococci* and *E. coli* and total coliforms using R, an open resource program to conduct the analysis (R Studio Team, 2020). The hypotheses are outlined below:

Hypothesis 1-
There is *Enterococci* present in all 10 sites along New River.

Hypothesis 2-
Enterococci concentrations will be higher at site 5 due to sewage outfalls.

Hypothesis 3-
There is *E. coli* present in all 10 sites along New River.

Hypothesis 4-
E. coli concentrations will be higher at site 5 due to sewage outfalls.

Hypothesis 5-
There are thermotolerant total coliforms present in all 10 sites along New River.

Hypothesis 6-
Thermotolerant total coliform concentrations will be higher at site 5 due to sewage outfalls.

METHODS

Based on preliminary data, nine sites within the Himmarshee Canal and one on New River were chosen (Figure 5). Figure 4 provides an overall map showing the ten sites, with site nine located at the Colee Hammock Park on New River. Three replicates were taken from each site as well as water temperature (Tables 3 and 4). Previous studies have also found that low tide is better for collecting FIBs (Santoro & Boehm, 2007; Louis, 2020). Water samples were collected by the roadside, as seen in Figures 6-12. Water samples were collected in ordinance with IDEXX, which is discussed further in the next section of these methods. Water samples had a max hold time of 6 hours. However, most were processed within 20-30 minutes after collection. IDEXX derived most of its methods from Standard Methods for the Examination of Water and Wastewater, which provides multiple avenues for researchers on how to properly examine, analyze, and dispose of their samples involved with, to name a few, drinking water, recreational water, swimming pools, and sewage (American Public Health Association et al., 2017). The standard methods also go into how to sample specific groups of microbes such as *Enterococci*, *E. coli*, total coliforms, and aerobic endospores (American Public Health Association et al., 2017). There are various ways to study them, but IDEXX was chosen due to its straightforwardness and faster processing time than other methods.

Table 3: Site Collections Enterolert®. All samples were collected during low tide by Catherine Bilodeau. Collection dates of each sample were written down, as well as coordinates for the site, water temperature, time, and weather conditions.

Date	Site	Coordinates	Temp (°C)	Time	Weather Conditions
9/14/22	1	26.121062 -80.135441	27.2	7:14 AM	Cloudy
9/15/22	2	26.120937 -80.135276	28.1	8:32 AM	Partly Sunny
9/19/22	3	26.120961 -80.132641	26.4	12:28 AM	Cloudy

9/20/23	4	26.120962 -80.132727	27.5	1:33 AM	Cloudy
9/25/22	5	26.120556 -80.132222	31.1	7:12 PM	Cloudy
9/29/22	6	26.119607 -80.131496	27.4	7:29 PM	Cloudy
10/3/22	7	26.119437 -80.131548	27.4	10:32 AM	Cloudy
10/4/22	10	26.117695 -80.130455	27.5	3:09 AM	Cloudy
10/10/22	8	26.11826 -80.13121	27.3	5:21 PM	Sunny
10/10/22	9	26.117004 -80.128397	28.7	6:18 PM	Sunny

Table 4: Site Collections Colilert-18®. All samples were collected during low tide by Catherine Bilodeau. Collection dates of each sample were written down, as well as coordinates for the site, water temperature, time, and weather conditions.

Date	**Site**	**Coordinates**	**Temp (℃)**	**Time**	**Weather Conditions**
11/17/22	1	26.121062 -80.135441	25.9	9:02 AM	Cloudy
11/17/22	2	26.120937 -80.135276	25.3	9:06 AM	Cloudy
11/28/22	3	26.120961 -80.132641	25.0	6:16 AM	Cloudy
11/28/22	5	26.120556 -80.132222	25.4	6:25 AM	Cloudy
11/28/22	6	26.119607 -80.131496	26.9	6:33 AM	Cloudy
11/30/22	7	26.119437 -80.131548	25.8	7:59 AM	Cloudy

11/30/22	4	26.120962 -80.132727	26.5	8:13 AM	Cloudy
11/30/22	9	26.117004 -80.128397	28.9	8:29 AM	Cloudy
1/20/23	10	26.117695 -80.130455	25.4	12:39 PM	Cloudy
2/3/23	8	26.11826 -80.13121	26.5	1:55 PM	Partly Sunny

Figure 6. SE 8th Ave by the Beginning of Himmarshee Canal. Site 1 is shown in a darker shade, with Site 2 on the other side of SE 2nd Street.

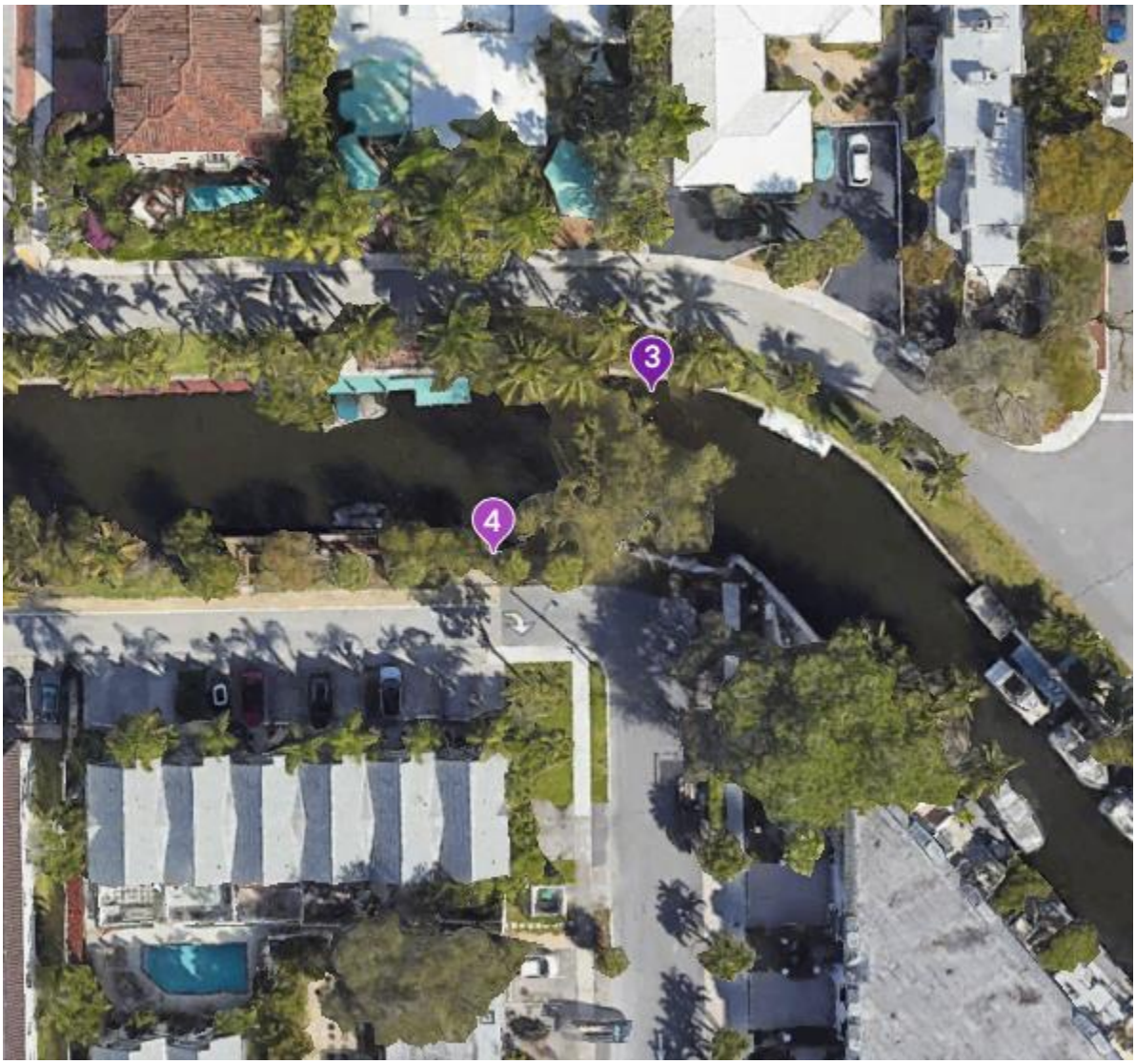

Figure 7: Site 3 and 4. One site, 3 in a darker marker, on the north side of SE 2nd Street. Site 4 on the south side of SE 2nd Street and corner of SE 10th Ter.

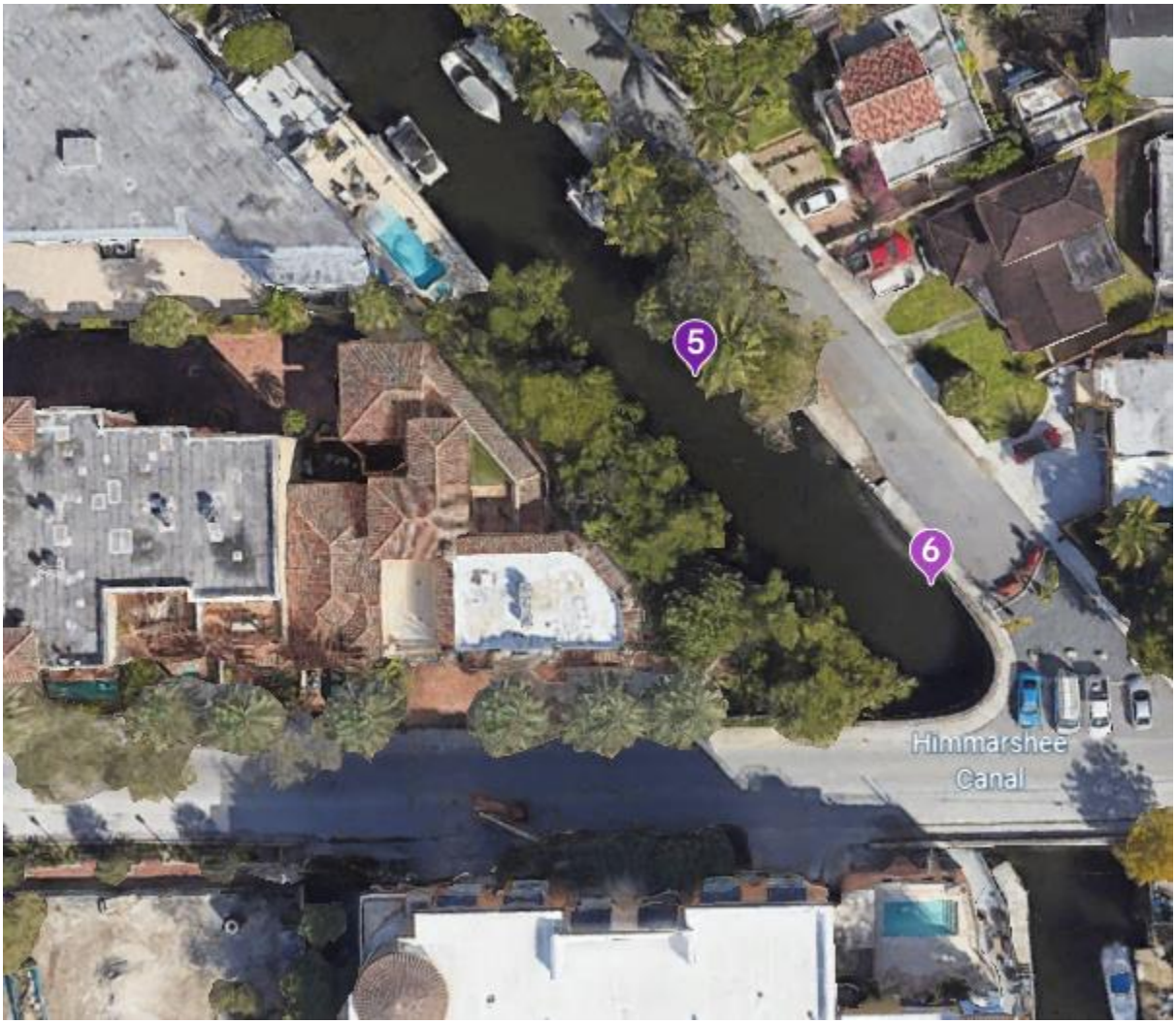

Figure 8: Site 5 and 6. Located along SE 2nd St, site 5 being the darker marker.

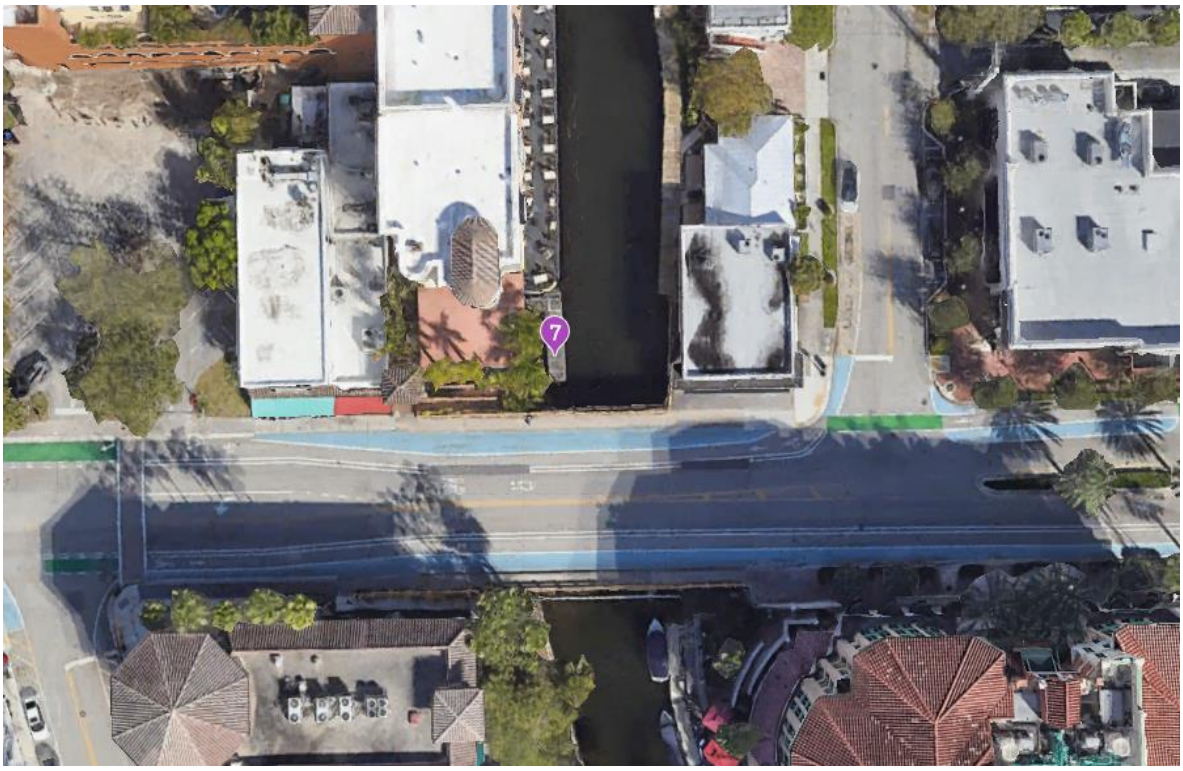

Figure 9: Site 7. Located off of E Las Olas Blvd along the southern side of the Himmarshee Canal.

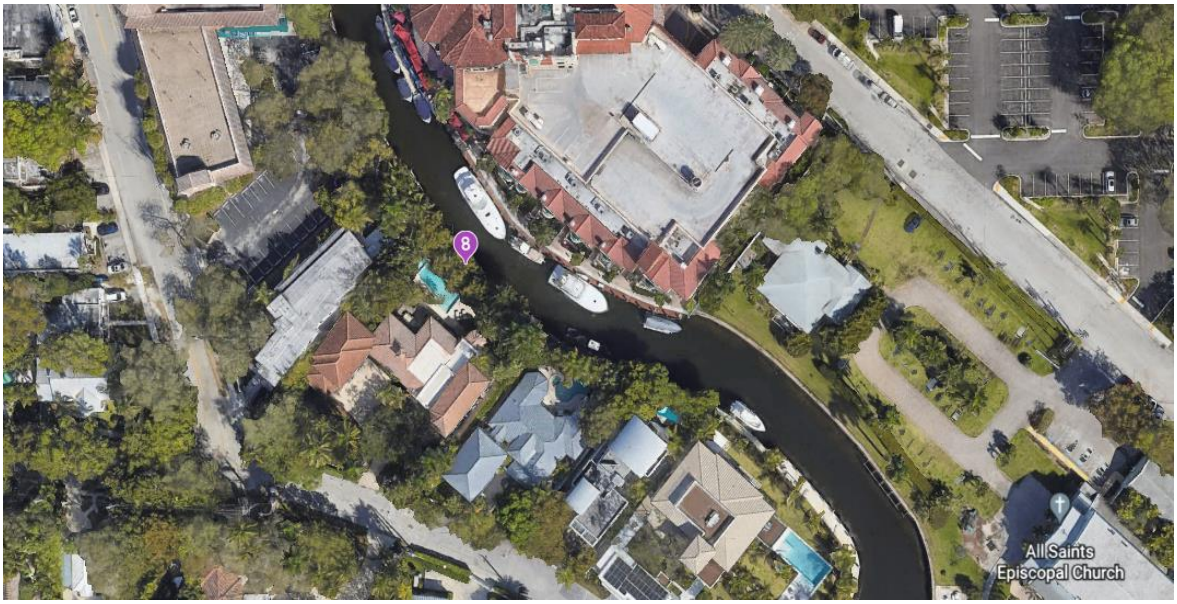

Figure 10: Site 8. Located towards the end of the Himmarshee Canal.

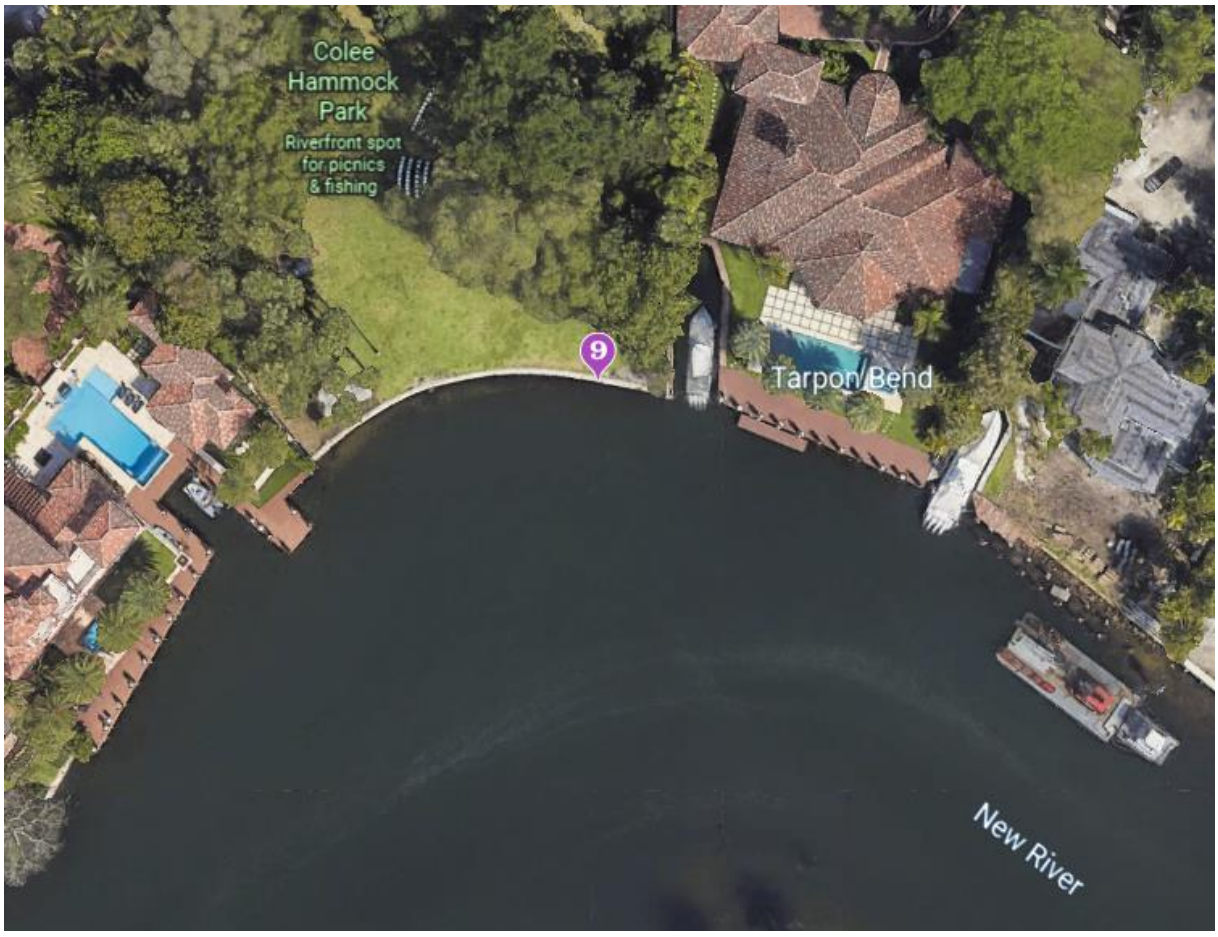

Figure 11: Site 9. Located at Colee Hammock Park, which is located along New River itself.

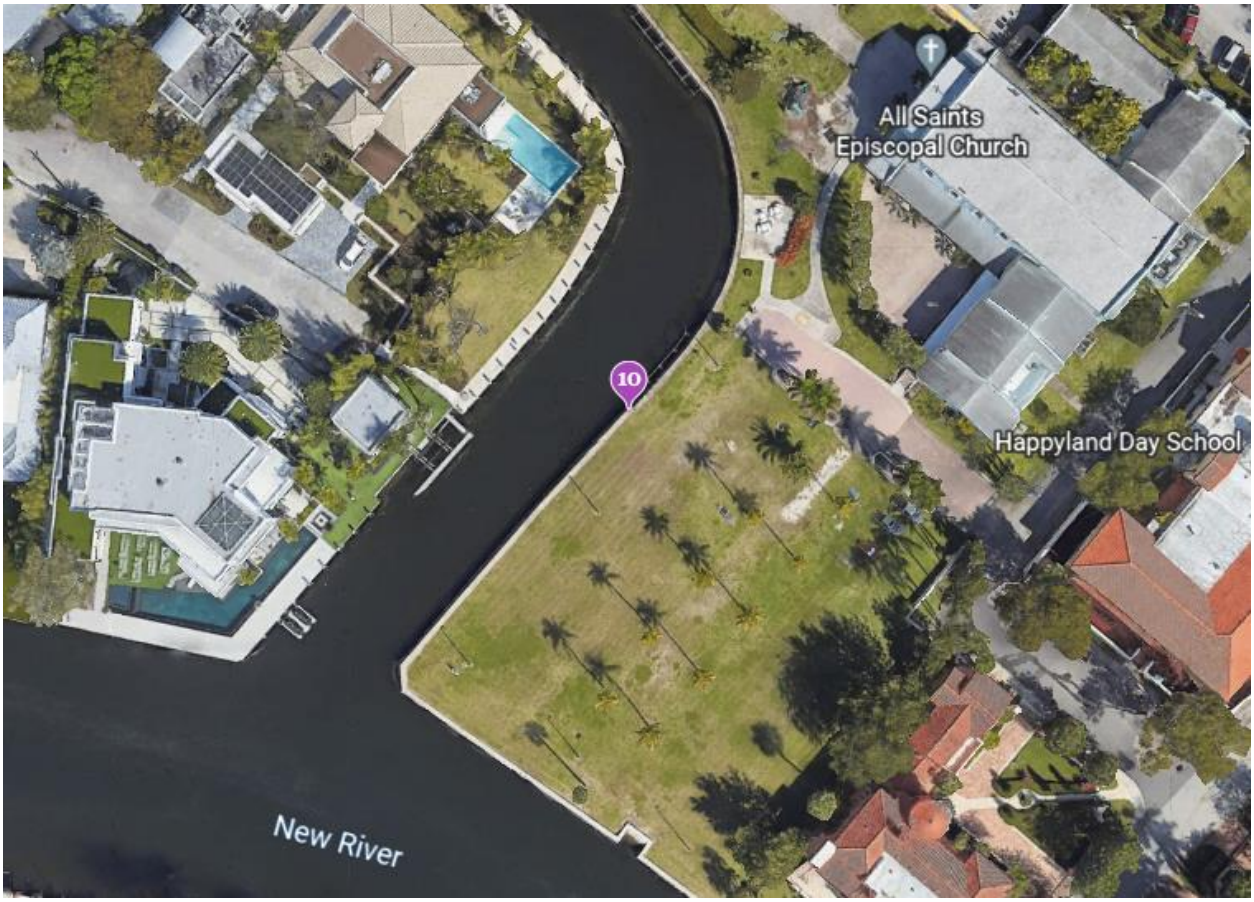

Figure 12: Site 10. Located at the All Saints Episcopal Church where Himmarshee Canal ends.

IDEXX Data Collection and Processing

All water samples were collected from the shore during low tide by putting a 100 mL vessel into the water while the collector wore latex gloves to avoid contamination. Samples were put on ice until it was transported to the Microbiology and Genetics lab at the Oceanographic Campus at Nova Southeastern University. The max hold time for the water samples was 6 hours, but most samples were processed within 20-30 minutes of collection. Then the samples were processed using two different IDEXX kits: Enterolert® and Colilert-18®. The site samples were diluted by 1:10 as advised by IDEXX as well as what a study on the North Fork of New River in 2000 had done (Solo-Gabriele et al., 2000). The reason was due to possible microbial interference from other bacteria mentioned before (i.e., *Bacillus*) (American Public Health Association et al., 2017). A powder pack of the reagent with each kit was added to each vessel, with three replicates for each site. The reagent was mixed until it was dissolved. The contents were poured into a 97-well IDEXX tray and then sealed using the Quanti-Tray®/2000 Sealer Model 2X (Figure 13). The 97-well tray is separated into 49 large wells and 48 small wells. Each kit used a different reagent that reacted to the target bacteria in a specific way.

Figure 13: Quanti-Tray® Sealer Model 2X. This is the sealer used with all of the quanti-trays from IDEXX, regardless of the number and size of the wells.

The first kit used in this experiment was Enterolert®, which targets *Enterococcus*. This kit required that after each sample was sealed in the tray, it would be incubated at 41 ± 0.5℃ for 24 hours. During the 24-hour incubation period, the inside temperature of the incubator was recorded twice (Table 5). Multiple trays from different sites were incubated at the same time. When incubation was completed, the trays were observed for results using a 6-watt, 365 nm UV flashlight. Results were taken by counting the wells that had a blue fluorescence. This is because Enterolert® uses DST to indicate when a target bacterium metabolizes the nutrient indicator. For the Enterolert® test, *Enterococcus* has an enzyme called glucosidase. When glucosidase is present in the sample, it will break down the nutrient indicator 4-methyl-umbelliferyl-β-glucoside. When 4-methylumbelliferyl is broken down, it releases 4-methyl-umbelliferone with a blue fluorescence (Figure 14). The counts were then compared to an MPN table (Appendix A) and multiplied by the dilution factor, resulting in *Enterococcus* counts of MPN/100 mL. An example of a positive well for *Enterococcus* can be seen in Figure 15. Then the second IDEXX kit followed these same methods; however, the targeted bacteria broke down different nutrient indicators.

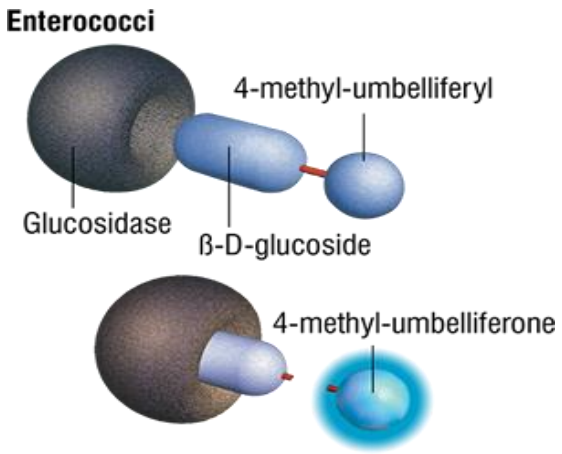

Figure 14: *Enterococci* Nutrient Indicator. This diagram depicts how the nutrient indicator is metabolized. When a 4-methyl-umbelliferyl is metabolized, a 4-methyl-umbelliferone is released, turning the well blue (Source: IDEXX).

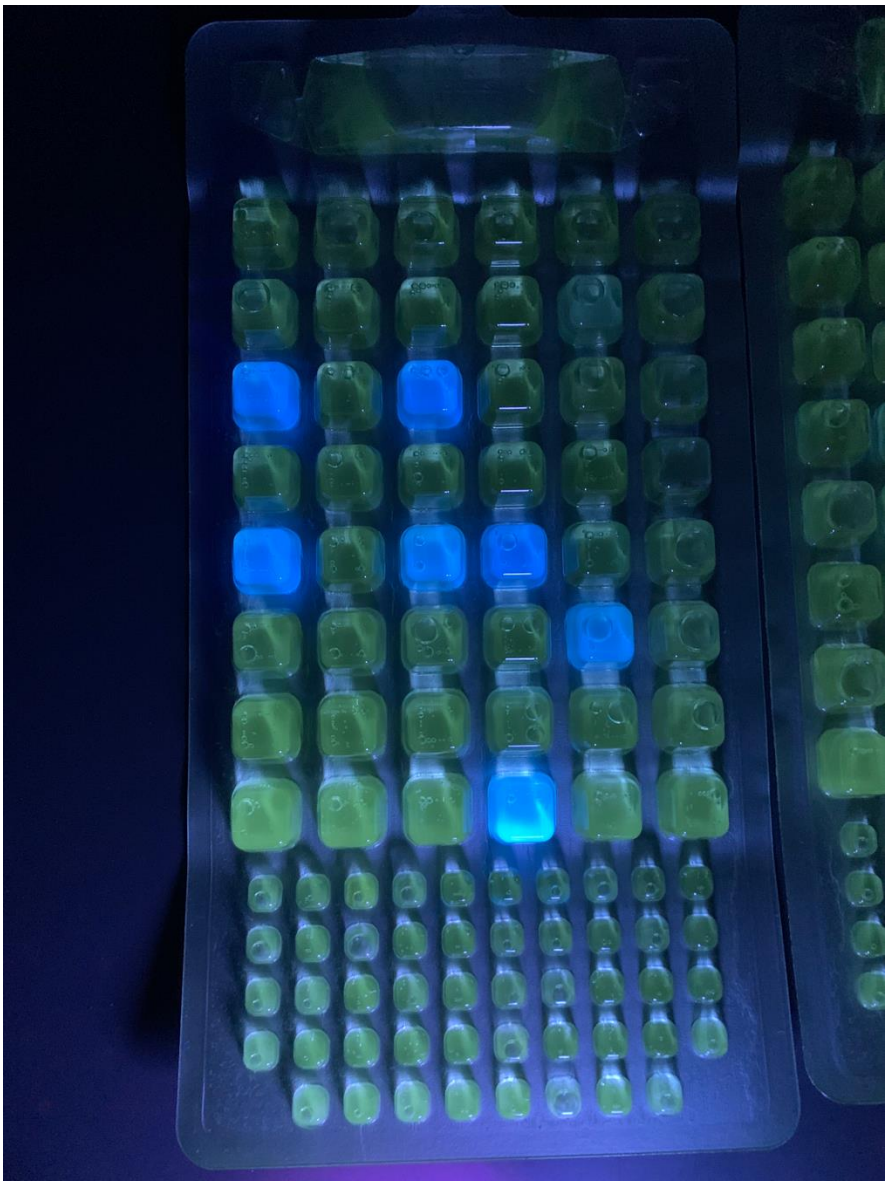

Figure 15: Enterolert® Quanti-Tray®/2000 Results. This picture shows site 9.2, which yielded only 7 positive large wells. This shows what *Enterococcus* does inside the well as it is being incubated. The positive blue fluorescence means that the enzyme 4-MUG has been metabolized.

Table 5: Incubation Timetable Enterolert®. Every sample was given a unique ID and recorded when it entered the incubator. The samples incubating were then checked twice during the incubation period of 24 hours. There was a digital thermometer located inside the incubator to verify the temperature.

Sample ID	Initial Incubation Time & Date	1st Check Time & Date	Temp (°C)	Initials 1st Check	2nd Check Time & Date	Temp (°C)	Initials 2nd Check
1.1 1.2 1.3	9/14/22 8:23 AM	9/14/22 3:34 PM	41.1111	MBH	9/15/22 8:00 AM	41.4	CMB
2.1 2.2 2.3	9/15/22 9:47 AM	9/15/22 3:14 PM	41.4	MBH	9/16/22 9:48 AM	41.4	CMB
3.1 3.2 3.3	9/19/22 1:56 AM	9/19/22 3:59 PM	41.4	MBH	9/20/22 1:59 AM	41.4	CMB
4.1 4.2 4.3	9/20/22 2:26 AM	9/20/22 4:13 PM	41.4	MBH	9/21/22 2:19 AM	41.5	CMB
5.1 5.2 5.3	9/25/22 8:40 PM	9/26/22 11:01 AM	41.2	PSS	9/26/22 7:32 PM	41.3	CMB
6.1 6.2 6.3	9/29/22 11:03 PM	9/30/22 10:00 AM	41.5	CMB	9/30/22 10:50 PM	41.5	CMB
7.1 7.2 7.3	10/3/22 12:02 AM	10/4/22 4:48 PM	41.1	MBH	10/4/22 9:30 PM	41.4	CMB
8.1 8.2 8.3	10/10/22 8:51 PM	10/11/22 10:06 AM	41.3	CMB	10/11/22 8:40 PM	41.3	CMB
9.1 9.2	10/10/22 8:51 PM	10/11/22 10:06 AM	41.3	CMB	10/11/22 8:40 PM	41.3	CMB

9.3							
10.1 10.2 10.3	10/8/22 4:37 AM	10/8/22 7:34 PM	41.4	CMB	10/9/22 4:35 AM	41.4	CMB

The second kit used in this experiment was Colilert-18®, which targeted total coliforms and *E. coli*. This kit required that after the sample was sealed in the tray, it would be incubated at 44.5 ± 0.5℃ for 22 hours. This would yield thermotolerant total coliforms because incubation would be at a higher temperature than the recommended 35°C. The trays were checked twice during the 22-hour incubation period (Table 6). During incubation, if coliforms are present in the sample that has an enzyme called β-galactosidase, that will break down a nutrient indicator called ONPG. When β-D-galactopryansodie is metabolized, it breaks down o-nitrophenyl, releasing o-nitrophenol. O-nitrophenol turns the well into a non-fluorescent yellow color (Figure 16). The nutrient indicator for *E. coli* is called MUG, which works similarly to *Enterococcus's* nutrient indicator. Once again, *E. coli* has an enzyme called β-glucuronidase that, when present, breaks down β-galactosidase with a 4-methyl-umbelliferyl attached to it. When 4-methyl-umbelliferyl is broken down, it releases 4-methyl-umbelliferone, which turns a blue fluorescence (Figure 17). As in the Enterolert® test, after 22 hours, the trays are counted for coliforms, and any wells that have turned yellow count as positive wells. The trays were then put under a 6-watt, 365 nm UV flashlight to count how many wells had blue fluorescence. For the well to be positive for *E. coli*, it must turn yellow and give off a blue fluorescence. The number of positive large and small wells were compared to the MPN table and then multiplied by the dilution factor, just as they were for the Enterolert® test.

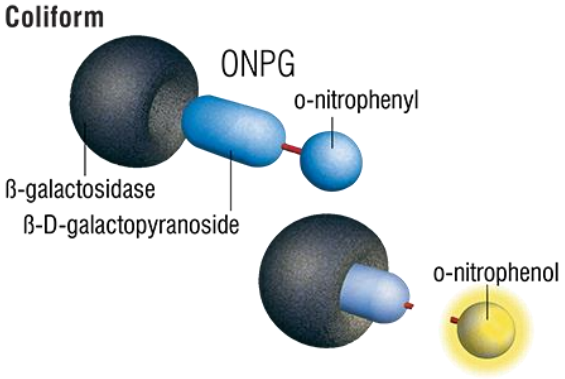

Figure 16: Coliform Nutrient Indicator. This diagram depicts how coliforms metabolize the nutrient indicator called ONPG, which turns a non-fluorescent yellow (Source: IDEXX).

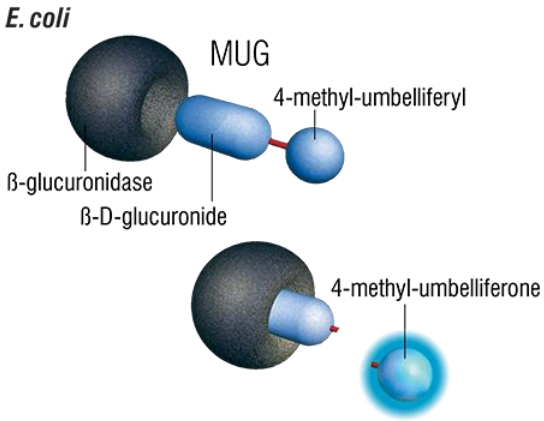

Figure 17: *E. coli* Nutrient Indicator. This diagram depicts how when *E. coli* metabolizes the MUG nutrient indicator, it turns a blue fluorescence (Source: IDEXX).

Table 6: Incubation Timetable Colilert-18®. Every sample was given a unique ID and recorded when it entered the incubator. The samples incubating were then checked twice during the incubation period of 22 hours. There was a digital thermometer located inside the incubator to verify the temperature. If the temperature was too high, it was adjusted and noted.

Sample ID	**Initial Time & Date**	**1st Check Time & Date**	**Temp (°C)**	**1st Check Initials**	**2nd Check Time & Date**	**Temp (°C)**	**2nd Check Initials**
1.1C-1.3C 2.1C-2.3C	11/17/22 10:30 AM	11/17/22 11:15 PM	44.7	CMB	11/18/22 8:15 AM	44.4	CMB
3.1C-3.3C 5.1C-5.3C 6.1C-6.3C	11/28/22 8:05 AM	11/28/22 2:39 PM	44.6	PSS	11/29/22 5:45 AM	44.4	CMB
4.1C-4.3C 7.1C-7.3C 9.1C-9.3C	11/30/22 10:49 AM	11/30/22 6:48 PM	44.9*	CMB	12/1/22 8 AM	44.3	CMB
10.1C- 10.3C	1/20/23 2:46 PM	1/20/23 8:28 PM	44.5	CMB	1/21/23 10:30 AM	44.5	CMB
8.1C-8.3C	2/3/23 7:43 PM	2/4/23 11:52 AM	44.8	CMB	2/4/23 4:30 PM	44.2	CMB

*Turned down and waited for the temperature to reach 44.4 before leaving.

Data Analysis

Three separate datasets were created from *Enterococcus*, total coliforms, and *E. coli*. All three datasets were run through R Studio (RStudio Team, 2020). RStudio has several different programs and packages; due to the nature of the data, a One-Way ANOVA was done for *Enterococcus* and fecal coliforms. A One-Way ANOVA analyzes the variances within the data when three or more factors or multiple samples are present (Kim, 2017). Before a One-Way ANOVA was done, the data went through tests to see if the data was normal. These tests were a Shapiro-Wilk test to see if the data was normal and Bartlett's test to see if the variances were homogenous (Logan, 2010). The datasets *Enterococcus* and total coliforms were transformed by

log(x) to bring normality and homogeny to the datasets. After a One-Way ANOVA was done for the two datasets, a Tukey Model was run to statistically find which variances, in this case, sites, were causing the difference in concentrations for the targeted bacteria. The Tukey Model does this by comparing the variances to each factor level, in this case, sites. Each site was compared to one another in the model to show, as mentioned before, which sites were driving the variation seen in the ANOVAs. For the *E.coli* dataset, a different test was done due to the data not being normal. This test is called a Kruskal-Wallis test, a non-parametric test that uses a ranking system of the observations in the data to calculate that statistic and is helpful when outliers are present in the data (Logan, 2010). After all the correct models and tests were run for the three datasets, a package from RStudio called "multcompLetters," was done to group the variances in the data by significance more visually. When a grouping of letters does not share the combination of letters or letter with another site, it means that the site is significantly different from the others.

RESULTS

All sites detected both *Enterococcus*, thermotolerant total coliforms, and *E. coli*, with the concentrations of each varying between locations. The site with the highest concentration of *Enterococcus* was site 3, with an average count of 22,029.50 MPN/100 mL. The EPA states that a count of more than 130 MPN/100 mL is a red flag, and site 3 greatly exceeds this recommendation (EPA, 2012). The highest concentration of thermotolerant total coliforms was at site 1, with an average count of 7,693.33 MPN/100 mL, and the highest concentration of *E. coli* was at site 1, with an average count of 5,846.33 MPN/100 mL (Table 7 & 8). The minimal amount of *Enterococcus* present was found at site 8, with an average count of 27 MPN/100 ml. While the lowest concentration of thermotolerant total coliforms was at site 5 with an average count of 132.67 MPN/100 mL, and then the lowest concentration of *E. coli* was at site 5 with an average count of 108.67 MPN/100 mL. These means of concentrations were compared in the One-Way ANOVA and the Kruskal Wilks Test.

A One-Way ANOVA done for *Enterococcus*, with a multiple comparison post hoc Tukey test being done after, showed significant differences between the sites. The One-Way ANOVA reported that sites significantly affected the concentration or measurements of *Enterococcus* ($F_{9,20} = 72.055$, $p = 2.854 \times 10^{-13}$). Sites 1 and 3 were significantly different from the other sites when

they were compared in a Tukey Model (p-value < 0.05). Site 2 was also significantly different, but was found to be alike other sites, 4 and 6 (p = 0.96 and 0.16). Figure 18 outlines these groupings in a plot showing how sites 5, 7, and 10 were grouped together under the lettering "de." These findings are also supported by the p values of sites 5, 7, and 10 when compared to one another (p-value > 0.05). For *Enterococcus*, sites 1 and 3, not site 5, are the source for high concentrations of bacteria in the genus *Enterococcus*. These same sites also showed significance in the fecal coliform data.

Once again, a One-Way ANOVA reported that the sites were affecting the measurements of total coliforms along all the sites ($F_{9,20}$ = 27.346, p = 2.456 x 10-9). Thermotolerant total coliform concentrations were significantly different between some of the sites. Site 1 was the only site significantly different from the others (p-value < 0.05). Some sites shared similarities, those being 2, 4, and 7. Site 5 was different from similar with some of the sites like 3, 8, and 10. These groupings can be seen in Figure 19, showing how different site 1 is from the others. The only dataset with a few groupings causing the variances in data was *E. coli*.

E. coli concentrations were found to be significantly different between only a few sites, unlike the other datasets. A One-Way ANOVA was not done for *E. coli* due to the data being non-homogenous. Instead, a Kruskal-Wallis Test was done, resulting in a p-value of 0.0014 (chi-squared = 26.921, df= 9), meaning sites once again were significantly affecting the concentration of bacteria. The non-parametric post hoc test revealed that the sites significantly different from the others were 1 and 2 with an "a" grouping and site 5 with a "b" grouping (Figure 20). The results also showed that sites 1, 2, and 5 are only significantly different from each other but are similar to the other sites. This data showed that there is *E. coli* present in the sites, but their concentration was similar between most of the sites.

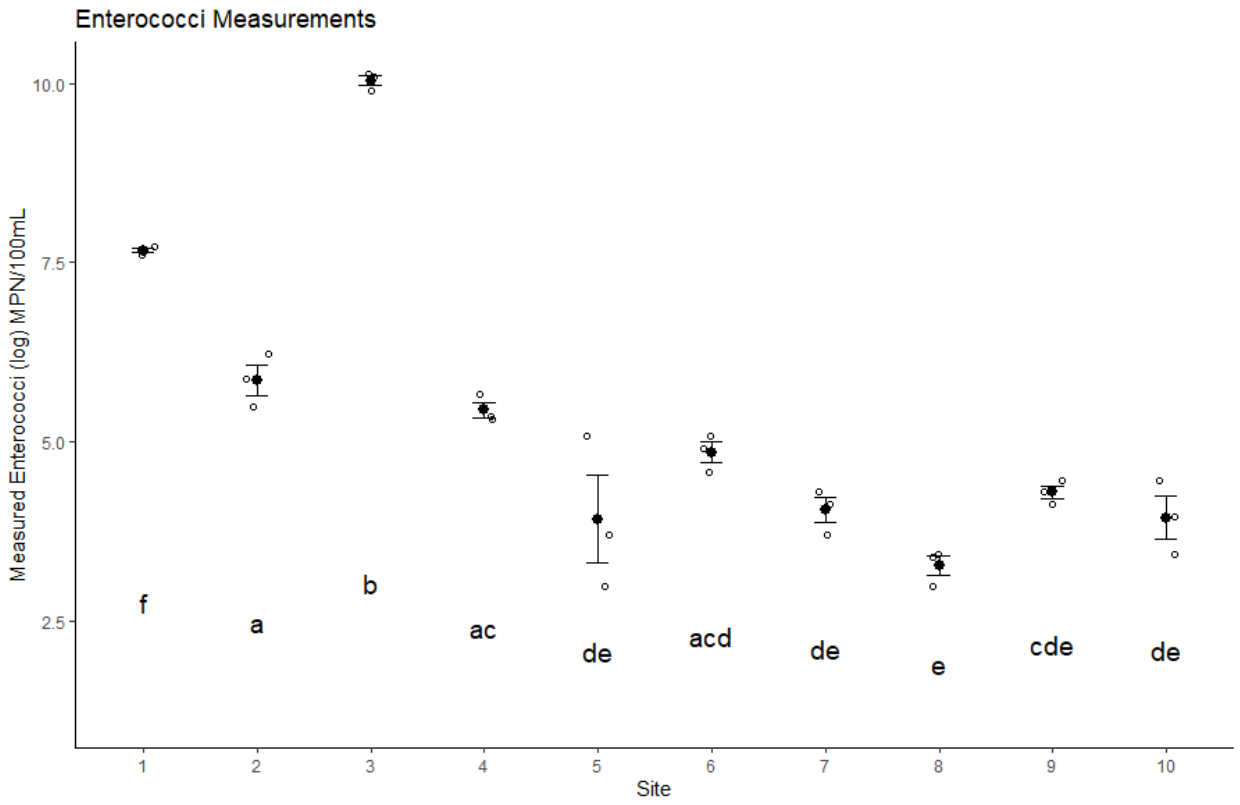

Figure 15: Enterolert Measurements. This plot shows the ANOVA and Tukey Model results, revealing the groupings and how significant specific sites are with one another. Site 1 and 3 are the most significant due to not sharing a common letter with other sites.

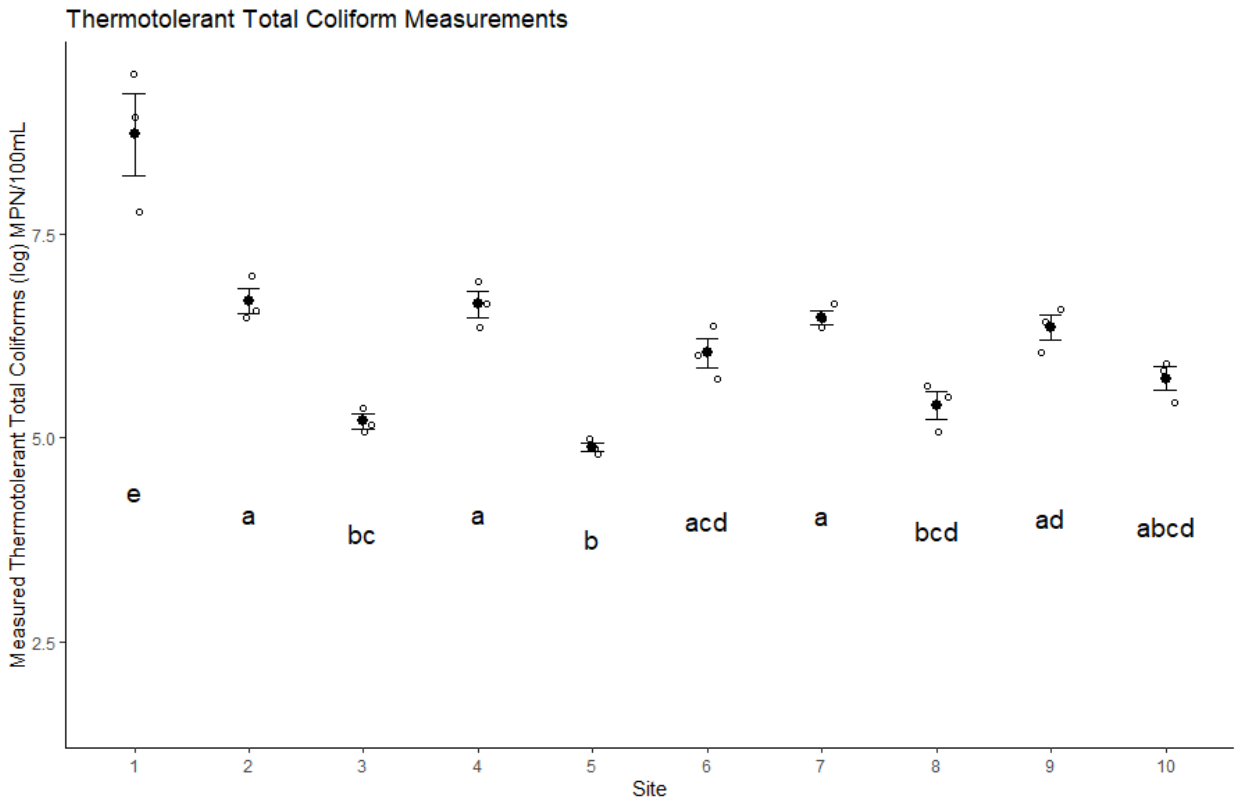

Figure 19: Thermotolerant Total Coliform Measurements. This plot shows the results of the ANOVA and Tukey Model revealing the sites' groupings and showing which is causing the variation in the data. Site 1, in this case, was found to be more significant when compared with the other sites due to not sharing a letter with any other grouping.

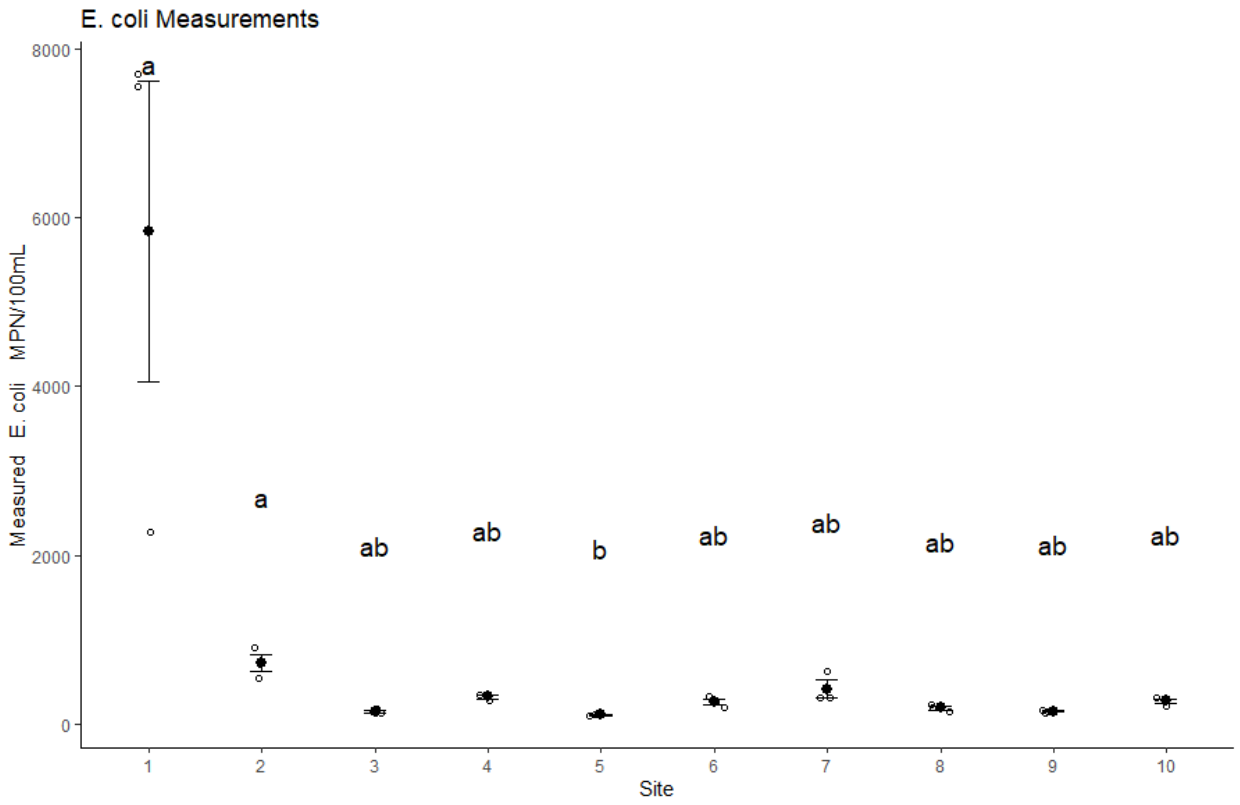

Figure 20: *E. coli* Measurements. This plot shows the results of the post non-parametric hoc test revealing sites 1 and 2 being statistically different from site 5. This is due to sites 1 and 2 having the grouping "a," while site 5 has a grouping of "b."

Table 7: Enterolert® Results. The date of the results was recorded with each sample as well as how many of the large wells and small wells gave a positive result. After the number of large and small positive wells were recorded, the MPN table was used. The value from the MPN was then multiplied by 10, the final recorded result in the MPN/100 mL column.

Date	**Sample ID Enterolert**	***Enterococcus***		
		Large Wells	**Small Wells**	**MPN/ 100 mL**
9/15/22	1.1	48	13	2,014
	1.2	48	15	2,187
	1.3	49	12	2,247
9/16/22	2.1	30	4	504
	2.2	18	2	243
	2.3	21	7	359
9/20/22	3.1	49	48	>24,196
	3.2	49	47	24,196
	3.3	49	46	19,863
9/21/22	4.1	12	6	204
	4.2	16	2	213
	4.3	20	3	288
9/26/22	5.1	4	0	41
	5.2	13	1	160

	5.3	2	0	20
9/30/22	6.1	12	0	135
	6.2	13	1	160
	6.3	9	0	98
10/4/22	7.1	6	0	63
	7.2	6	1	74
	7.3	4	0	41
10/11/22	8.1	2	0	20
	8.2	2	1	30
	8.3	3	0	31
10/11/22	9.1	5	1	63
	9.2	7	0	75
	9.3	8	0	86
10/9/22	10.1	3	0	31
	10.2	4	1	52
	10.3	8	0	86

Table 8: Colilert-18® Results. The date of the results was recorded for each sample as well as how many positive results there were for the large and small wells. Thermotolerant total coliforms and *E. coli* each had their own set of positive large or small wells hence the two different columns. After the combination of large and small wells was recorded, the MPN table was used to find the MPN number for each sample. That value was then multiplied by 10 and, then that final number was recorded.

Date	**Sample ID Colilter-18**	**Thermotolerant total coliforms**			***E. coli***		
		Large Wells	**Small Wells**	**MPN/ 100 mL**	**Large Wells**	**Small Wells**	**MPN/ 100 mL**
11/18/22	1.1C	49	42	12,997	48	42	7,556
	1.2C	49	34	7,701	49	34	7,701
	1.3C	48	17	2,382	48	16	2,282
11/18/22	2.1C	33	8	657	29	8	545
	2.2C	37	4	712	37	4	712
	2.3C	43	7	1,081	41	5	906
11/29/22	3.1C	13	1	160	11	0	122
	3.2C	17	1	216	14	1	173
	3.3C	15	0	175	12	0	135
12/1/22	4.1C	41	9	1,014	21	1	279
	4.2C	31	7	581	23	3	341
	4.3C	39	3	767	24	2	345
11/29/22	5.1C	12	1	146	11	0	122

	5.2C	10	1	121	8	1	97
	5.3C	9	3	131	7	3	107
11/29/22	6.1C	22	2	309	15	2	199
	6.2C	26	4	414	18	3	256
	6.3C	35	1	586	24	1	331
12/1/22	7.1C	39	3	767	35	3	624
	7.2C	34	2	576	23	1	313
	7.3C	36	2	637	23	1	313
2/4/23	8.1C	19	1	246	16	0	189
	8.2C	22	0	282	19	0	233
	8.3C	13	1	160	12	1	146
12/1/22	9.1C	28	2	426	12	0	135
	9.2C	33	6	620	11	2	145
	9.3C	39	1	722	14	0	161
1/21/23	10.1C	26	1	369	22	1	295
	10.2C	18	1	231	17	1	216
	10.3C	23	3	341	21	3	305

DISCUSSION

Many regions across the United States strive for more ways to monitor the waters around them for pollution to protect water quality, public health, and ecotourism. Fecal contamination has caused public health issues in third-world countries; however, human exposure to FIBs and other viral microorganisms is increasing with global climate change (Teixeira et al., 2020). The forensic assessment on the Himmarshee Canal, located off SE 2nd Street in Fort Lauderdale, Florida, yielded findings showing that the canal is still plagued by fecal pollution. Sites 1 and 2 were a factor in concentrations of thermotolerant total coliforms, *Enterococcus,* and *E. coli*; however, it is essential to point out that all bacteria were detected in high levels across all the sites. EPA guidelines state that any samples with a quantity of 130 per 100 mL for *Enterococci* and 410 per 100 mL for *E. coli* are a raise for concern (EPA, 2012). The guideline for total coliforms is 200 per 100 mL (EPA, 1986). Due to this guideline, of the 60 samples taken from September 15th, 2022, to February 3rd, 2023, 63% were under the recommended levels (38/60). However, most sites are heavily polluted by thermotolerant total coliforms, which raises much concern. More concern is raised when the levels of *Enterococcus* and *E. coli* found in the sediment samples from 2020 are compared to the water samples collected in this current study.

Sediment samples back in 2020 showed high levels of enteric bacteria, with possible contamination of *Enterococcus* and *E. coli* being the most notable. As mentioned, sites 10 and 5 yielded the most data due to a sewage break in that area, which occurred just before the samples were collected in 2020. *Bacteroidetes* in site 10 had counts of 23.27, but then 35.43 counts of *Firmicutes,* while site 5 had 13.63 counts of *Bacteroidetes* and 14.35 counts of *Firmicutes*. *Firmicutes* is a dominant gut phylum where bacteria in this group make up 90% of the human gut microbiome (Rinninella et al., 2019). An example of a bacteria from this phylum would be *E. faecalis*, a human pathogen; however, some bacteria in the phylum *Firmicutes* are beneficial for a healthy gut (Kulagina et al., 2012). The fact that they are present in the Himmarshee Canal is still a cause of concern regardless of if they are not pathogenic bacteria because their presence means there is a likelihood of fecal contamination. A visual representation of the other phyla in the Himmarshee Canal and New River can be seen in Figure 18. The 16s RNA sequencing, however, could go down to order and family to give a better profile of the bacteria present in the Himmarshee Canal. Figure 19 outlines orders found in the canal, with sites 10 and 5 having the most enteric

bacteria groups. This time the order *Enterobacterales* shows high levels, with site 10 having 12.45 and site 5 having 6.33 (Figure 19). Figures 20 A-C provide a legend for the other orders found in the samples. An important bacterium to note that is from the order *Enterobacterales* is *E. coli*. A similarity between the sediment and water samples is starting to form due to both samples and data finding high levels of enteric bacteria, specifically *E. coli*. Figure 21 represents the families found in the canal, and once again, *E. coli* is represented at high levels being from the family *Enterobacteriaceae*. Another interesting finding is that when comparing the outcome of both datasets, sites 10 and 5 were significantly similar in 2023 with *Enterococcus* and *E. coli*. In 2020, these sites both had high levels of *Bacteroidetes*. Another study should be done to look at the microbial profile of the canal through sediment data, with consideration given to doing HF183 and using dPCR.

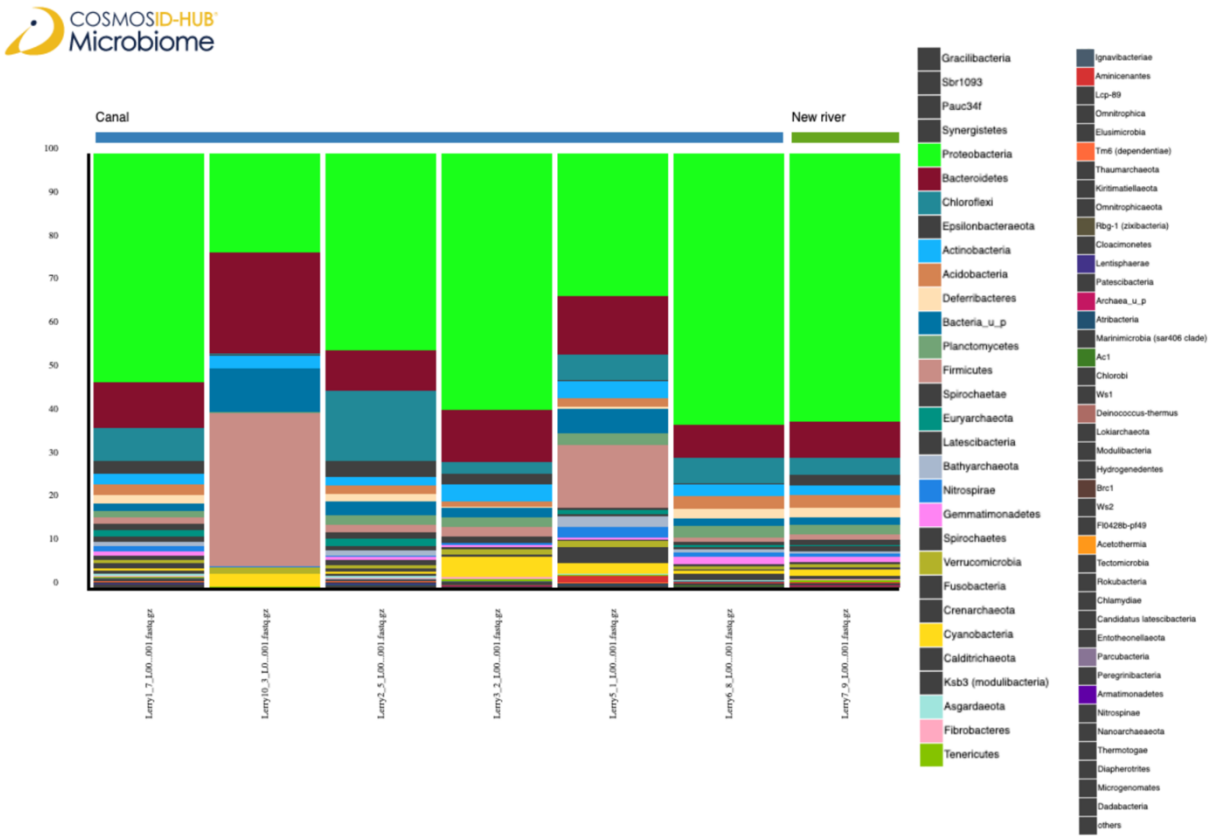

Figure 21: Stacked Bar Chart of Bacterial Phyla Represented in Sediment Samples along the Himmarshee Canal and New River. This bar chart represents the phyla of bacteria found in sediment samples along the Himmarshee Canal and New River. (Source: CosmosID Metagenomics Cloud).

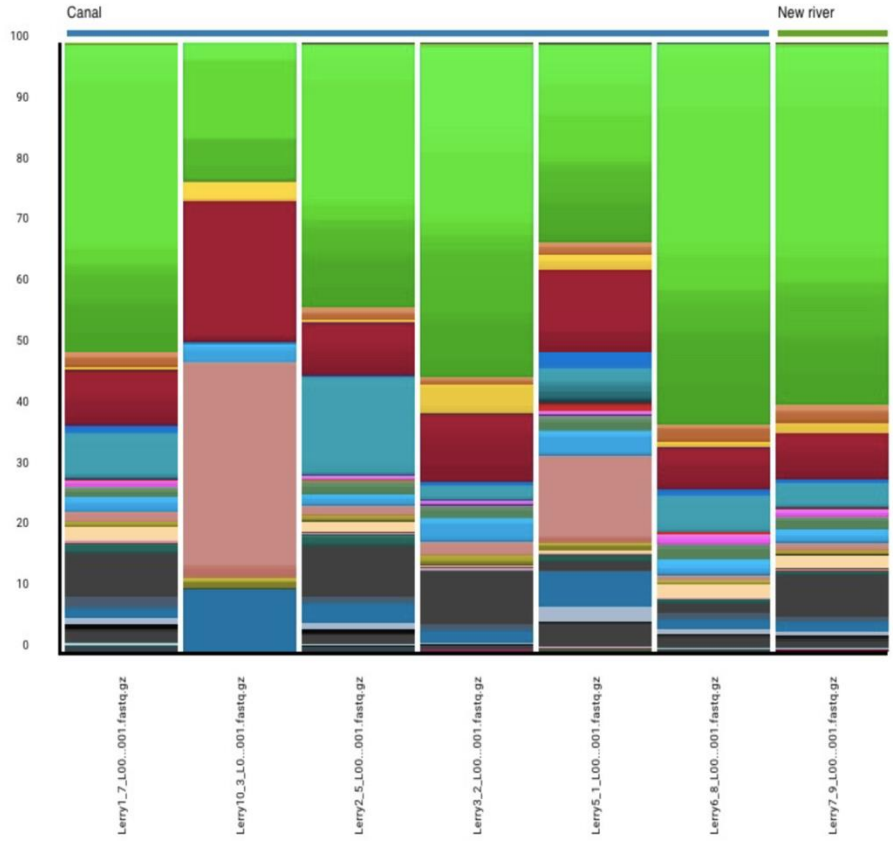

Figure 22: Stacked Bar Chart of Bacterial Order Represented in Sediment Samples along the Himmarshee Canal and New River. The order *Bacteroidales* and *Enterobacterales* are the highest. See Figures 23 A-C, for the legend for this stacked bar chart. (Source: CosmosID Metagenomics Cloud).

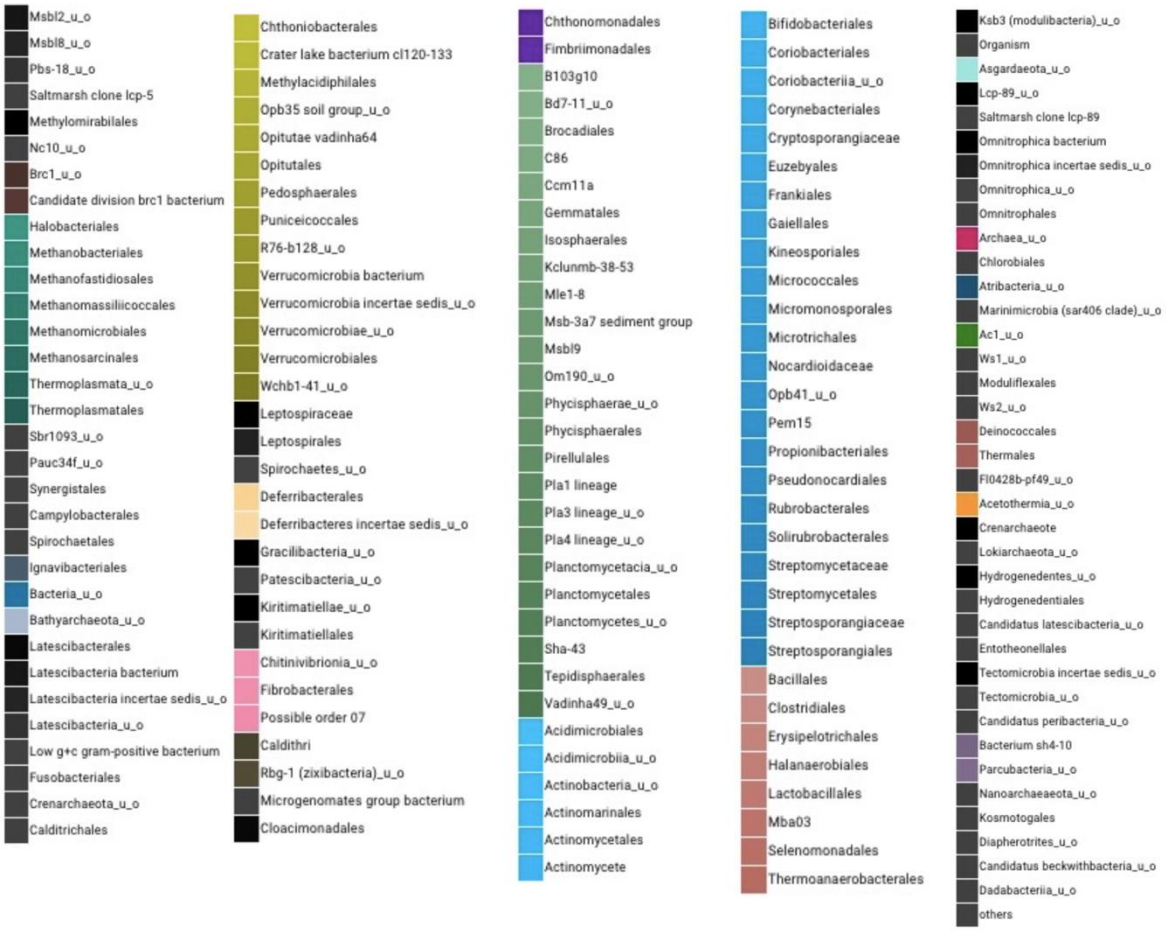

Figure 23. A: Key to Orders of Bacteria Represented in Sediment Samples. (Source: CosmosID Metagenomics Cloud).

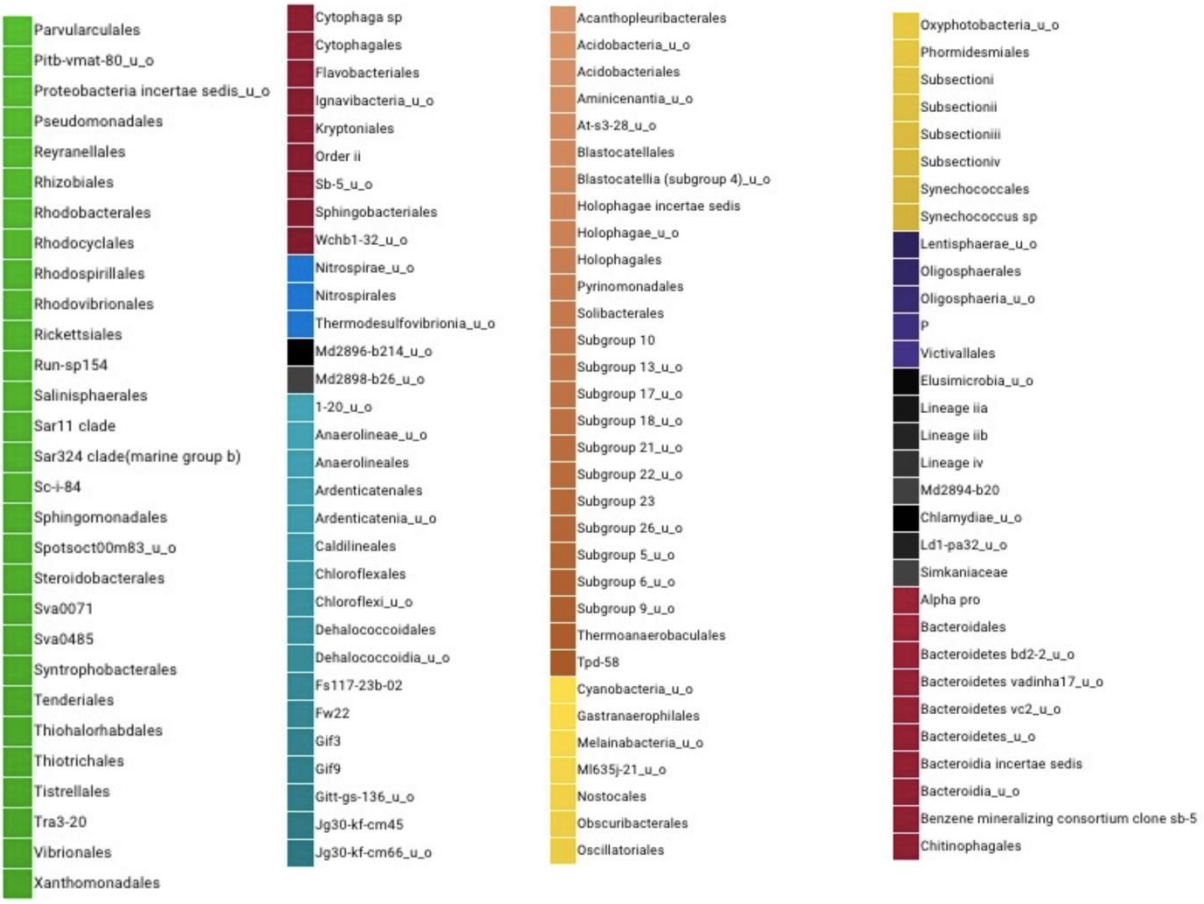

Figure 23. B: Key to Orders of Bacteria Represented in Sediment Samples. (Source: CosmosID Metagenomics Cloud).

Figure 23. C: Key to Orders of Bacteria Represented in Sediment Samples. (Source: CosmosID Metagenomics Cloud).

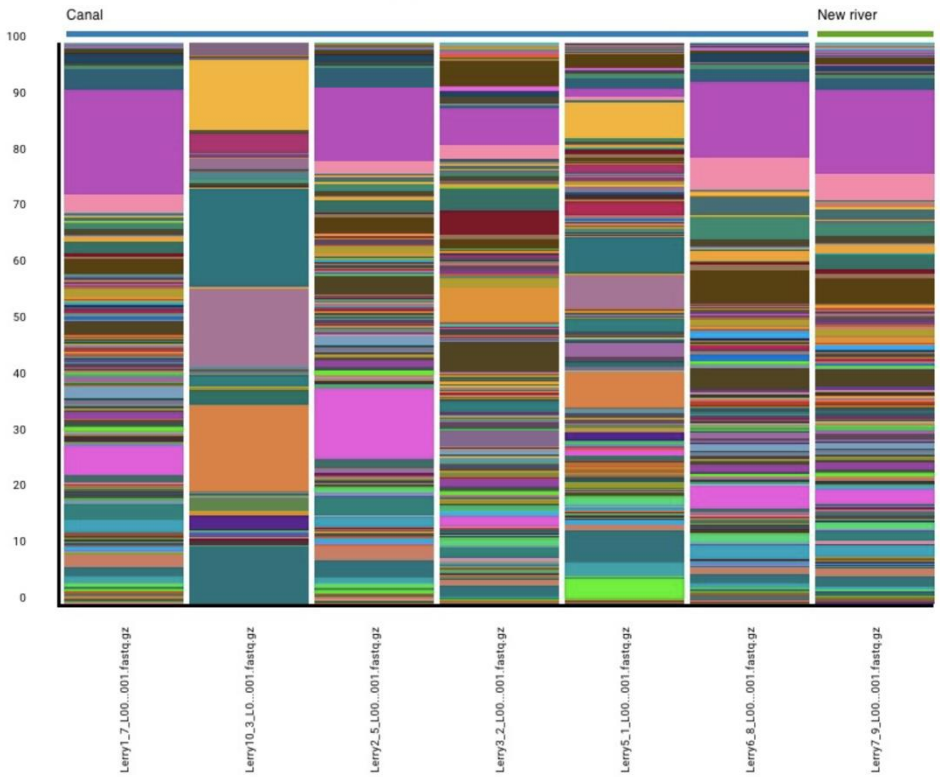

Figure 24: Stacked Bar Chart of Family Represented in Sediment Samples along the Himmarshee Canal and New River. Once again, the highest families are bacteria like HF183, *E. coli*, and other total coliforms in the families *Bacteroidaceae* and *Enterobacteriaceae*, respectively. See Figure 25. A-C, for the legend for this stacked bar chart. (Source: CosmosID Metagenomics Cloud).

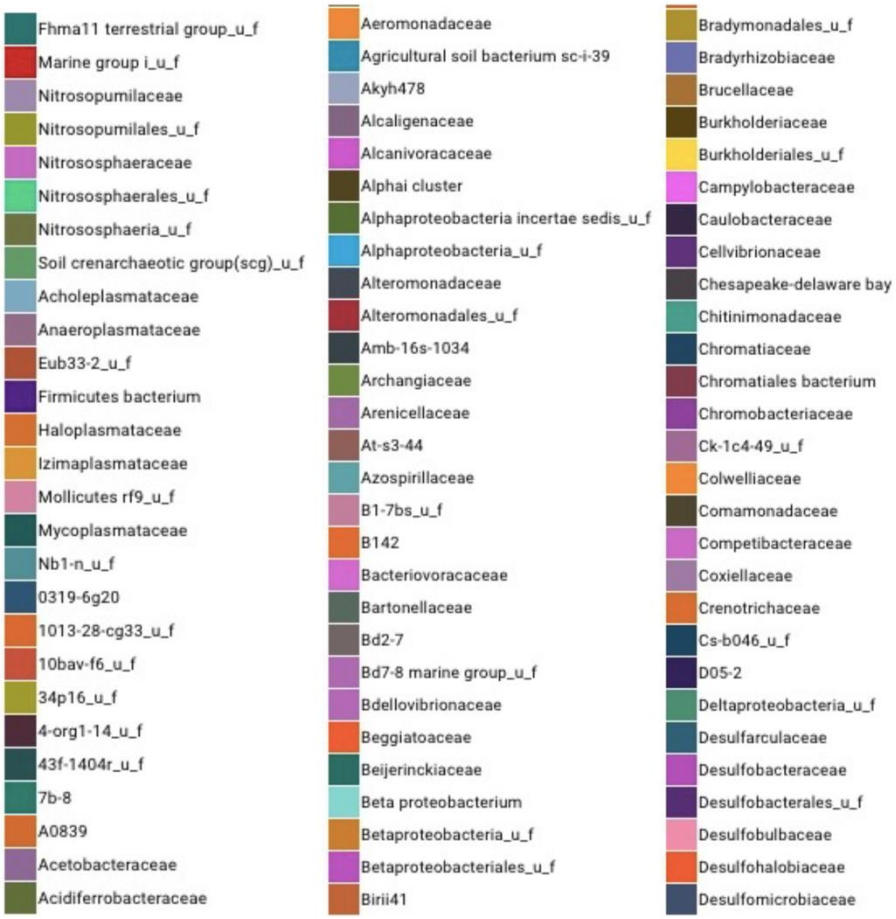

Figure 25. A: Key to Family of Bacteria Represented in Sediment Samples. (Source: CosmosID Metagenomics Cloud).

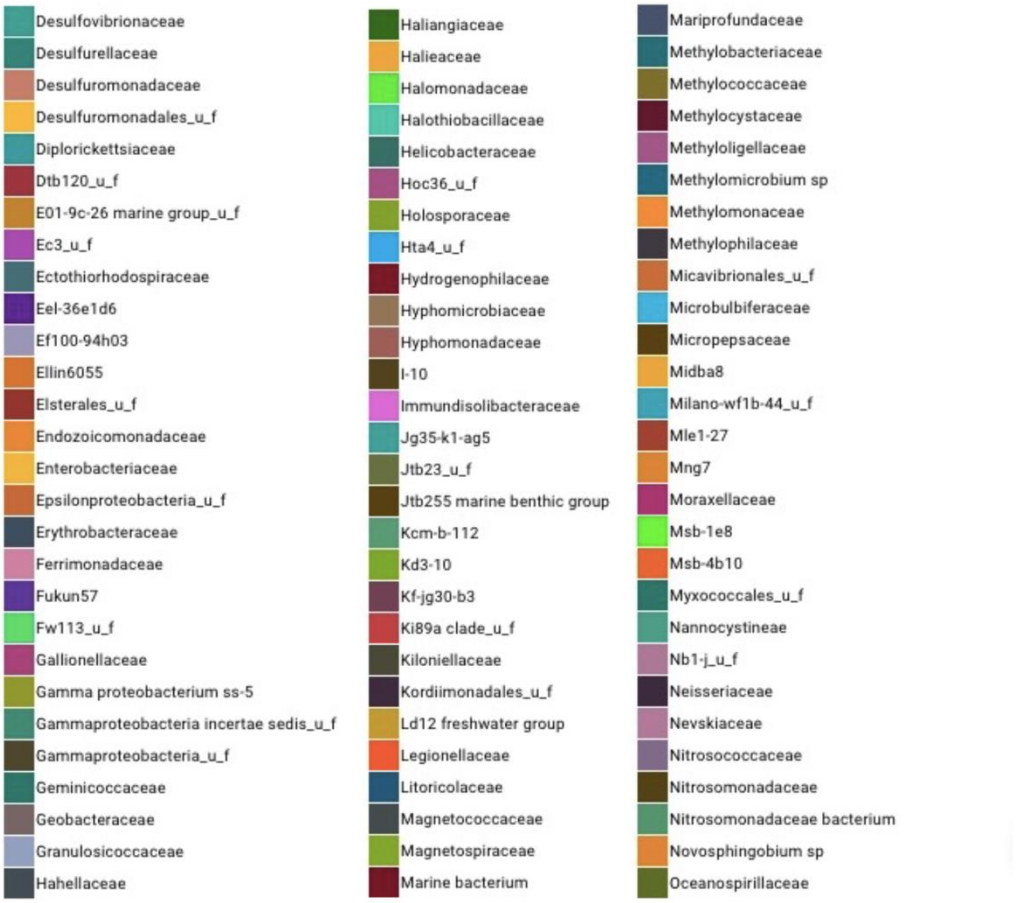

Figure 25. B: Key to Family of Bacteria Represented in Sediment Samples. (Source: CosmosID Metagenomics Cloud).

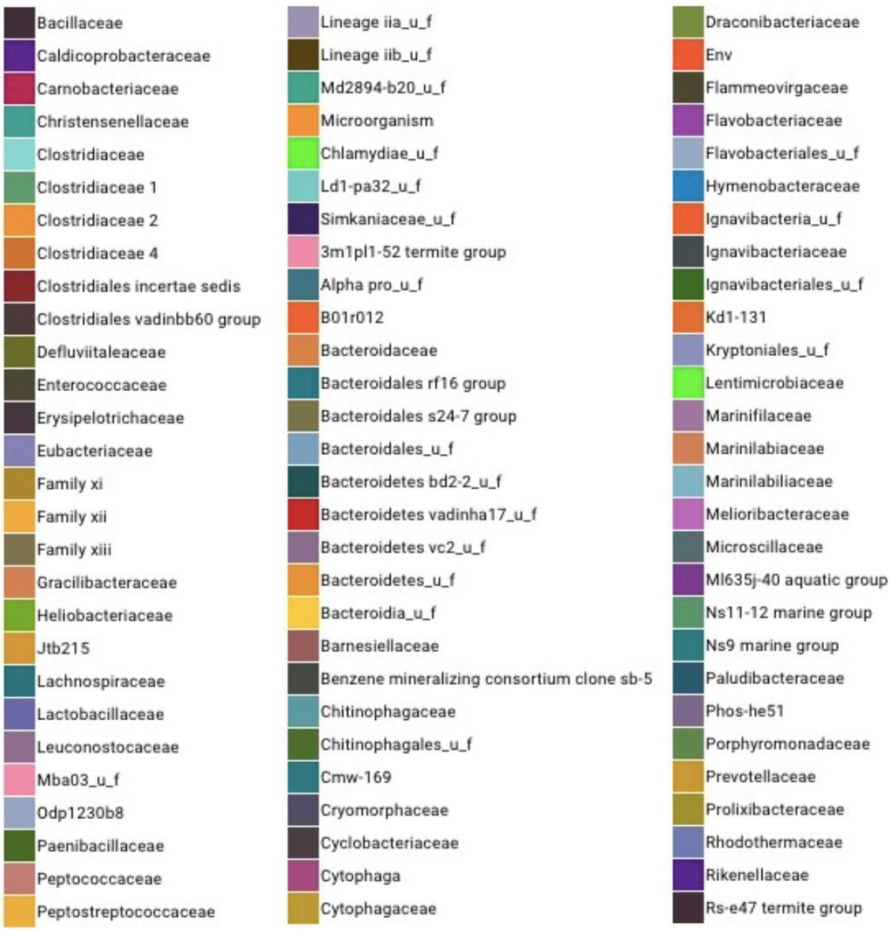

Figure 25. C: Key to Family of Bacteria Represented in Sediment Samples. (Source: CosmosID Metagenomics Cloud).

Researchers can properly distinguish whether these targeted bacteria are from humans or other sources, i.e., dogs, cats, ducks, etc., by using dPCR. dPCR uses nanoplate technology and targeted specific assays to quantify the targeted subject. A future study must use dPCR to distinguish possible sources of *Enterococcus* and total coliforms. By using that technique, it can be confirmed who is the source of the high-leveled enteric bacteria found in Himmarshee Canal. There are also many advantages to using dPCR over other PCR techniques.

Researchers argue that dPCR provides more accurate data since it has more resistance to inhibition, the ability to find low quantifications in a sample, and higher reproducibility than qPCR (Cao et al., 2015; Hindson et al., 2013; Strain et al., 2013). dPCR uses assays and probes to find biomarkers, which for fecal coliforms in humans would be HF183. It is important to note that the Broward County Monitoring Lab is already tracking HF183 levels; however, more sites must be added to track FIBs adequately. Broward County Monitoring Lab is using HF183 and IDEXX together. There is more recent data in July 2022, which showed high levels of *Enterococci* at Colee Hammock Park, as well as the beginning of the Himmarshee Canal with an MPN/100 ml of 2,420.00 (Broward County Environmental Monitoring Lab, 2022). A study mentioned earlier by Shahin et al., 2022, used the Colilert-18® IDEXX kit and dPCR together. They found that IDEXX resulted in low concentrations of *E. coli*; however, using dPCR along with IDEXX yielded better results showing high HF183 concentrations around densely populated areas (Shahin et al., 2022). dPCR has also been used in agricultural studies on food to find HF183, again due to its high reproducibility (Bartsch et al., 2018; Merino-Mascorro et al., 2018; Sun et al., 2019). These studies were done by Merino-Moascorro et al. (2018), Bartsch et al. (2018), and Sun et al. (2019) all used berries and strawberries, one used tomatoes as well, to track fecal contamination which used HF183 as a marker. A recent study conducted by FIU using strawberries found how sensitive dPCR can be with specific assays (Fernandez-Tejero et al., 2022). The study conducted by FIU reveals how well dPCR could work for Himmarshee Canal and other waterways plagued by fecal pollution.

In 2022, FIU conducted a set of tests on strawberries where a few members from NSU volunteered their resources to see how dPCR could be used in fecal detection. As stated before, the study found that dPCR is much more sensitive than qPCR, which required the researchers to dilute their samples before running them through the QIAcuity® Digital PCR System from Qiagen (Fernandez-Tejero et al., 2022). After each run, the machine outputted a 1-D scatterplot depicting

how many wells were positive for HF183 (Figure 26). The program uses Poisson distribution to calculate the quantity of the target. The sensitivity of dPCR would allow researchers to properly determine what species the fecal coliforms are coming from in the Himmarshee Canal and allow Broward County to find ways to prevent fecal contamination from getting out of hand. Broward County has already put in stipulations as of 2021, with protein skimmers at the beginning of the canal.

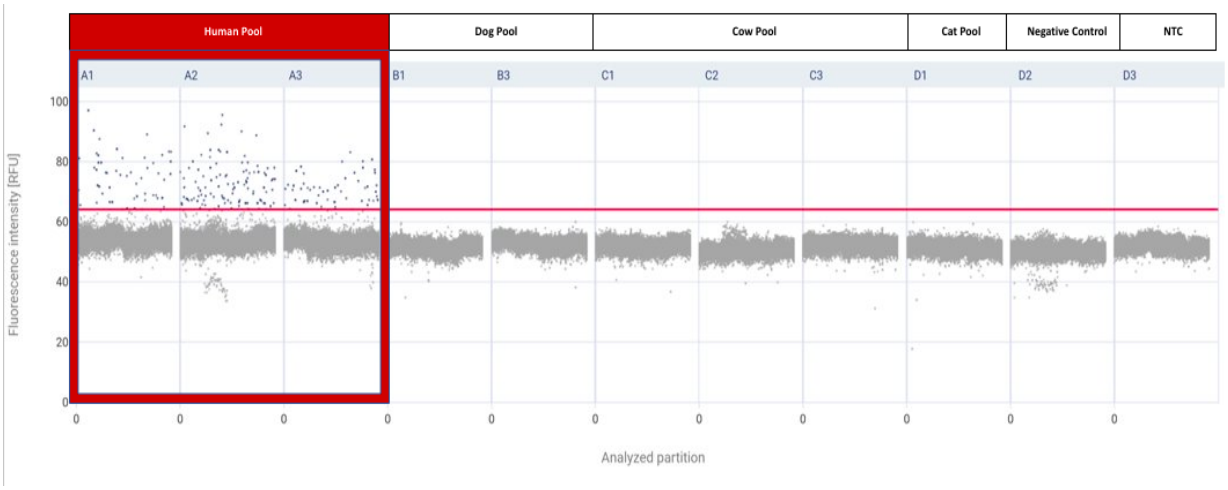

Figure 26: 1-D Scatterplot from the HF183 Assay. This figure depicts what data the QIAcuity® Digital PCR System from Qiagen outputs from each run. The data outlined in red are the human fecal samples used in the assay to see how well it picked up HF183. Some of the dog fecal samples contained HF183. However, this could be due to the sample being contaminated.

Broward County, in 2021, implemented protein skimmers in the Himmarshee Canal to help clean up the waterways due to fecal contamination. However, as the results of this study have shown, these protein skimmers are not enough to clean up the waters. Fecal contamination is still entering the water due to poor infrastructure and will worsen if something is not done. Protein skimmers are being used due to how well they work in aquarium settings. Protein skimmers remove organic matter, in this case, fecal material, from the water and replace it with nitrogenous waste (Rahman et al., 2012). A study was done in 2012, and after 87 days, researchers measured how much of a few abalones' feces were left in an enclosed system (Rahman et al., 2012). They found that the water quality was much better in the protein skimmer system than the one without

and noticed that the protein skimmer system had more dissolved oxygen. This study explains why Broward County decided to put protein skimmers into the Himmarshee Canal; however, more solutions need to be implemented so the system is not just working by itself and especially updating the infrastructure plagued by many pipe bursts, as mentioned before, and more recently, a major flood event.

On April 12th, 2023, Southern Florida suffered from over two feet of rainfall in less than 12 hours. Due to how quickly and fast the rain came down, large parts of the cities of Fort Lauderdale, Hollywood, and Dania Beach were under a couple of feet of water. This flooding overflowed septic tanks in many areas (Kearney, 2023). As a result, high levels of fecal bacteria were detected in those areas, including the Himmarshee Canal. These levels were 144 over the recommended EPA guidelines and progressively worsened over the days leading up to the flooding (Kearney, 2023). Miami Waterkeeper (MWK), a non-profit group (https://www.miamiwaterkeeper.org/), took measurements using the IDEXX Enterolert® test. They have it in their protocol that they resample the area when levels are over 70 (Kearney, 2023). Coincidentally on April 11, MWK tested the Himmarshee Canal and reported an MPN of 1,935 MPN/100 mL. Since this exceeded the recommended guidelines, the Miami Waterkeepers returned the next day to collect more samples which was the flash flood event. MWK suggested the high numbers were due to septic tank overflow rather than water draining from parks where there can be some canine fecal matter and possible human (Kearney, 2023). Other studies have shown that when *Enterococci* and other FIBs are present, it is a sign of possible septic tank failures (Ahmed et al., 2005). When these bacteria get into the water, they last for about two weeks, raising much concern when these areas are susceptible to heavy flooding and occur in populated areas (Kearney, 2023; Wickes, 2018). In 2018, Wickes found various species of bacteria that are naturally occurring in the South Florida area; the most abundant groups of bacteria were the family *Campylobacterales* and the genus *Bacillus* (Wickes, 2018). *Campylobacterales* do cause infectious diseases, as well as being able to thrive outside of the gut, as does *Bacillus*. This recent news, along with articles with news of South Florida's aging sewage pipe and frequent bursts (REF), and previous studies have shown that intercoastal canals and waterways are not improving. The community, and perhaps with the help of the federal government or other non-profits, should find a way to renovate the deteriorated infrastructure in these areas.

In conclusion, small waterways that board highly populated neighborhoods are susceptible to the pollution that stems from fecal pollution due to poor infrastructure, sewage runoff, and natural means. More studies need to be done to look into what techniques could be used to help clean up the waterways and continue to monitor them by any means necessary to flag any high levels of contamination. By continuing to monitor the waterways, especially after new treatments are implemented, the county can effectively screen for fecal contamination and thus implement whatever treatment works best to protect their local waters for public and marine health.

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APPENDIX

Appendix A: IDEXX Quanti-Tray®/2000 MPN Table

APPENDIX A: IDEXX QUANTI-TRAY®/2000 MPN TABLE

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																								
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
0	<1	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.1	15.1	16.1	17.1	18.1	19.1	20.2	21.2	22.2	23.3	24.3
1	1.0	2.0	3.0	4.0	5.0	6.0	7.1	8.1	9.1	10.1	11.1	12.1	13.2	14.2	15.2	16.2	17.3	18.3	19.3	20.4	21.4	22.4	23.5	24.5	25.6
2	2.0	3.0	4.1	5.1	6.1	7.1	8.1	9.2	10.2	11.2	12.2	13.3	14.3	15.4	16.4	17.4	18.5	19.5	20.6	21.6	22.7	23.7	24.8	25.8	26.9
3	3.1	4.1	5.1	6.1	7.2	8.2	9.2	10.3	11.3	12.4	13.4	14.5	15.5	16.5	17.6	18.6	19.7	20.8	21.8	22.9	23.9	25.0	26.1	27.1	28.2
4	4.1	5.2	6.2	7.2	8.3	9.3	10.4	11.4	12.5	13.5	14.6	15.6	16.7	17.8	18.8	19.9	21.0	22.0	23.1	24.2	25.3	26.3	27.4	28.5	29.6
5	5.2	6.3	7.3	8.4	9.4	10.5	11.5	12.6	13.7	14.7	15.8	16.9	17.9	19.0	20.1	21.2	22.2	23.3	24.4	25.5	26.6	27.7	28.8	29.9	31.0
6	6.3	7.4	8.4	9.5	10.6	11.6	12.7	13.8	14.9	16.0	17.0	18.1	19.2	20.3	21.4	22.5	23.6	24.7	25.8	26.9	28.0	29.1	30.2	31.3	32.4
7	7.5	8.5	9.6	10.7	11.8	12.8	13.9	15.0	16.1	17.2	18.3	19.4	20.5	21.6	22.7	23.8	24.9	26.0	27.1	28.3	29.4	30.5	31.6	32.8	33.9
8	8.6	9.7	10.8	11.9	13.0	14.1	15.2	16.3	17.4	18.5	19.6	20.7	21.8	22.9	24.1	25.2	26.3	27.4	28.6	29.7	30.8	32.0	33.1	34.3	35.4
9	9.8	10.9	12.0	13.1	14.2	15.3	16.4	17.6	18.7	19.8	20.9	22.0	23.2	24.3	25.4	26.6	27.7	28.9	30.0	31.2	32.3	33.5	34.6	35.8	37.0
10	11.0	12.1	13.2	14.4	15.5	16.6	17.7	18.9	20.0	21.1	22.3	23.4	24.6	25.7	26.9	28.0	29.2	30.3	31.5	32.7	33.8	35.0	36.2	37.4	38.6
11	12.2	13.4	14.5	15.6	16.8	17.9	19.1	20.2	21.4	22.5	23.7	24.8	26.0	27.2	28.3	29.5	30.7	31.9	33.0	34.2	35.4	36.6	37.8	39.0	40.2
12	13.5	14.6	15.8	16.9	18.1	19.3	20.4	21.6	22.8	23.9	25.1	26.3	27.5	28.6	29.8	31.0	32.2	33.4	34.6	35.8	37.0	38.2	39.5	40.7	41.9
13	14.8	16.0	17.1	18.3	19.5	20.6	21.8	23.0	24.2	25.4	26.6	27.8	29.0	30.2	31.4	32.6	33.8	35.0	36.2	37.5	38.7	39.9	41.2	42.4	43.6
14	16.1	17.3	18.5	19.7	20.9	22.1	23.3	24.5	25.7	26.9	28.1	29.3	30.5	31.7	33.0	34.2	35.4	36.7	37.9	39.1	40.4	41.6	42.9	44.2	45.4
15	17.5	18.7	19.9	21.1	22.3	23.5	24.7	25.9	27.2	28.4	29.6	30.9	32.1	33.3	34.6	35.8	37.1	38.4	39.6	40.9	42.2	43.4	44.7	46.0	47.3
16	18.9	20.1	21.3	22.6	23.8	25.0	26.2	27.5	28.7	30.0	31.2	32.5	33.7	35.0	36.3	37.5	38.8	40.1	41.4	42.7	44.0	45.3	46.6	47.9	49.2
17	20.3	21.6	22.8	24.1	25.3	26.6	27.8	29.1	30.3	31.6	32.9	34.1	35.4	36.7	38.0	39.3	40.6	41.9	43.2	44.5	45.9	47.2	48.5	49.8	51.2
18	21.8	23.1	24.3	25.6	26.9	28.1	29.4	30.7	32.0	33.3	34.6	35.9	37.2	38.5	39.8	41.1	42.4	43.8	45.1	46.5	47.8	49.2	50.5	51.9	53.2
19	23.3	24.6	25.9	27.2	28.5	29.8	31.1	32.4	33.7	35.0	36.3	37.6	39.0	40.3	41.6	43.0	44.3	45.7	47.1	48.4	49.8	51.2	52.6	54.0	55.4
20	24.9	26.2	27.5	28.8	30.1	31.5	32.8	34.1	35.4	36.8	38.1	39.5	40.8	42.2	43.6	44.9	46.3	47.7	49.1	50.5	51.9	53.3	54.7	56.1	57.6
21	26.5	27.9	29.2	30.5	31.8	33.2	34.5	35.9	37.3	38.6	40.0	41.4	42.8	44.1	45.5	46.9	48.4	49.8	51.2	52.6	54.1	55.5	56.9	58.4	59.9
22	28.2	29.5	30.9	32.3	33.6	35.0	36.4	37.7	39.1	40.5	41.9	43.3	44.8	46.2	47.6	49.0	50.5	51.9	53.4	54.8	56.3	57.8	59.3	60.8	62.3
23	29.9	31.3	32.7	34.1	35.5	36.8	38.3	39.7	41.1	42.5	43.9	45.4	46.8	48.3	49.7	51.2	52.7	54.2	55.6	57.1	58.6	60.2	61.7	63.2	64.7
24	31.7	33.1	34.5	35.9	37.3	38.8	40.2	41.7	43.1	44.6	46.0	47.5	49.0	50.5	52.0	53.5	55.0	56.5	58.0	59.5	61.1	62.6	64.2	65.8	67.3
25	33.6	35.0	36.4	37.9	39.3	40.8	42.2	43.7	45.2	46.7	48.2	49.7	51.2	52.7	54.3	55.8	57.3	58.9	60.5	62.0	63.6	65.2	66.8	68.4	70.0
26	35.5	36.9	38.4	39.9	41.4	42.8	44.3	45.9	47.4	48.9	50.4	52.0	53.5	55.1	56.7	58.2	59.8	61.4	63.0	64.7	66.3	67.9	69.6	71.2	72.9
27	37.4	38.9	40.4	42.0	43.5	45.0	46.5	48.1	49.6	51.2	52.8	54.4	56.0	57.6	59.2	60.8	62.4	64.1	65.7	67.4	69.1	70.8	72.5	74.2	75.9
28	39.5	41.0	42.6	44.1	45.7	47.3	48.8	50.4	52.0	53.6	55.2	56.9	58.5	60.2	61.8	63.5	65.2	66.9	68.6	70.3	72.0	73.7	75.5	77.3	79.0
29	41.7	43.2	44.8	46.4	48.0	49.6	51.2	52.8	54.5	56.1	57.8	59.5	61.2	62.9	64.6	66.3	68.0	69.8	71.5	73.3	75.1	76.9	78.7	80.5	82.4
30	43.9	45.5	47.1	48.7	50.4	52.0	53.7	55.4	57.1	58.8	60.5	62.2	64.0	65.7	67.5	69.3	71.0	72.9	74.7	76.5	78.3	80.2	82.1	84.0	85.9
31	46.2	47.9	49.5	51.2	52.9	54.6	56.3	58.1	59.8	61.6	63.3	65.1	66.9	68.7	70.5	72.4	74.2	76.1	78.0	79.9	81.8	83.7	85.7	87.6	89.6
32	48.7	50.4	52.1	53.8	55.6	57.3	59.1	60.9	62.7	64.5	66.3	68.2	70.0	71.9	73.8	75.7	77.6	79.5	81.5	83.5	85.4	87.5	89.5	91.5	93.6
33	51.2	53.0	54.8	56.5	58.3	60.2	62.0	63.8	65.7	67.6	69.5	71.4	73.3	75.2	77.2	79.2	81.2	83.2	85.2	87.3	89.3	91.4	93.6	95.7	97.8
34	53.9	55.7	57.6	59.4	61.3	63.1	65.0	67.0	68.9	70.8	72.8	74.8	76.8	78.8	80.8	82.9	85.0	87.1	89.2	91.4	93.5	95.7	97.9	100.2	102.4
35	56.8	58.6	60.5	62.4	64.4	66.3	68.3	70.3	72.3	74.3	76.3	78.4	80.5	82.6	84.7	86.9	89.1	91.3	93.5	95.7	98.0	100.3	102.6	105.0	107.3
36	59.8	61.7	63.7	65.7	67.7	69.7	71.7	73.8	75.9	78.0	80.1	82.3	84.5	86.7	88.9	91.2	93.5	95.8	98.1	100.5	102.9	105.3	107.7	110.2	112.7
37	62.9	65.0	67.0	69.1	71.2	73.3	75.4	77.6	79.8	82.0	84.2	86.5	88.8	91.1	93.4	95.8	98.2	100.6	103.1	105.6	108.1	110.7	113.3	115.9	118.6
38	66.3	68.4	70.6	72.7	74.9	77.1	79.4	81.6	83.9	86.2	88.6	91.0	93.4	95.8	98.3	100.8	103.4	105.9	108.6	111.2	113.9	116.6	119.4	122.2	125.0
39	70.0	72.2	74.4	76.7	78.9	81.3	83.6	86.0	88.4	90.9	93.4	95.9	98.4	101.0	103.6	106.3	109.0	111.8	114.6	117.4	120.3	123.2	126.1	129.2	132.2
40	73.8	76.2	78.5	80.9	83.3	85.7	88.2	90.8	93.3	95.9	98.5	101.2	103.9	106.7	109.5	112.4	115.3	118.2	121.2	124.3	127.4	130.5	133.7	137.0	140.3
41	78.0	80.5	83.0	85.5	88.0	90.6	93.3	95.9	98.7	101.4	104.3	107.1	110.0	113.0	116.0	119.1	122.2	125.4	128.7	132.0	135.4	138.8	142.3	145.9	149.5
42	82.6	85.2	87.8	90.5	93.2	96.0	98.8	101.7	104.6	107.6	110.6	113.7	116.9	120.1	123.4	126.7	130.1	133.6	137.2	140.8	144.5	148.3	152.2	156.1	160.2
43	87.6	90.4	93.2	96.0	99.0	101.9	105.0	108.1	111.2	114.5	117.8	121.1	124.6	128.1	131.7	135.4	139.1	143.0	147.0	151.0	155.2	159.4	163.8	168.2	172.8
44	93.1	96.1	99.1	102.2	105.4	108.6	111.9	115.3	118.7	122.3	125.9	129.6	133.4	137.4	141.4	145.5	149.7	154.1	158.5	163.1	167.9	172.7	177.7	182.9	188.2
45	99.3	102.5	105.8	109.2	112.6	116.2	119.8	123.6	127.4	131.4	135.4	139.6	143.9	148.3	152.9	157.6	162.4	167.4	172.6	178.0	183.5	189.2	195.1	201.2	207.5
46	106.3	109.8	113.4	117.2	121.0	125.0	129.1	133.3	137.6	142.1	146.7	151.5	156.5	161.6	167.0	172.5	178.2	184.2	190.4	196.8	203.5	210.5	217.8	225.4	233.3
47	114.3	118.3	122.4	126.6	130.9	135.4	140.1	145.0	150.0	155.3	160.7	166.4	172.3	178.5	185.0	191.8	198.9	206.4	214.2	222.4	231.0	240.0	249.5	259.5	270.0
48	123.9	128.4	133.1	137.9	143.0	148.3	153.9	159.7	165.8	172.2	178.9	186.0	193.5	201.4	209.8	218.7	228.2	238.2	248.9	260.3	272.3	285.1	298.7	313.0	328.2
49	135.5	140.8	146.4	152.3	158.5	165.0	172.0	179.3	187.2	195.6	204.6	214.3	224.7	235.9	248.1	261.3	275.5	290.9	307.6	325.5	344.8	365.4	387.3	410.6	435.2

09-63235-01

IDEXX Quanti-Tray®/2000 MPN Table (per 100ml)

# Large Wells Positive	# Small Wells Positive																							
	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
0	25.3	26.4	27.4	28.4	29.5	30.5	31.5	32.6	33.6	34.7	35.7	36.8	37.8	38.9	40.0	41.0	42.1	43.1	44.2	45.3	46.3	47.4	48.5	49.5
1	26.6	27.7	28.7	29.8	30.8	31.9	32.9	34.0	35.0	36.1	37.2	38.2	39.3	40.4	41.4	42.5	43.6	44.7	45.7	46.8	47.9	49.0	50.1	51.2
2	27.9	29.0	30.0	31.1	32.2	33.2	34.3	35.4	36.5	37.5	38.6	39.7	40.8	41.9	43.0	44.0	45.1	46.2	47.3	48.4	49.5	50.6	51.7	52.8
3	29.3	30.4	31.4	32.5	33.6	34.7	35.8	36.8	37.9	39.0	40.1	41.2	42.3	43.4	44.5	45.6	46.7	47.8	48.9	50.0	51.2	52.3	53.4	54.5
4	30.7	31.8	32.8	33.9	35.0	36.1	37.2	38.3	39.4	40.5	41.6	42.8	43.9	45.0	46.1	47.2	48.3	49.5	50.6	51.7	52.9	54.0	55.1	56.3
5	32.1	33.2	34.3	35.4	36.5	37.6	38.7	39.9	41.0	42.1	43.2	44.4	45.5	46.6	47.7	48.9	50.0	51.2	52.3	53.5	54.6	55.8	56.9	58.1
6	33.5	34.7	35.8	36.9	38.0	39.2	40.3	41.4	42.6	43.7	44.8	46.0	47.1	48.3	49.4	50.6	51.7	52.9	54.1	55.2	56.4	57.6	58.7	59.9
7	35.0	36.2	37.3	38.4	39.6	40.7	41.9	43.0	44.2	45.3	46.5	47.7	48.8	50.0	51.2	52.3	53.5	54.7	55.9	57.1	58.3	59.4	60.6	61.8
8	36.6	37.7	38.9	40.0	41.2	42.3	43.5	44.7	45.9	47.0	48.2	49.4	50.6	51.8	53.0	54.1	55.3	56.5	57.7	59.0	60.2	61.4	62.6	63.8
9	38.1	39.3	40.5	41.6	42.8	44.0	45.2	46.4	47.6	48.8	50.0	51.2	52.4	53.6	54.8	56.0	57.2	58.4	59.7	60.9	62.1	63.4	64.6	65.8
10	39.7	40.9	42.1	43.3	44.5	45.7	46.9	48.1	49.3	50.6	51.8	53.0	54.2	55.5	56.7	57.9	59.2	60.4	61.7	62.9	64.2	65.4	66.7	67.9
11	41.4	42.6	43.8	45.0	46.3	47.5	48.7	49.9	51.2	52.4	53.7	54.9	56.1	57.4	58.6	59.9	61.2	62.4	63.7	65.0	66.3	67.5	68.8	70.1
12	43.1	44.3	45.6	46.8	48.1	49.3	50.6	51.8	53.1	54.3	55.6	56.8	58.1	59.4	60.7	62.0	63.2	64.5	65.8	67.1	68.4	69.7	71.0	72.4
13	44.9	46.1	47.4	48.6	49.9	51.2	52.5	53.7	55.0	56.3	57.6	58.9	60.2	61.5	62.8	64.1	65.4	66.7	68.0	69.3	70.7	72.0	73.3	74.7
14	46.7	48.0	49.3	50.5	51.8	53.1	54.4	55.7	57.0	58.3	59.6	60.9	62.3	63.6	64.9	66.3	67.6	68.9	70.3	71.6	73.0	74.4	75.7	77.1
15	48.6	49.9	51.2	52.5	53.8	55.1	56.4	57.8	59.1	60.4	61.8	63.1	64.5	65.8	67.2	68.5	69.9	71.3	72.6	74.0	75.4	76.8	78.2	79.6
16	50.5	51.8	53.2	54.5	55.8	57.2	58.5	59.9	61.2	62.6	64.0	65.3	66.7	68.1	69.5	70.9	72.3	73.7	75.1	76.5	77.9	79.3	80.8	82.2
17	52.5	53.9	55.2	56.6	58.0	59.3	60.7	62.1	63.5	64.9	66.3	67.7	69.1	70.5	71.9	73.3	74.8	76.2	77.6	79.1	80.5	82.0	83.5	84.9
18	54.6	56.0	57.4	58.8	60.2	61.6	63.0	64.4	65.8	67.2	68.6	70.1	71.5	73.0	74.4	75.9	77.3	78.8	80.3	81.8	83.3	84.8	86.3	87.8
19	56.8	58.2	59.6	61.0	62.4	63.9	65.3	66.8	68.2	69.7	71.1	72.6	74.1	75.5	77.0	78.5	80.0	81.5	83.1	84.6	86.1	87.6	89.2	90.7
20	59.0	60.4	61.9	63.3	64.8	66.3	67.7	69.2	70.7	72.2	73.7	75.2	76.7	78.2	79.8	81.3	82.8	84.4	85.9	87.5	89.1	90.7	92.2	93.8
21	61.3	62.8	64.3	65.8	67.3	68.8	70.3	71.8	73.3	74.9	76.4	77.9	79.5	81.1	82.6	84.2	85.8	87.4	89.0	90.6	92.2	93.8	95.4	97.1
22	63.8	65.3	66.8	68.3	69.8	71.4	72.9	74.5	76.1	77.6	79.2	80.8	82.4	84.0	85.6	87.2	88.9	90.5	92.1	93.8	95.5	97.1	98.8	100.5
23	66.3	67.8	69.4	71.0	72.5	74.1	75.7	77.3	78.9	80.5	82.2	83.8	85.4	87.1	88.7	90.4	92.1	93.8	95.5	97.2	98.9	100.6	102.4	104.1
24	68.9	70.5	72.1	73.7	75.3	77.0	78.6	80.3	81.9	83.6	85.2	86.9	88.6	90.3	92.0	93.8	95.5	97.2	99.0	100.7	102.5	104.3	106.1	107.9
25	71.7	73.3	75.0	76.6	78.3	80.0	81.7	83.3	85.1	86.8	88.5	90.2	92.0	93.7	95.5	97.3	99.1	100.9	102.7	104.5	106.3	108.2	110.0	111.9
26	74.6	76.3	78.0	79.7	81.4	83.1	84.8	86.6	88.4	90.1	91.9	93.7	95.5	97.3	99.2	101.0	102.9	104.7	106.6	108.5	110.4	112.3	114.2	116.2
27	77.6	79.4	81.1	82.9	84.6	86.4	88.2	90.0	91.9	93.7	95.5	97.4	99.3	101.2	103.1	105.0	106.9	108.8	110.8	112.7	114.7	116.7	118.7	120.7
28	80.8	82.6	84.4	86.3	88.1	89.9	91.8	93.7	95.6	97.5	99.4	101.3	103.3	105.2	107.2	109.2	111.2	113.2	115.2	117.3	119.3	121.4	123.5	125.6
29	84.2	86.1	87.9	89.8	91.7	93.7	95.6	97.5	99.5	101.5	103.5	105.5	107.5	109.5	111.6	113.7	115.7	117.8	120.0	122.1	124.2	126.4	128.6	130.8
30	87.8	89.7	91.7	93.6	95.6	97.6	99.6	101.6	103.7	105.7	107.8	109.9	112.0	114.2	116.3	118.5	120.6	122.8	125.1	127.3	129.5	131.8	134.1	136.4
31	91.6	93.6	95.6	97.7	99.7	101.8	103.9	106.0	108.2	110.3	112.5	114.7	116.9	119.1	121.4	123.6	125.9	128.2	130.5	132.9	135.3	137.7	140.1	142.5
32	95.7	97.8	99.9	102.0	104.2	106.3	108.5	110.7	113.0	115.2	117.5	119.8	122.1	124.5	126.8	129.2	131.6	134.0	136.5	139.0	141.5	144.0	146.6	149.1
33	100.0	102.2	104.4	106.6	108.9	111.2	113.5	115.8	118.2	120.5	122.9	125.4	127.8	130.3	132.8	135.3	137.8	140.4	143.0	145.6	148.3	150.9	153.7	156.4
34	104.7	107.0	109.3	111.7	114.0	116.4	118.9	121.3	123.8	126.3	128.8	131.4	134.0	136.6	139.2	141.9	144.6	147.4	150.1	152.9	155.7	158.6	161.5	164.4
35	109.7	112.2	114.6	117.1	119.6	122.2	124.7	127.3	129.9	132.6	135.3	138.0	140.8	143.6	146.4	149.2	152.1	155.0	158.0	161.0	164.0	167.1	170.2	173.3
36	115.2	117.8	120.4	123.0	125.7	128.4	131.1	133.9	136.7	139.5	142.4	145.3	148.3	151.3	154.3	157.3	160.5	163.6	166.8	170.0	173.3	176.6	179.9	183.3
37	121.3	124.0	126.8	129.6	132.4	135.3	138.2	141.2	144.2	147.3	150.3	153.5	156.7	159.9	163.1	166.5	169.8	173.2	176.7	180.2	183.7	187.3	191.0	194.7
38	127.9	130.8	133.8	136.8	139.9	143.0	146.2	149.4	152.6	155.9	159.2	162.6	166.1	169.6	173.2	176.8	180.4	184.2	188.0	191.8	195.7	199.7	203.7	207.7
39	135.3	138.5	141.7	145.0	148.3	151.7	155.1	158.6	162.1	165.7	169.4	173.1	176.9	180.7	184.7	188.7	192.7	196.8	201.0	205.3	209.6	214.0	218.5	223.0
40	143.7	147.1	150.6	154.2	157.8	161.5	165.3	169.1	173.0	177.0	181.1	185.2	189.4	193.7	198.1	202.5	207.1	211.7	216.4	221.1	226.0	231.0	236.0	241.1
41	153.2	157.0	160.9	164.8	168.9	173.0	177.2	181.5	185.8	190.3	194.8	199.5	204.2	209.1	214.0	219.1	224.2	229.4	234.8	240.2	245.8	251.5	257.2	263.1
42	164.3	168.6	172.9	177.3	181.9	186.5	191.3	196.1	201.1	206.2	211.4	216.7	222.2	227.7	233.4	239.2	245.2	251.3	257.5	263.8	270.3	276.9	283.6	290.5
43	177.5	182.3	187.3	192.4	197.6	202.9	208.4	214.0	219.8	225.8	231.8	238.1	244.5	251.0	257.7	264.6	271.7	278.9	286.3	293.8	301.5	309.4	317.4	325.7
44	193.6	199.3	205.1	211.0	217.2	223.5	230.0	236.7	243.6	250.8	258.1	265.6	273.3	281.2	289.4	297.8	306.3	315.1	324.1	333.3	342.8	352.4	362.3	372.4
45	214.1	220.9	227.9	235.2	242.7	250.4	258.4	266.7	275.3	284.1	293.3	302.6	312.3	322.3	332.5	343.0	353.8	364.9	376.2	387.9	399.8	412.0	424.5	437.4
46	241.5	250.0	258.9	268.2	277.8	287.8	298.1	308.8	319.9	331.4	343.3	355.5	368.1	381.1	394.5	408.3	422.5	437.1	452.0	467.4	483.3	499.6	516.3	533.5
47	280.9	292.4	304.4	316.9	330.0	343.6	357.8	372.5	387.7	403.4	419.8	436.6	454.1	472.1	490.7	509.9	529.8	550.4	571.7	593.8	616.7	640.5	665.3	691.0
48	344.1	360.9	378.4	396.8	416.0	436.0	456.9	478.6	501.2	524.7	549.3	574.8	601.5	629.4	658.6	689.3	721.5	755.6	791.5	829.7	870.4	913.9	960.6	1011.2
49	461.1	488.4	517.2	547.5	579.4	613.1	648.8	686.7	727.0	770.1	816.4	866.4	920.8	980.4	1046.2	1119.9	1203.3	1299.7	1413.6	1553.1	1732.9	1986.3	2419.6	>2419.6

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