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Pilot Project for Future Fast and Accurate Detection of the Human Fecal Marker HF183 for Presence of Human Pollution in the New River

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NOVA SOUTHEATERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

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By

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Abstract

One type of pollution that has a negative impact on the environmental waters originates from fecal contamination. Identifying the source of pollution is an important step in effective resource management, mitigation, and reducing risk to human health. Microbial source tracking (MST) can be used to identify fecal pollution and to identify specific microbes in the environment and the quantitative polymerase chain reaction (qPCR) can be utilized in this analysis. In this pilot study the New River waterway, running through the City of Fort Lauderdale, was tested for a human specific bacteria *Bacteroids*, using the TaqMan HF183 qPCR assay (HF183/BFDrev). The water samples from six sites along the New River, South Fork New River, and North New River Canal were tested for the presence of HF183 from grab samples taken every other day for a 30-day period between March 11 through April 10th 2019. In this preliminary study, the quantity of HF183 was estimated to be greater than 100 targets/100mL, which is the risk threshold for human illness and indicates a need for further study of the New River waterways. It is recommended to take samples along the New River, South Fork of the New River and North New River Canal under various conditions such as at times of the year with different water temperatures, daylight versus evening along with wet versus dry conditions.

Key Words: fecal pollution, PCR, qPCR, HF183

Introduction

Several sources of pollution, such as ship pollution and land-based sources are causing ocean and coastal waters to deteriorate (EPA, 2017). Many environmental waters such as lakes, canals, and waterways are compromised by fecal pollution, which can originate from many sources such as wildlife, agricultural, natural, human activities, leaking sewer lines, faulty septic systems and combined sewer overflows (Cao et al, 2018; Wright, Solo-Gabriele, Elmir and Fleming, 2009; Soller, 2010b; Budowle, Schutzer and Morse, 2020). The use of fecal indicator bacteria (FIB) is a common method to detect this form of pollution, FIB consists of bacteria which indicate the potential presence or absence of disease-causing microbes. This can be useful in quantifying the amount of feces in the water but some FIBs can originate from a wide range of animals making it a poor choice when trying to determine the source (Boehm, 2018). It is important to understand where the source of pollution is originating from if possible.

Tracing human fecal material in bodies of water is classified in two general categories; point and non-point. Point sources are much easier to identify than nonpoint sources. Point sources can be localized to one particular or a series of outfalls such as effluent discharges from water treatment plants as well as many other sources like drain waters from the land. Non-point sources are much harder to pinpoint as they may be from many different origins such as rainfall, environmental events such as hurricanes and floods. It follows that because there are a large number of local and regional management systems in the South Florida area, the probability of pathogens from local septic tanks, sewage spills, wildlife and pet waste, as well as many other sources cannot not be localized to one specific area (Shanks, 2020).

The Broward County Florida area in particular has seen many spills in the last two years as reported by multi-fin.com as well as many news articles in the local newspaper The Sun Sentinel (Bryan, 2020). See Figure 1 below.

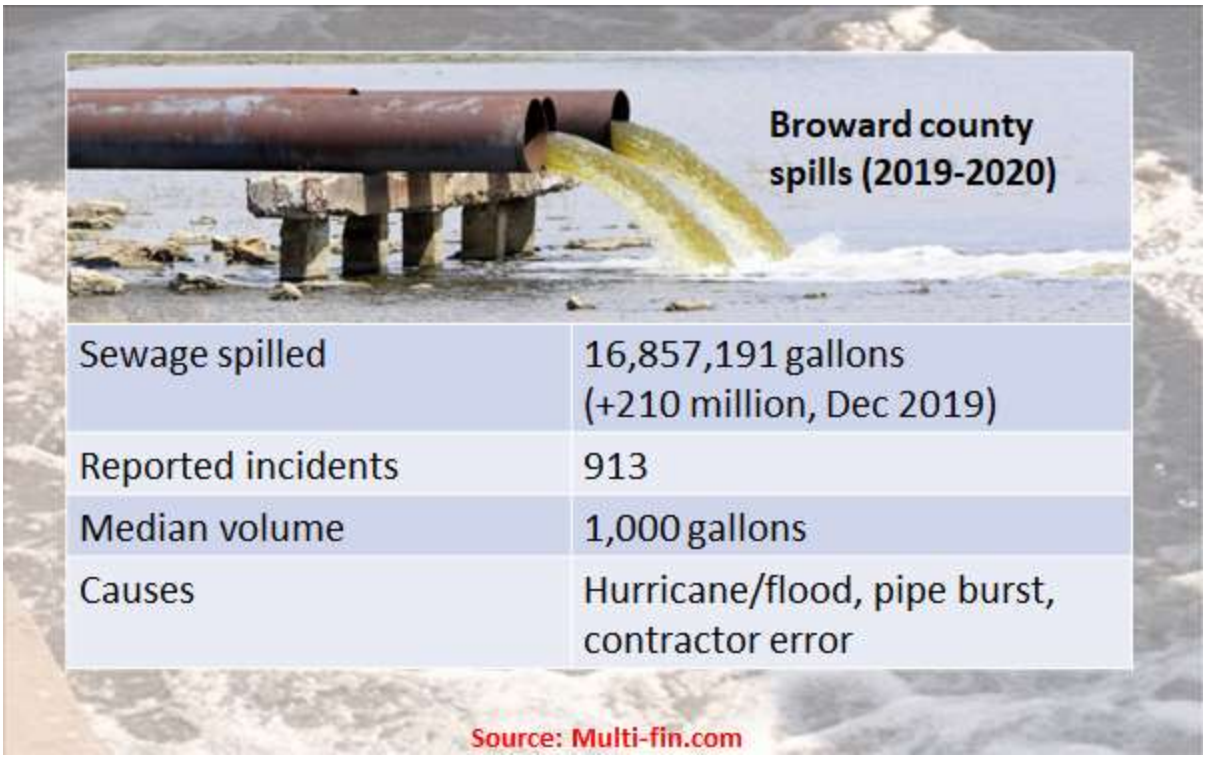

Figure 1: A two-year compilation of reported incidents of flooding, pipe breakage from various causes. (Dr. Eben Gehring, NSU, Personal communication with permission from the author).

Possible exposure to disease causing microorganisms is a major public health concern in waters that have fecal contamination (Napier, et al., 2017). Humans may come in contact with disease-causing microorganisms that can be found in recreational waters (Ahmed et al., 2018a). Water contamination with human feces is considered a greater risk to human health due to the potential of human-specific enteric pathogens (Scott, et al., 2002). Human polluted recreational water has the potential presence of pathogenic organisms such as bacteria, protozoa, helminths (Ahmed, Hughes & Harwood, 2016), waterborne diseases (Shanks et al, 2009) and viruses highly specific to humans, such as enteric viruses, (Soller, 2010a). The presence of human-specific enteric viruses represents an even greater risk to human health than animal feces contamination (Ahmed et al., 2018a). The high density and range of potential pathogenic microorganisms found in sewage is the reason why human fecal contamination is a greater health risk than non-human sources (Field, 2007). Diarrhea, abdominal pain, cramping, nausea and vomiting can be caused by fecal pathogens humans may have come in contact with (Ahmed et al., 2018a).

Recently the FIB used for the assessment of the safety of recreational waters in the State of Florida include fecal coliform, Escherichia coli, and enterococci. The issue is that these possible

pathogens are used for screening and are not specific for human feces (Soller et al 2010b). In the year 2000, the Beach Water Sampling Program was extended to 30 of Florida's coastal counties through state legislation, Senate Bill 1412 and House Bill 2145, as well as through funding. At this point in time sampling under this new program included fecal coliform as well as enterococci bacteria, (Florida Health, 2020).

Enterococci consist of enteric bacteria which usually occupy the intestinal tract of humans and animals as normal inhabitants of the gastrointestinal tract. Pollution due to animal and human pollution is not only a Florida problem but a nationwide issue. There are an estimated $1x10^9$ tons of fecal material produced in the U.S. each year and a small amount from humans. See Figure 2. (Shanks, 2014).

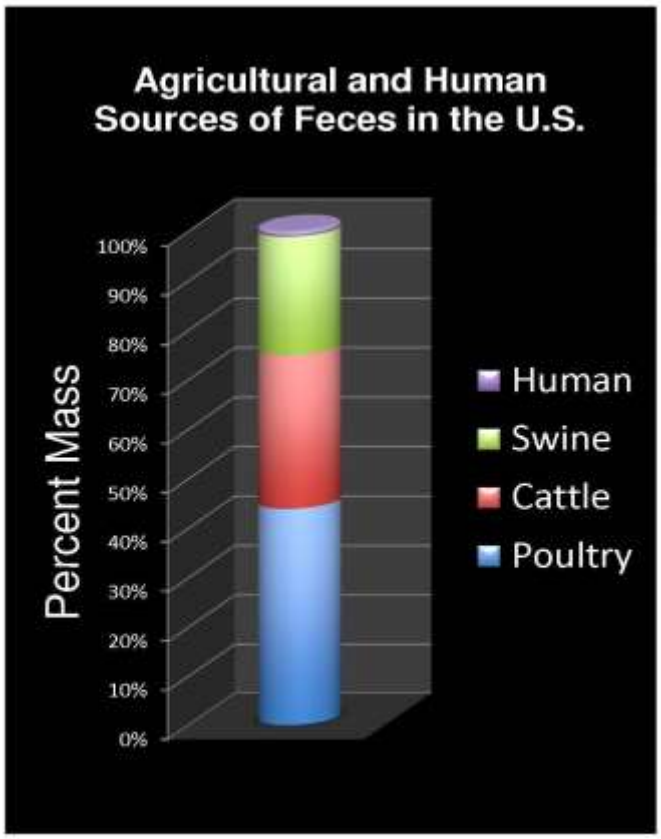

Figure 2: There are an estimated $1x10^9$ tons of fecal material produced in the U.S. each year. Humans produce a very small amount of material (0.01%) with Poultry, Cattle, Swine, and contributions from other agricultural animals and wildlife were not included in the figure (Shanks, 2014).

The present Florida testing program tests for enterococci by recommendation of the United States Environmental Protection Agency (EPA) as a saltwater quality indicator. The EPA states that enterococci have a greater correlation with swimming-associated gastrointestinal illness in both marine and fresh waters than other bacterial indicator organisms. Most other organisms are less likely to be killed in a saltwater environment. The EPA suggests if an enterococci (70 colony forming units per 100 milliliters) of water are sampled and a resampling exceeds this value, an Advisory would be issued for the sampling location (EPA, 2012). The EPA revised their 1986 standards for accessing water Quality and formed new criteria in 2012. These criteria are listed Table 1. They suggested Enterococci should be used as guidance in Table 1 for States and communities to follow. Enterococci should be used for both fresh salt water and E. coli for fresh water only (EPA, 2012).

Table 1: Recommendations from the EPA 2012. GM is the geometric mean and STV is the statistical threshold value. The exact definition is "the approximate 90th percentile of a water quality distribution not to be exceeded by more than 10% of all samples taken".

CRITERIA ELEMENTS	Recommendation 1 Estimated Illness Rate 36/1,000		Recommendation 2 Estimated Illness Rate 32/1,000	
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

Mitigation and reducing risk to human health is difficult when the source of contamination is unknown (Ahmed, Payyappat, Cassidy and Besley, 2019). The water quality safety of the systems used for recreation, along with drinking and seafood harvesting waters is important, as contamination can be a high risk to human health and can result in economic loss (Scott et al., 2002). In order to assess associated health risks and remedy these effects, the origin of fecal pollution is critical (Scott et al., 2002). Knowing the source of pollution is critical in effective resource management and in ultimately solving the problem of waterway contamination (Bernhard and Field, 2000a). The first step in initiating remediation efforts and reducing human health risks is identification of the fecal pollution source (Ahmed, Hughes & Harwood, 2016). Identification of the source of pollution is the first step in effective water management.

Microbial source tracking (MST) is one approach to identify fecal pollution. MST-methods are often based on different phenotypic and genotypic characteristics that differ between

microorganism populations (Seurinck et al, 2005). MST initially used library-dependent methods where isolation and typing from human and animal feces were used as a comparison to those found in the environmental water to identify the source of fecal pollution. Library-dependent methods are culture based; samples are taken from numerous fecal sources as well as from the water. Bacteria from both sets of samples are cultured. The bacteria cultures then are compared and matched to identify the source (Stoeckel, 2007). Now, host-specific genes or markers can be identified and quantified using PCR and qPCR, library-independent, techniques as a more rapid MST method (Ahmed, Hughes & Harwood, 2016). One such MST tool is using a host-specific fecal indicator bacteria (FIB). FIB uses microorganisms associated with the gastrointestinal tract found in specific animal groups to provide information on the potential source of fecal pollution (Ahmed, Hughes & Harwood, 2016). The FIB markers target genes of host-associated bacteria that are specific to the gastrointestinal tract of the host species and are common in the host species (Nguyen et al., 2018). General FIB cannot discriminate between humans and animals or between animal groups, making it difficult to determine the origin of the fecal pollution (Shanks, et al., 2016). Indicator organisms are useful because this eliminates the need to assay for every pathogen that might be present in the water (Scott et al., 2002).

Recently, the application of polymerase chain reaction (PCR) is being used to study specific microbes in environmental samples (Bej and Mahbubani, 1992). PCR is a common technique because it can be used to selectively target a human-associated gene and amplify trace quantities from polluted water samples (Shanks et al, 2010). In 1985, two research groups hypothesized that some specific culturable Bacteroides spp. could be associated with human fecal waste. This would mean that this bacterium could be used to distinguish human from non-human pollution sources (Allsop and Stickler, 1985; Fiksdal et al., 1985). The first qPCR-based HF183 method was published in 2005 by Seurinck (Seurinck et al, 2005).

Bernhard and Field (2000a) identified a human-specific gene cluster, notably a fecal marker from *Bacteroides-Prevotella* species that could be recovered from both freshwater and saltwater samples. This marked a landmark paper by identifying a culture independent strategy that may work for the identification of a human identification locus in the HF8 Bacteroides spp. 16s rRNA cluster. This meant that there might be specific Bacteroides that might be human specific. This of course needed much more research and validation. Later that same year Bernhard and Field (2000b) used fecal and water clone sequences to develop a cluster-specific primer that

can discriminate between human and ruminant feces. In a further study conducted by Seurinck et al (2005), a real-time PCR assay was developed using SYBR Green I to quantify the human-specific HF183 *Bacteroides* 16S rRNA genetic marker from human feces and freshwater samples. Recently, k (first order decay rate constants) values have been used in quantitative microbial risk assessments (QMRA) to simulate gastrointestinal illness risk associated with swimming in water with aged sewage contamination (Boehm, 2018). Sewage associated marker concentrations can translate into health risks (Ahmed et al., 2018a). PCR-based methods to detect genetic markers for fecal indicator bacteria is useful for rapid prediction of health risks that are associated with exposure to fecal pollution found in surface water where recreational activities occur (Haugland et al, 2010). QMRA uses human-associated fecal indicator HF183 as an index for sewage presence and thereby provides insight into how risk relates to HF183 concentrations in surface water (Boehm, 2018). This allows scientists to quantify and monitor how much fecal pollution is contaminating environmental waters.

As the MST techniques are growing and FIB has become better at discriminating between animal groups, there is a need for a mainstream water quality management protocol (Shanks et al., 2016). *Bacteroides* are useful due to their high abundance in feces and their low potential for re-growth in the environment (Haugland et al, 2010). Other advantages of *Bacteroides* is the short-term survival rate in water, exclusively to the gut of warm-blooded animals and constituents of a larger portion of fecal bacteria compared to another FIB used, such as *E. Coli* and *Enterococcus* spp (Ahmed, Hughes & Hayword, 2016). *Bacteroides* spp. is an anaerobic bacterium that is less likely to reproduce once it is introduced into the environment, making it a useful marker (Scott et al., 2002). The divergence and redundancy of the 16S rRNA sequences that occur between operons within the same genome allow for more specificity when detecting bacterial presence (Acinas, 2004). HF183 assays show high sensitivity in detecting samples that have a human origin and have low or undetectable cross-reactivity with feces from other animals (Napier, et al., 2017).

Ahmed, Goonetilleke, Powell, Chauhan, and Gardner conducted a study comparing process limit of detection (PLOD) values using human-specific *Bacteroides* HF183, *E. faecium esp*, adenoviruses and polyomaviruses assays to detect fresh sewage pollution in sewage spiked freshwater, seawater and distilled water samples from Austria; HF183 was found to be the most sensitive. A Florida study conducted by Staley, Gordon, Schoen, and Harwood, HF183 also demonstrated to be more sensitive than the human polyomaviruses (HPyVs) marker. Staley,

Gordon, Schoen, and Harwood determined PLOD values using HF183 in various surface water types to be generally sufficient to detect sewage in ambient waters at dilutions that indicate a potential human health risk based on the QMRA assumption.

The New River is a waterway that runs through the city of Fort Lauderdale and is the basis for this study. As per Rule 62-302.400 Classification of Surface Waters, Usage, Reclassification, Classified Waters in the Surface Water Quality Standards Chapter from the Florida Department of State, The New River is classified as a Class III waterbody. The New River is roughly 30 miles long, splitting into the North Fork and South Fork (Broward County Florida, 2018). See Figure 3). The aim of this study was to quantify and compare the levels of human fecal contamination among six sites along the New River, South Fork New River and North New River Canal, using the HF183 *Bacteroides* 16S rRNA genetic marker. A known HF183 sequence standard was tested by amplification and quantification of samples using a Qiagen Rotor-Gene Q. The Qiagen Rotor-Gene Q is a dedicated real-time PCR cycler (Qiagen, 2018). Once the tests were validated, the water samples from the six sites along the New River, South Fork New River and North New River Canal were tested for the presence of HF183. The samples at each site were compared to the standard sample of HF183 and r^2 values and qPCR efficiencies obtained.

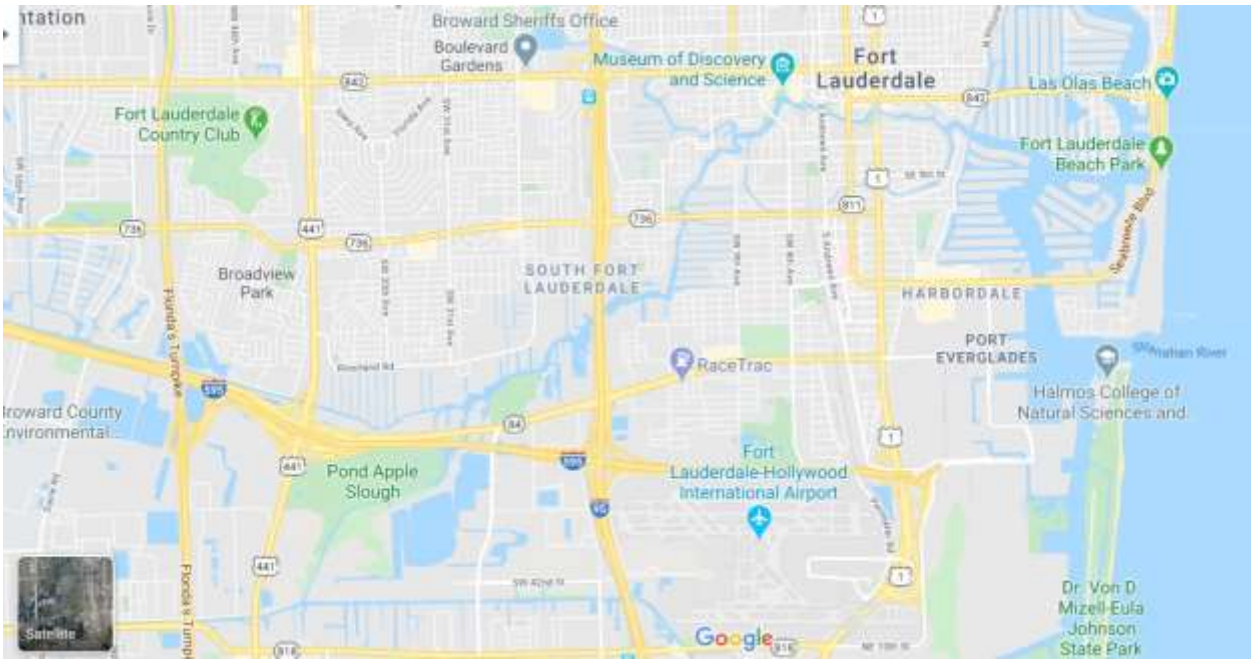

Figure 3: A capture of Google Maps (with permission) showing the mouth of the New River near Fort Lauderdale Beach Park and entering into a canal next to State Road 595 (South Fork).

Materials

Mastermix; Qiagen QuantiTect Probe PCR Kit cat. no. 204341 is a mastermix to be used in the qPCR process. The QuantiTect Probe PCR Kits is optimized, and a master mix for highly specific and sensitive real-time quantification of gDNA which has been designed for use with sequence-specific probes; in this case this dealt with the hydrolysis probe TaqMan®.
The primers were ordered from Sigma-Aldrich with the below sequences;
Forward primer-(HF-183): 5'- ATCATGAGTTCACATGTCCG -3'
Reverse primer-(HF-183)): 5'- CTTCCTCTCAGAACCCCTATCC -3'
The TaqMan probe was obtained from ThermoFisher
MGB. FAM-CTA ATG GAA CGC ATC CC-MGBNFQ
The standard HF-183 standard was from Invitrogen, GeneArt® Strings® DNA Fragments
Sense strand, 5' - 3' of 319 bp standard
CGTCAGGTTTGTTTCGGTATTGAGTATCGAAAATCTCACGGATTAACTCTTGTGTACG
CTCTCGAGGACCAGCTAATGCATATAAATAAGTTACGTGATGAGACCGGCCAACCT
GCCGTCTACTCTTGGCCAGCCTTCTGAAAGGAAGATTAATCCAGGATGGGATCATGA
GTTCACATGTCCGCATGATTAAAGGTATTTTCCGGTAGACGATGGGGATGCGTTCCA
TTAGCTCGAGATAGTAGGCGGGGTAACGGCCCACCTAGTCAACGATGGATAGGGGT
TCTGAGAGGAAGG

qPCR amplification

Amplification was performed in a Qiagen RotorGene Q (Qiagen). Reaction mixtures contained 1× TaqMan® Qiagen QuantiTect Probe PCR Master Mix, 0.2 mg/ml, 1M of each primer (except where, 80nM FAM® labeled TaqMan®probe (Applied Biosystems) and test sample DNA extracts (containing DNA from variable amounts of total DNA from river water samples) or 10 to 4×10^4 target gene copies (genomic)ds) in a total reaction volume of 25 uL (See Figure 4). Reaction mixtures were prepared in the same manner each run. Thermal cycling conditions were 2min at 95 ◦C, followed by 40 cycles of 5 s at 95 ◦C and 30 s at 60 ◦C. Data was analyzed by Qiagen on-board software. The threshold determination was automatically determined by the instrument software. Threshold cycle (CT) values were exported to Microsoft Excel for further analysis.

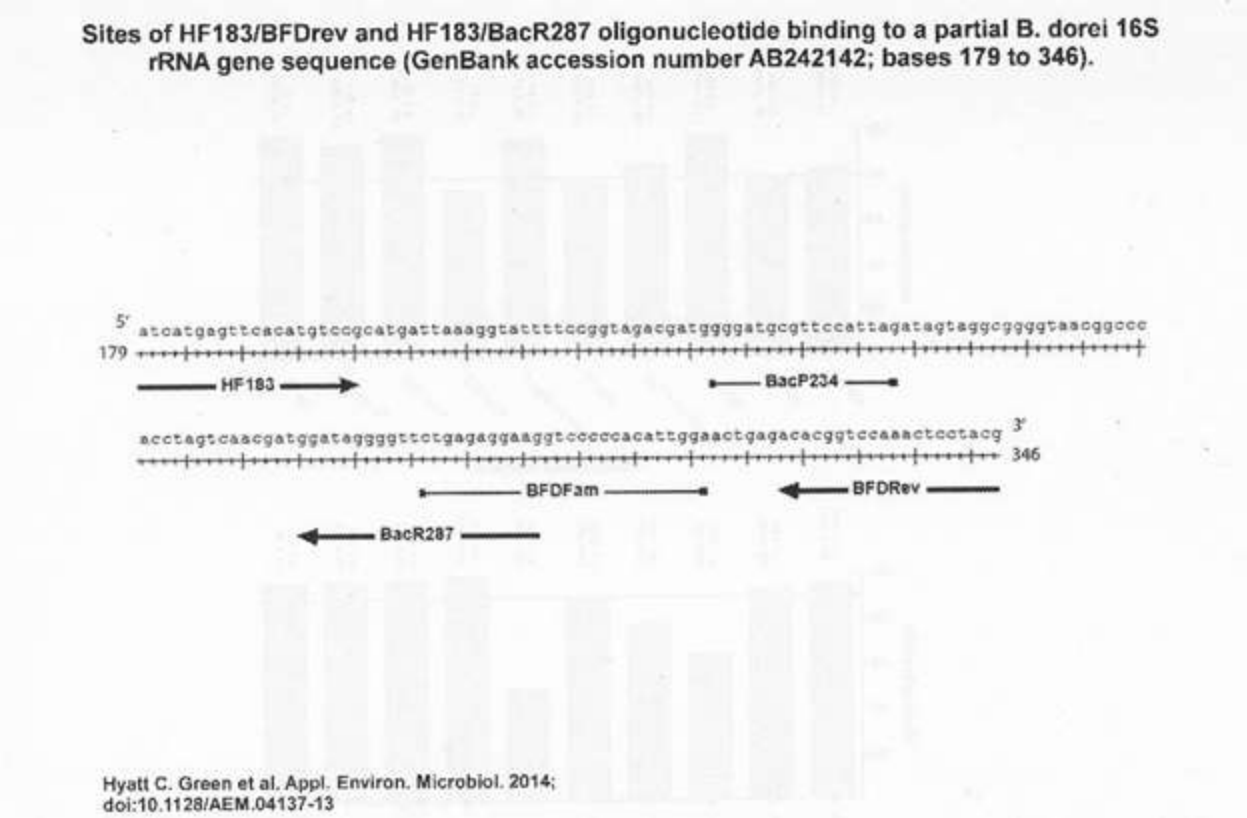

Figure 4: This represents the TaqMan HF183/BFDrev assay which highlights an improved assay as performed in this study. HF183 forward primer starts at the 5' end (forward arrow) and the reverse primer is at the 3' end with a reverse arrow (Green et al, 2014).

Methods for this pilot study

Grab samples were taken every other day for a 30-day period between March 11 through April 10th 2019. Samples were collected along the New River at the following locations; the beginning of the New River, the New River, New River fork, two sites along the South Fork New River and the North New River Canal. Water temperature and weather conditions (raining, cloudy, sunny) were taken at the time of sampling. The collection from land for each of these locations along with the corresponding site number and abbreviations used can be found in Table 2. Figure 5 through 10 shows the locations for each site. Figure 11 shows the location of all 6 sites with respect to one another.

All samples were taken at low tide. In an experiment conducted by Santoro and Boehm, it was found that FIB abundance was significantly affected by tide levels. It is recommended by Crim that in tidal areas water samples should be taken during slack tide conditions. Slack periods

occur during high or low tides (Leonardi, Kolker and Fagherazzi, 2015). Low tide was chosen over high tide because it occurs between the falling and rising tides where any microorganisms, including *Bacteroides* that may be towards to bottom can be collected, this allows the remaining particles not taken out with the tide to be sampled and there is less potential of dilution caused by runoff or recent rain. Another factor in the decision to sample at low tide comes from the study conducted by Santoro and Boehm where the effects of microbial pollution was studied in tidal variability. It was found that total and fecal coliform and enterococci were greater during low tide at several of their sample locations. Similarly, in a study by Johnston, Dorsey and Saez, higher than average concentrations of FIB were found during slack-water low periods and ebb flows compared to other times in the tidal cycle.

The low tide predictions were obtained from the NOAA website for 8722937, Ft. Lauderdale, Andrews Avenue Bridge, FL. This is the only location along the New River where predictions were available. The collection time was the same for all six sites and was taken as close to the low tide predictions as possible. There were two low tides per day, the low tide time used was the time that occurred during the hours of operations for all of the Parks where the samples were being collected. Table 3 contains the low tide prediction used for the sample collection time frame and the predicted tide heights generated relative to the standard tidal or geodetic reference datum. Table 4 contains the hours of operation for all the Parks used for sampling.

Volunteers to aid in samples collection were solicited from the Oceanographic Center graduate distribution list (OCEANSTUDENTS@list.acast.nova.edu), the marine biology and environmental science undergraduate distribution list (UG-OceanStudents@nova.edu), along with non-student volunteers. The participating sample collectors were: Aubrey Anthony, Miranda Brahman, Cynthia Cleveland, Caileigh Craddock, Dr. George Duncan, Ron Honse, John Leon, Skylar Muller and myself, Angie Louis.

Volunteers for sites 1, 2, 3, 4 and 6 were given a collection pole with the sterilized collection sample bottle and thermometer attached, a screwdriver, a cooler bag, several ice packs and a data collection sheet. The collection pole consisted of a PVC pole cut to the length needed for each of the five sites, two stainless steel adjustable clamps used to attach the sterilized collection sample bottle and a Marina Aquarium Floating Thermometer. The collection pole sizes are in Table 5. Volunteers for site 5 were given the collection sample bottle, a thermometer, a

cooler bag, several ice packs and a data collection sheet. The data collection sheet had the following information to fill out: Name of Collector, Site, Time, Weather Conditions (raining/overcast/sunny) and Water Temperature. The quantity of ice packs were two ice packs per collection sample bottle.

The collection sample bottles were sterilized by rinsing the bottles with 10% hydrochloric acid (HCL), rinsing 3 times using deionized (DI) water and then a final rinse with either 95% or 100% ethanol.

Volunteers for all six sites were told to take samples from just below the water surface, slightly deeper when it was raining during the time of collection or when collections occurred after it had been raining prior to collection. Water samples could not be taken 1 ft below the water surface for all sites, which is why just below the water surface was used for all sites. Volunteers for sites 1, 2, 3, 4 and 6 were told to take the sample by pushing the pole with the collection sample bottle directly into the water until the collection bottle was completely submerged vertically just below the water surface. Once the collection bottle was completely submerged and filled to the top, the collection pole was taken out of the water, the collection bottle was capped, the adjustable clamps were then unscrewed and the collected sample was placed into the cooler bag with ice packs. Once the sample was cooling, the volunteer then stuck the collection pole back into the water where the sample was taken for a few minutes to get an accurate reading on the thermometer. Volunteers were told to mark the temperate as either degrees Fahrenheit or Celsius.

Volunteers for site 5 were told to collect the water sample by entering the water to the point where it was deep enough to be able to get the water sample bottle just below. They were told to dip the collection bottle into the water until it was completely submerged, then dump the water out of the collection bottle, then completely submerge the collection bottle again and dump the water a second time. This was done to rinse the collection bottle with sample water prior to collection. Once the collection sample bottle was rinsed twice, the third submersion of the collection bottle was used as the collected sample. Once the sample had been taken, the collection bottle was capped and the collected sample was placed into the cooler bag with ice packs. The volunteer went back into the water where the sample was taken to place the thermometer in the water for a few minutes to get an accurate reading on the thermometer. Table 6 contains the sample collection date, location, collector, collection time, weather conditions and water temperature.

Once all the samples had been collected for the day, the water samples were kept cool until they could be filtered. Filtration occurred within 24 hours of collection. The holding time of ambient water samples should be within 24 hours of sampling. Although the microbiological samples should be processed as soon as possible to avoid unpredictable changes in the sample (Aulenbach 2010). This is consistent with the Florida Department of Environmental Protections Standard Operating Procedure (SOP) FS 1000 General Sampling Procedures and was the maximum holding time for this study.

Filtration was completed using a 300ml magnetic filter funnel, sterilized in an autoclave prior to use, attached to PVC pipes hooked up to two filtering flasks and a vacuum pump, see Figure 12. 0.45 μm, 47mm diameter sterile Nitrocellulose (NC) Membrane black and white grid disk filters were used to collect bacteria from the water samples. In a study done by Ahmed, Goonetilleke, Powell, Chauhan and Gardner where real-time PCR minimum detection limits of human-specific *Bacteroides* HF183, *E. faecium esp*, adenoviruses and polyomaviruses assays to detect fresh sewage pollution in sewage spiked freshwater, seawater and distilled water samples were evaluated, each sewage spiked water sample was filtered through a 0.45 μm pore size membrane. A 0.45 μm pore size filter was also used in a study done by Jiang et al where a field ready human fecal diagnostic based on a LAMP-OSD assay was designed to target the same *Bacteroides* sequence cluster that the HF183 TaqMan qPCR targets. This is consistent with the Florida Department of Environmental Protection SOP PCR-4.0- 1.3 Preparation of samples for qPCR analysis and was the filter size used in this study.

Sterilized forceps, sterilized using either 95% or 100% ethanol, were used to place the filter onto the filter funnel. Once the vacuum and filtering apparatus was set up, the samples were poured into the filter until the water level reached 300ml and the vacuum was turned on. The vacuum was set to 10psi. A total of 1000ml of water was collected for each sample taken and two filters were utilized to filter approximately 500ml of water samples per filter. Filters that had less than 500ml of sample water run through it were marked and the reason was noted. In between samples, the funnel was cleaned with ultrapure (type 1) water. Once the samples were filtered, the filters were removed using sterilized forceps and placed into a labeled sterile 1.5ml centrifuge tube for storage. Samples were stored in a -80°C freezer. Samples A was stored in Dr. Duncan's lab freezer and samples B were stored in Dr. Lopez's lab freezer. Table 7 contains the collection date, location, filtration start time, volume filtered for sample A and B referred to as Filter A and Filter

B, holding time in hour and minutes between collection time and filtration start time and any notes taken will water samples were being filtered. Symbol ~ indicates approximate amounts and + indicates slightly above the filtration line as filter volumes were in 50ml increments.

DNA extraction was conducted using the DNeasy PowerSoil Kit Procedure provided with the DNeasy PowerSoil Kit. All Solutions and tubes for the extraction process were provided in the DNeasy PowerSoil Kit, Solutions were labeled C1 through C6. In a study conducted by Staley, Reckhow, Lukasik and Harwood, DNA was extracted from water samples using the MoBio PowerSoil DNA kit and was carried out following the manufacturer's instructions. The ThermoFisher Scientific DNeasy PowerSoil Kit was used to extract DNA from a membrane, according to the protocol provided by the manufacturer, for sewage samples collected in a study from Ahmed et al in 2018a.

Half a filter from Filter A was used during the extraction process. Sterilized forceps and sterilized scissors, sterilized using 99% isopropyl alcohol, were used. The sample filter was taken out of the 1.5ml centrifuge tube and unfolded using two sterilized forceps. Once unfolded, the sample filter was then cut down the center using the sterilized scissors. One half of the sample filter was placed back into the 1.5ml centrifuge tube and the other sample filter half was placed in a PowerBead Tube. The sample filter in the 1.5ml centrifuge tube was placed back in the -80°C freezer. DNA extraction was conducted with the sample filter in the PowerBead Tube.

The sample PowerBead Tube was gently vortexed for 5 seconds to mix. 60µl of Solution C1 was added and the PowerBead Tube was inverted 3 times then vortexed for 5 seconds. The Mo BIO PowerLyzer 24 was used in place of the Vortex Adapter, as both instruments are used to mix centrifuge tubes. The PowerBead Tubes was run using Program 1, this runs for 45 secs at 20°C for 1 cycle at 4000 min^{-1}. The sample PowerBead Tube was then placed in the Centrifuge and run at 10,000xg for 30 seconds. The Eppendorf Centrifuge 5414 was used and the units for this instrument are revolutions per minute. As per the Eppendorf Micro Centrifuge 5415 C Instruction Manual the conversion from centrifuge g-force (xg) and revolutions per minute (min^{-1}) is between 11,000 and 12,000 min^{-1}, 11,000 min^{-1} was used as the equivalent to 10,000xg. 11,000 min^{-1} will be the units used for the remainder of this paper. Approximately 500µl of supernatant was transferred over to a clean 2ml collection tube. 250µl of Solution C2 was added, vortex for 5 seconds and put in a 4°C refrigerator to incubate for 5 minutes. The supernatant 2ml collection tube was placed in the Centrifuge and ran at 11,000 min^{-1} for 1 minute. 600µl

Supernatant was transferred to a clean 2ml collection tube. 200µl of Solution C3 was added, vortex for 5 seconds and put in a 4°C refrigerator to incubate for 5 minutes. The supernatant 2ml collection tube was placed in the Centrifuge and ran at 11,000 min^{-1} for 1 minute. 750µl supernatant was transferred to a clean 2ml collection tube. Solution C4 was shaken to mix, 1200µl of C4 was added to the supernatant and vortex for 5 seconds. Between 650µl and 600µl of supernatant was added to an MB Spin Column and centrifuge at 11,000 min^{-1} for 1 minute. The flow through was discarded and for a second time between 650µl and 600µl of supernatant was added to an MB Spin Column and centrifuge at 11,000 min^{-1} for 1 minute. The flow through was discarded and the remaining supernatant was added to the MB Spin Column and centrifuge at 11,000 min^{-1} for 1 minute. The flow through was discarded, 500µl of Solution C5 was added and Centrifuge at 11,000 min^{-1} for 30 seconds. The flow through was discarded, and centrifuge again at 11,000 min^{-1} for 1 minute. The MB Spin Column was wiped with a Kimwipe and transferred to a LoBind 1.5ml tube. 100µl of Solution C6 was added to the center of the white filter membrane and Centrifuge at 11,000 min^{-1} for 30 seconds. The MB Spin Column was discarded and the LoBind 1.5ml tube, containing the extracted DNA, was placed into a -20°C freezer. Table 8 contains the collection date, location, DNA extraction date and any notes taken will extraction was conducted.

All the samples for March, Table 9, were given to the Broward County Environmental Lab to conduct PCR analyzes to be run following the Primer/Probe Mix Preparation, cycling condition and Simplex Mastermix reaction from The California Microbial Source Identification Manual: A Tiered Approach to Identifying Fecal Pollution Sources to Beaches from the Southern California Coastal Water Research Project (SCCWRP). National Oceanic and Atmospheric Administration protocol was taken into account for the Simplex Mastermix reaction.

The samples that were not run at the Broward County Environmental Lab, Table 10, along with a second run for each sample was conducted at the Schure building at the Nova Southeastern Oceanographic Center in Dr. George Duncan's lab.

The qPCR (Quantitative Polymerase Chain Reaction) is a procedure by which the amount of DNA amplified in each PCR cycle is measured using specific fluorescent dyes to determine the original amount of DNA in a sample. The target sequence is the sequence that is amplified by the primers and a specific probe which anneals to the gene sequence in each PCR cycle. This procedure is known as a TaqMan assay.

The most important part of qPCR is the creation of a quantitation standard curve. This was performed by a serial dilution of known standards which are provided with known concentrations. This calibration curve was constructed by running the serial dilution of the standards. On the Qiagen Rotorgene Q, the qPCR instrument plotted a graph with the log of the known concentrations on the x-axis (abscissa) and the threshold cycle (Ct) value of each dilution on the y-axis (ordinate) to create a standard curve after the run. The standard curve was used to determine the concentrations of unknown samples and the performance of the reaction using several parameters including the slope and r squared values (r^2) of the standards on the graph.

One of the many important aspects of the qPCR analysis is the efficiency of PCR reaction. The efficiency essentially tells us if the reaction has been the precise estimation of PCR efficiency: (1) one robust standard curve with at least 3–4 qPCR replicates at each concentration shall be generated, (2) the efficiency is instrument dependent, but reproducibly stable on one platform, and (3) using a larger volume when constructing serial dilution series reduces sampling error and enables calibration across a wider dynamic range. Under ideal conditions the efficiency (E) of a PCR should be 100%. This translates to for each cycle the amount of product doubles (E=2). This efficiency is calculated from the slope(s) of the standard curve according to the following formulas: E = 10(-1/slope)-1 Log E = (-1/slope)log 10 – log 1 Log 2 = (-1/slope) x1 – 0 (because E=2, log 1= 0 and log10 = 1) Slope= -1/log2 (after multiplying both sides by (slope/log2) Slope = -3.32 For an efficiency of 100%, the slope is -3.32. A good reaction should have an efficiency between 90% and 110%, which corresponds to a slope between -3.58 and -3.10 (Bustin et al, 2009).

Table 2: Collection Sites

Table 2			
Location along the New River	**Collection location from land**	**Site Number**	**Abbreviation**
Beginning of the New River	Idlewyld Dr.	1	I
New River	Colee Hammock Park	2	CH
New River Fork	Cooley's Landing Park	3	CL
South Fork New River	Lewis Landing Park	4	LL
South Fork New River	Bill Keith Preserve Park	5	BKP
North New River Canal	N side of 84, W of S Pine Island Rd	6	84

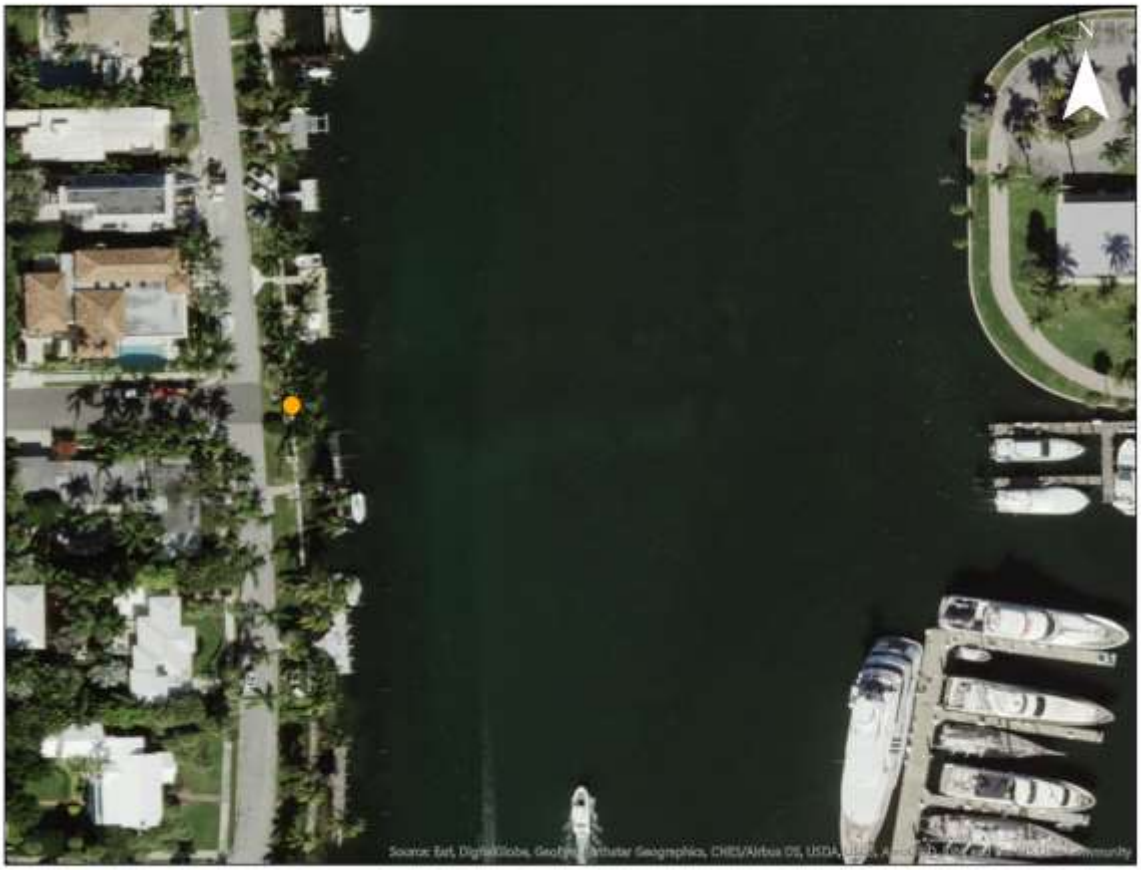

Figure 5: The beginning of the New River. Location of collection off of Idlewyld Dr.

Figure 6: The New River. Location of collection at Colee Hammock Park.

Figure 7: New River Fork. Location of collection at Cooley's Landing Park.

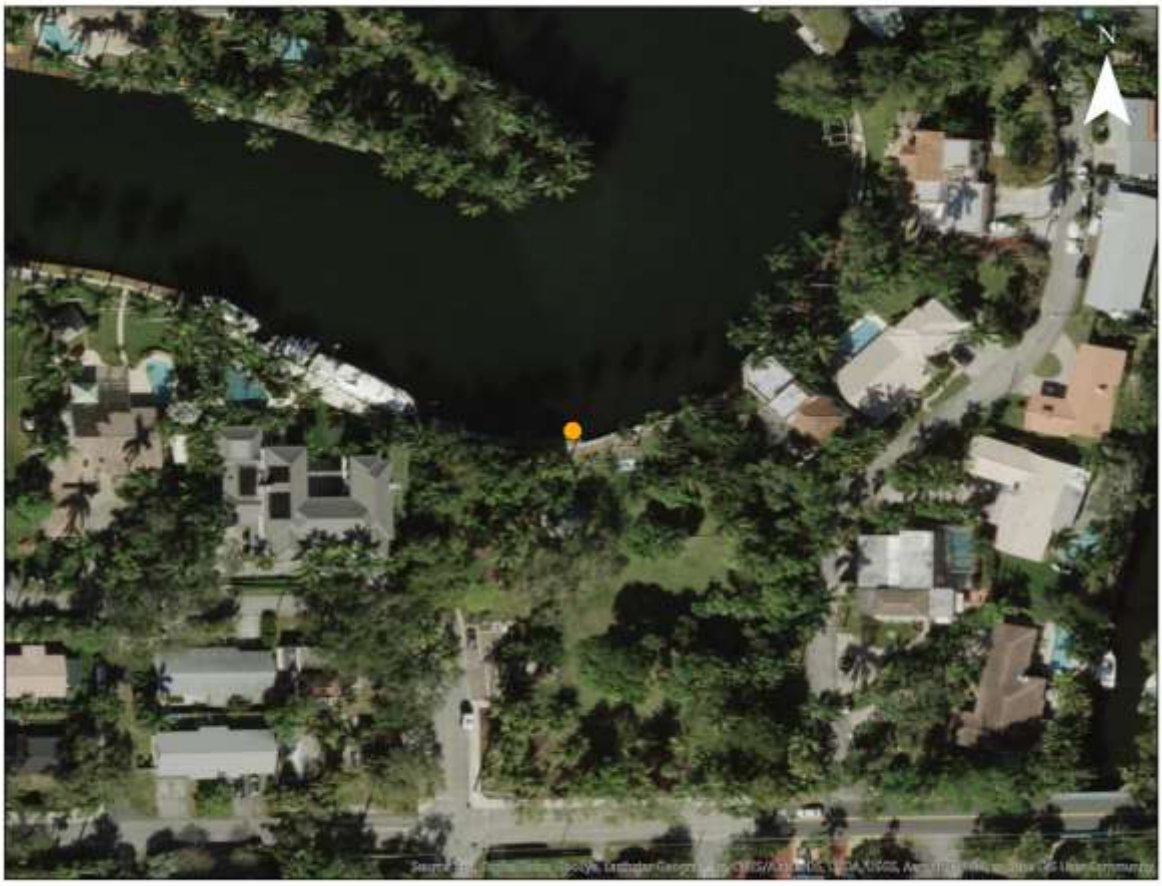

Figure 8: South Fork New River. Location of collection at Lewis Landing Park.

Figure 9: South Fork New River. Location of collection at Bill Keith Preserve Park.

Figure 10: North New River Canal. Location of collection on the north side of 84, west of South Pine Island Rd.

Figure 11: Sites 1 through 6 locations.

Table 3: Low Tide predictions from NOAA website

Table 3				
Date	**Day of the Week**	**Time (LST/LDT)**	**Predicted (cm)**	**Low Tide**
3/11/2019	Mon	7:06 PM	-3	L
3/13/2019	Wed	8:25 AM	9	L
3/15/2019	Fri	10:39 AM	9	L
3/17/2019	Sun	12:51 PM	0	L
3/19/2019	Tue	2:42 PM	-12	L
3/21/2019	Thu	4:22 PM	-21	L
3/23/2019	Sat	5:58 PM	-18	L

Table 3				
Date	**Day of the Week**	**Time (LST/LDT)**	**Predicted (cm)**	**Low Tide**
3/25/2019	Mon	7:38 PM	-9	L
3/27/2019	Wed	9:06 AM	12	L
3/29/2019	Fri	11:10 AM	15	L
3/31/2019	Sun	1:01 PM	12	L
4/2/2019	Tue	2:27 PM	6	L
4/4/2019	Thu	3:38 PM	0	L
4/6/2019	Sat	4:46 PM	-6	L
4/8/2019	Mon	6:00 PM	-6	L
4/10/2019	Wed	7:34 PM	-3	L

Table 4: Park Hours for Collection sites

Table 4	
Collection location from land	**Hour (all collections were at low tide)**
Colee Hammock Park	6am – 9pm
Cooley's Landing Park	6am – 9pm
Lewis Landing Park	6am – 9pm
Bill Keith Preserve Park	Sunset to Sunrise

Table 5: Pole size for collection poles

Table 5		
Collection location from land	**Pole Size (m)**	**Pole Size (ft)**
Idlewyld Dr.	3.048	10
Colee Hammock Park	2.286	7.5
Cooley's Landing Park	2.1336	7
Lewis Landing Park	2.286	7.5
Bill Keith Preserve Park	None	None
N side of 84, W of S Pine Island Rd	1.524	5

Table 6: Collection data

Table 6					
Collection Date	**Location**	**Collector**	**Collection Time**	**Weather Conditions**	**Water Temperature (Degree Celsius)**
3/11/2019	I	Angie Louis	7:12pm	Sunny	26
3/11/2019	CH	Angie Louis	7:01pm	Sunny	26
3/11/2019	CH	Caileigh Craddock	7:06pm	Sunny	26
3/11/2019	LL	Aubrey Anthony	7:07pm	Sunny	26
3/11/2019	BKP	Dr. George Duncan	7:10pm	Sunny	26
3/11/2019	84	Ron Honse	7:06pm	Sunny	26
3/13/2019	I	Dr. George Duncan	7:15pm	Overcast	26
3/13/2019	CH	Dr. George Duncan	7:25pm	Overcast	26
3/13/2019	CH	Dr. George Duncan	7:40pm	Overcast	26
3/13/2019	LL	Ron Honse	8:23am	Overcast	26
3/13/2019	BKP	Ron Honse	8:37am	Overcast	26
3/13/2019	84	Ron Honse	9:01am	Sunny	24
3/15/2019	I	Angie Louis	9:34am	Overcast	25
3/15/2019	CH	Angie Louis	9:49am	Overcast	26
3/15/2019	CH	Angie Louis	10:06am	Overcast	27
3/15/2019	LL	Angie Louis	10:17am	Overcast	27
3/15/2019	BKP	Angie Louis	10:37am	Overcast	27
3/15/2019	84	Angie Louis	11:09am	Overcast	26
3/17/2019	I	Dr. George Duncan	12:48pm	Overcast	27
3/17/2019	CH	Dr. George Duncan	12:58pm	Overcast	27
3/17/2019	CH	Johan Leon	12:55pm	Sunny	27
3/17/2019	LL	Johan Leon	1:10pm	Sunny	27
3/17/2019	BKP	Angie Louis	12:51pm	Sunny	27
3/17/2019	84	Angie Louis	1:21pm	Sunny	28

Table 6					
Collection Date	**Location**	**Collector**	**Collection Time**	**Weather Conditions**	**Water Temperature (Degree Celsius)**
3/19/2019	I	Angie Louis	2:25pm	Rain	25
3/19/2019	CH	Angie Louis	2:37pm	Rain	26
3/19/2019	CH	Angie Louis	2:55pm	Rain	25
3/19/2019	LL	Angie Louis	3:07pm	Rain	25
3/19/2019	BKP	Angie Louis	3:19pm	Rain	25
3/19/2019	84	Angie Louis	3:49pm	Rain	25
3/21/2019	I	Angie Louis	4:18pm	Overcast	26
3/21/2019	CH	Angie Louis	4:29pm	Overcast	26
3/21/2019	CH	Ron Honse	4:18pm	Overcast	26
3/21/2019	LL	Ron Honse	4:28pm	Overcast	24
3/21/2019	BKP	Dr. George Duncan	4:15pm	Overcast	26
3/21/2019	84	Dr. George Duncan	5:00pm	Overcast	25
3/23/2019	I	Angie Louis	5:51pm	Sunny	25
3/23/2019	CH	Angie Louis	6:09pm	Sunny	25
3/23/2019	CH	Cynthia Cleveland	5:49pm	Sunny	25
3/23/2019	LL	Skylar Muller	5:58pm	Overcast	25
3/23/2019	BKP	Skylar Muller	6:15pm	Overcast	24
3/23/2019	84	Ron Honse	5:58pm	Sunny	26
3/25/2019	I	Dr. George Duncan	8:00pm	Overcast	26
3/25/2019	CH	Dr. George Duncan	8:20pm	Overcast	26
3/25/2019	CH	Caileigh Craddock	7:36pm	Overcast	26
3/25/2019	LL	Aubrey Anthony	7:58pm	Overcast	19
3/25/2019	BKP	Miranda Brahman	7:39pm	Overcast	26
3/25/2019	84	Angie Louis	7:38pm	Sunny	25
3/27/2019	I	Dr. George Duncan	9:15am	Overcast	24

Table 6					
Collection Date	**Location**	**Collector**	**Collection Time**	**Weather Conditions**	**Water Temperature (Degree Celsius)**
3/27/2019	CH	Dr. George Duncan	9:30am	Overcast	25
3/27/2019	CH	Miranda Brahman	8:53pm	Overcast	24
3/27/2019	LL	Miranda Brahman	9:05am	Overcast	23
3/27/2019	BKP	Miranda Brahman	9:15am	Overcast	23
3/27/2019	84	Ron Honse	9:07am	Overcast	23
3/29/2019	I	Angie Louis	10:49am	Overcast	25
3/29/2019	CH	Angie Louis	11:01am	Overcast	25
3/29/2019	CH	Angie Louis	11:17am	Overcast	25
3/29/2019	LL	Angie Louis	11:26am	Overcast	25
3/29/2019	BKP	Angie Louis	11:39am	Overcast	25
3/29/2019	84	Angie Louis	12:07pm	Rain	25
3/31/2019	I	Angie Louis	12:55pm	Sunny	27
3/31/2019	CH	Angie Louis	1:05pm	Sunny	27
3/31/2019	CH	Cynthia Cleveland	12:43pm	Sunny	26
3/31/2019	LL	Cynthia Cleveland	12:58pm	Sunny	25
3/31/2019	BKP	Cynthia Cleveland	1:16pm	Sunny	25
3/31/2019	84	Ron Honse	1:01pm	Overcast	26
4/2/2019	I	Angie Louis	1:52pm	Overcast	27
4/2/2019	CH	Angie Louis	2:04pm	Overcast	27
4/2/2019	CH	Angie Louis	2:27pm	Overcast	27
4/2/2019	LL	Angie Louis	2:40pm	Overcast	27
4/2/2019	BKP	Angie Louis	2:54pm	Overcast	27
4/2/2019	84	Angie Louis	3:24pm	Overcast	27
4/4/2019	I	Angie Louis	3:28pm	Overcast	25
4/4/2019	CH	Angie Louis	3:38pm	Overcast	25

Table 6					
Collection Date	**Location**	**Collector**	**Collection Time**	**Weather Conditions**	**Water Temperature (Degree Celsius)**
4/4/2019	CH	Ron Honse	3:34pm	Overcast	26
4/4/2019	LL	Ron Honse	3:48pm	Overcast	25
4/4/2019	BKP	Ron Honse	4:04pm	Overcast	24
4/4/2019	84	Dr. George Duncan	3:40pm	Overcast	25
4/6/2019	I	Angie Louis	5:01pm	Sunny	27
4/6/2019	CH	Angie Louis	4:48pm	Sunny	27
4/6/2019	CH	Skylar Muller	4:36pm	Sunny	27
4/6/2019	LL	Skylar Muller	4:52pm	Sunny	27
4/6/2019	BKP	Skylar Muller	5:10pm	Sunny	26
4/6/2019	84	Dr. George Duncan	4:50pm	Overcast	25
4/8/2019	I	Dr. George Duncan	8:30pm	Overcast	26
4/8/2019	CH	Dr. George Duncan	8:40pm	Overcast	27
4/8/2019	CH	Caileigh Craddock	6:00pm	Overcast	26
4/8/2019	LL	Angie Louis	6:38pm	Overcast	27
4/8/2019	BKP	Miranda Brahman	6:05pm	Overcast	27
4/8/2019	84	Ron Honse	6:03pm	Sunny	27
4/10/2019	I	Dr. George Duncan	7:10pm	Overcast	27
4/10/2019	CH	Dr. George Duncan	7:20pm	Overcast	27
4/10/2019	CH	Angie Louis	7:34pm	Overcast	27
4/10/2019	LL	Caileigh Craddock	7:34pm	Overcast	27
4/10/2019	BKP	Caileigh Craddock	7:51pm	Overcast	26
4/10/2019	84	Ron Honse	7:34pm	Clear	26

Figure 12: two 300ml magnetic filter funnels attached to PVC pipes hooked up to two filtering flasks and a vacuum pump

Table 7: Filtration Data

Table 7						
Collection Date	**Location**	**Filtration Start Time**	**Filter A Volume (ml)**	**Filter B Volume (ml)**	**Holding Time (hour: minutes)**	**Notes**
3/11/2019	I	2:50pm	~450+	~450+	19:38	
3/11/2019	CH	3:25pm	~500	~500	20:24	Went here first do to what looked like an accident on the road
3/11/2019	CH	3:43pm	~450+	~450+	20:37	
3/11/2019	LL	4:00pm	~450+	~450+	20:53	
3/11/2019	BKP	4:14pm	~450+	~450+	21:04	
3/11/2019	84	4:30pm	350+	350+	21:24	lost 300mL from filter A initially due to tear after putting the flask
3/13/2019	I	3:31pm	~450+	~450+	8:16	

Table 7						
Collection Date	**Location**	**Filtration Start Time**	**Filter A Volume (ml)**	**Filter B Volume (ml)**	**Holding Time (hour: minutes)**	**Notes**
3/13/2019	CH	3:48pm	~450+	~450+	8:23	Filter B looked ripped after putting it into 1.5 ml tube, may have ripped when folding
3/13/2019	CH	4:05pm	~450+	~450+	8:25	
3/13/2019	LL	4:19pm	~450+	~450+	7:56	
3/13/2019	BKP	4:34pm	~450+	~450+	7:57	
3/13/2019	84	4:48pm	500	500	7:47	
3/15/2019	I	12:19pm	~450+	~450+	2:45	
3/15/2019	CH	12:34pm	~450+	~450+	2:45	
3/15/2019	CH	12:47pm	500	500	2:41	
3/15/2019	LL	1:02pm	~450+	~450+	2:45	
3/15/2019	BKP	1:16pm	500	500	2:39	
3/15/2019	84	1:29pm	500	500	2:20	
3/17/2019	I	5:26pm	~450+	~450+	4:38	
3/17/2019	CH	5:40pm	~450+	~450+	4:42	
3/17/2019	CH	8:24am	~450+	~450+	19:29	
3/17/2019	LL	8:35am	~450+	~450+	19:25	
3/17/2019	BKP	6:02pm	350	350	5:11	lost about 200mL
3/17/2019	84	6:13pm	~450+	~450+	4:52	
3/19/2019	I	6:44am	~500	~500	16:19	
3/19/2019	CH	6:56am	500+	500+	16:19	
3/19/2019	CH	7:12am	500+	500+	16:17	
3/19/2019	LL	7:24am	500+	500+	16:17	
3/19/2019	BKP	7:39am	500	500	16:20	

Table 7						
Collection Date	**Location**	**Filtration Start Time**	**Filter A Volume (ml)**	**Filter B Volume (ml)**	**Holding Time (hour: minutes)**	**Notes**
3/19/2019	84	7:53am	500+	500+	16:04	
3/21/2019	I	2:13pm	500+	500+	21:55	dirty filter
3/21/2019	CH	2:26pm	~500	~500	21:57	light
3/21/2019	CH	2:39pm	~450+	~450+	22:21	
3/21/2019	LL	2:53pm	~450+	~450+	22:25	
3/21/2019	BKP	3:13pm	~450+	~450+	22:58	
3/21/2019	84	3:20pm	~450+	~450+	22:20	Filter A finished filtering at 4:35pm, filter B finished filtering 4:25pm
3/23/2019	I	10:18am	500	500	16:27	
3/23/2019	CH	10:30am	500	500	16:21	
3/23/2019	CH	10:44am	~500	~500	16:55	
3/23/2019	LL	10:56am	500	500	16:58	
3/23/2019	BKP	11:09am	~500	~500	16:54	
3/23/2019	84	11:26am	500	500	17:28	Filter A finished filtering at 1:11pm, filter B finished filtering 1:10pm
3/25/2019	I	2:11pm	~450	~450	18:11	
3/25/2019	CH	2:26pm	~450	~450	18:06	
3/25/2019	CH	2:38pm	~425	~425	19:02	
3/25/2019	LL	2:48pm	500	500	18:50	
3/25/2019	BKP	3:01pm	500	500	19:22	
3/25/2019	84	3:12pm	500	500	19:34	Filter A finished filtering at 5:28pm, filter B

Table 7						
Collection Date	**Location**	**Filtration Start Time**	**Filter A Volume (ml)**	**Filter B Volume (ml)**	**Holding Time (hour: minutes)**	**Notes**
						finished filtering 5:30pm
3/27/2019	I	1:38pm	450	450	4:23	
3/27/2019	CH	1:49pm	~500	~500	4:19	
3/27/2019	CH	2:01pm	500	500	5:08	
3/27/2019	LL	2:12pm	500	500	5:07	
3/27/2019	BKP	2:21pm	500	500	5:06	
3/27/2019	84	2:30pm	500	500	5:23	Filter A finished filtering at 2:51pm, filter B finished filtering 2:54pm
3/29/2019	I	12:58pm	500	500	2:09	
3/29/2019	CH	1:13pm	500	500	2:12	
3/29/2019	CH	1:16pm	500	500	1:59	
3/29/2019	LL	1:25pm	500	500	1:59	
3/29/2019	BKP	1:34pm	500	500	1:55	
3/29/2019	84	1:45pm	500	500	1:38	Filter A finished filtering at 2:09pm, filter B finished filtering 2:06pm
3/31/2019	I	2:25pm	500	500	1:30	
3/31/2019	CH	2:36pm	500	500	1:31	
3/31/2019	CH	2:44pm	500	500	2:01	
3/31/2019	LL	2:53pm	500	500	1:55	
3/31/2019	BKP	3:03pm	500	500	1:47	
3/31/2019	84	3:12pm	500	500	2:11	Filter A finished filtering at

Table 7						
Collection Date	**Location**	**Filtration Start Time**	**Filter A Volume (ml)**	**Filter B Volume (ml)**	**Holding Time (hour: minutes)**	**Notes**
						3:24pm, filter B finished filtering 3:24pm
4/2/2019	I	5:26am	500	500	15:34	
4/2/2019	CH	5:37am	500	500	15:33	
4/2/2019	CH	5:48am	500	500	15:21	
4/2/2019	LL	6:00am	500	500	15:20	
4/2/2019	BKP	6:11am	~500	~500	15:17	
4/2/2019	84	6:22am	500	500	14:58	Filter A finished filtering at 6:37am, filter B finished filtering 6:38am
4/4/2019	I	1:29pm	500	500	22:01	
4/4/2019	CH	1:43pm	500	500	22:05	
4/4/2019	CH	1:52pm	500	500	22:18	
4/4/2019	LL	2:03pm	500	500	22:15	
4/4/2019	BKP	2:13pm	500	500	22:09	
4/4/2019	84	2:25pm	500	500	22:45	Filter A finished filtering at 2:40pm, filter B finished filtering 2:40pm
4/6/2019	I	10:06am	500	500	17:05	dirty filter
4/6/2019	CH	10:17am	500	500	17:29	
4/6/2019	CH	10:28am	500	500	17:52	filter B looked ripped
4/6/2019	LL	10:40am	500	500	17:48	
4/6/2019	BKP	10:51am	500	500	17:41	
4/6/2019	84	11:04am	500	500	18:14	Filter A finished

Table 7						
Collection Date	**Location**	**Filtration Start Time**	**Filter A Volume (ml)**	**Filter B Volume (ml)**	**Holding Time (hour: minutes)**	**Notes**
						filtering at 11:18am, filter B finished filtering 11:17am
4/8/2019	I	2:08pm	500	500	17:38	dirty filter
4/8/2019	CH	2:13pm	500	500	17:33	
4/8/2019	CH	2:28pm	~400	~400	20:28	collection was less
4/8/2019	LL	2:36pm	500	500	19:58	
4/8/2019	BKP	2:47pm	500	500	20:42	
4/8/2019	84	2:58pm	500	500	20:55	Filter A finished filtering at 3:10pm, filter B finished filtering 3:10pm
4/10/2019	I	2:09pm	500	500	18:59	
4/10/2019	CH	2:19pm	500	500	18:59	
4/10/2019	CH	2:29pm	500	500	18:55	
4/10/2019	LL	2:40pm	450	450	19:06	collection was less
4/10/2019	BKP	2:51pm	450	450	19:00	collection was less
4/10/2019	84	3:01pm	500	500	19:27	Filter A finished filtering at 3:41pm, filter B finished filtering 3:39pm

Table 8: DNA Extraction Data

Table 8			
Date	**Location**	**DNA Extraction Date**	**Notes**
3/11/2019	I	9/21/2019	
3/11/2019	CH	9/21/2019	
3/11/2019	CH	9/21/2019	pink after extraction
3/11/2019	LL	9/21/2019	
3/11/2019	BKP	9/21/2019	
3/11/2019	84	9/21/2019	
3/13/2019	I	9/21/2019	
3/13/2019	CH	9/21/2019	
3/13/2019	CH	9/21/2019	
3/13/2019	LL	9/21/2019	
3/13/2019	BKP	9/21/2019	
3/13/2019	84	9/21/2019	
3/15/2019	I	9/23/2019	
3/15/2019	CH	9/23/2019	pink after extraction
3/15/2019	CH	9/23/2019	
3/15/2019	LL	9/23/2019	
3/15/2019	BKP	9/23/2019	
3/15/2019	84	9/23/2019	
3/17/2019	I	9/23/2019	
3/17/2019	CH	9/23/2019	
3/17/2019	CH	9/23/2019	
3/17/2019	LL	9/23/2019	
3/17/2019	BKP	9/23/2019	
3/17/2019	84	9/23/2019	
3/19/2019	I	9/25/2019	

Table 8			
Date	**Location**	**DNA Extraction Date**	**Notes**
3/19/2019	CH	9/25/2019	
3/19/2019	CH	9/25/2019	
3/19/2019	LL	9/25/2019	
3/19/2019	BKP	9/25/2019	
3/19/2019	84	9/25/2019	
3/21/2019	I	9/25/2019	
3/21/2019	CH	9/25/2019	
3/21/2019	CH	9/25/2019	
3/21/2019	LL	9/25/2019	
3/21/2019	BKP	9/25/2019	
3/21/2019	84	9/25/2019	
3/23/2019	I	9/28/2019	
3/23/2019	CH	9/28/2019	
3/23/2019	CH	9/28/2019	pink after extraction
3/23/2019	LL	9/28/2019	
3/23/2019	BKP	9/28/2019	
3/23/2019	84	9/28/2019	
3/25/2019	I	9/28/2019	
3/25/2019	CH	9/28/2019	
3/25/2019	CH	9/28/2019	
3/25/2019	LL	9/28/2019	
3/25/2019	BKP	9/28/2019	
3/25/2019	84	9/28/2019	
3/27/2019	I	9/30/2019	
3/27/2019	CH	9/30/2019	
3/27/2019	CH	9/30/2019	

Table 8			
Date	**Location**	**DNA Extraction Date**	**Notes**
3/27/2019	LL	9/30/2019	
3/27/2019	BKP	9/30/2019	
3/27/2019	84	9/30/2019	
3/29/2019	I	9/30/2019	
3/29/2019	CH	9/30/2019	
3/29/2019	CH	9/30/2019	
3/29/2019	LL	9/30/2019	
3/29/2019	BKP	9/30/2019	
3/29/2019	84	9/30/2019	
3/31/2019	I	10/2/2019	
3/31/2019	CH	10/2/2019	
3/31/2019	CH	10/2/2019	pink after extraction
3/31/2019	LL	10/2/2019	
3/31/2019	BKP	10/2/2019	
3/31/2019	84	10/2/2019	
4/2/2019	I	10/2/2019	
4/2/2019	CH	10/2/2019	
4/2/2019	CH	10/2/2019	
4/2/2019	LL	10/2/2019	
4/2/2019	BKP	10/2/2019	
4/2/2019	84	10/2/2019	
4/4/2019	I	10/5/2019	
4/4/2019	CH	10/5/2019	
4/4/2019	CH	10/5/2019	
4/4/2019	LL	10/5/2019	
4/4/2019	BKP	10/5/2019	

Table 8			
Date	**Location**	**DNA Extraction Date**	**Notes**
4/4/2019	84	10/5/2019	
4/6/2019	I	10/5/2019	
4/6/2019	CH	10/5/2019	
4/6/2019	CH	10/5/2019	
4/6/2019	LL	10/5/2019	
4/6/2019	BKP	10/5/2019	
4/6/2019	84	10/5/2019	
4/8/2019	I	10/6/2019	
4/8/2019	CH	10/6/2019	pink after extraction
4/8/2019	CH	10/6/2019	
4/8/2019	LL	10/6/2019	
4/8/2019	BKP	10/6/2019	
4/8/2019	84	10/6/2019	
4/10/2019	I	10/6/2019	
4/10/2019	CH	10/6/2019	
4/10/2019	CH	10/6/2019	
4/10/2019	LL	10/6/2019	
4/10/2019	BKP	10/6/2019	
4/10/2019	84	10/6/2019	pink around the edges after extraction

Table 9: Samples given to the Broward County Environmental Lab for PCR analyzes

Table 9		
Date	**Location**	**Abbreviation**
3-11-2019	I	311 I
3-11-2019	CH	311 CH
3-11-2019	CL	311 CL

Table 9		
Date	**Location**	**Abbreviation**
3-11-2019	LL	311 LL
3-11-2019	BKP	311 BKP
3-11-2019	84	311 84
3-13-2019	I	313 I
3-13-2019	CH	313 CH
3-13-2019	CL	313 CL
3-13-2019	LL	313 LL
3-13-2019	BKP	313 BKP
3-13-2019	84	313 84
3-15-2019	I	315 I
3-15-2019	CH	315 CH
3-15-2019	CL	315 CL
3-15-2019	LL	315 LL
3-15-2019	BKP	315 BKP
3-15-2019	84	315 84
3-17-2019	I	317 I
3-17-2019	CH	317 CH
3-17-2019	CL	317 CL
3-17-2019	LL	317 LL
3-17-2019	BKP	317 BKP
3-17-2019	84	317 84
3-19-2019	I	319 I
3-19-2019	CH	319 CH
3-19-2019	CL	319 CL
3-19-2019	LL	319 LL
3-19-2019	BKP	319 BKP
3-19-2019	84	319 84

Table 9		
Date	**Location**	**Abbreviation**
3-21-2019	I	321 I
3-21-2019	CH	321 CH
3-21-2019	CL	321 CL
3-21-2019	LL	321 LL
3-21-2019	BKP	321 BKP
3-21-2019	84	321 84
3-23-2019	I	323 I
3-23-2019	CH	323 CH
3-23-2019	CL	323 CL
3-23-2019	LL	323 LL
3-23-2019	BKP	323 BKP
3-23-2019	84	323 84
3-25-2019	I	325 I
3-25-2019	CH	325 CH
3-25-2019	CL	325 CL
3-25-2019	LL	325 LL
3-25-2019	BKP	325 BKP
3-25-2019	84	325 84
3-27-2019	I	327 I
3-27-2019	CH	327 CH
3-27-2019	CL	327 CL
3-27-2019	LL	327 LL
3-27-2019	BKP	327 BKP
3-27-2019	84	327 84
3-29-2019	I	329 I
3-29-2019	CH	329 CH
3-29-2019	CL	329 CL

Table 9		
Date	**Location**	**Abbreviation**
3-29-2019	LL	329 LL
3-29-2019	BKP	329 BKP
3-29-2019	84	329 84
3-31-2019	I	331 I
3-31-2019	CH	331 CH
3-31-2019	CL	331 CL
3-31-2019	LL	331 LL
3-31-2019	BKP	331 BKP
3-31-2019	84	331 84

Table 10: Samples not send to Broward County Environmental Lab for PCR analyzes

Table 10		
Date	**Location**	**Abbreviation**
4-2-2019	I	42 I
4-2-2019	CH	42 CH
4-2-2019	CL	42 CL
4-2-2019	LL	42 LL
4-2-2019	BKP	42 BKP
4-2-2019	84	42 84
4-4-2019	I	44 I
4-4-2019	CH	44 CH
4-4-2019	CL	44 CL
4-4-2019	LL	44 LL
4-4-2019	BKP	44 BKP
4-4-2019	84	44 84
4-6-2019	I	46 I
4-6-2019	CH	46 CH

Table 10		
Date	**Location**	**Abbreviation**
4-6-2019	CL	46 CL
4-6-2019	LL	46 LL
4-6-2019	BKP	46 BKP
4-6-2019	84	46 84
4-8-2019	I	48 I
4-8-2019	CH	48 CH
4-8-2019	CL	48 CL
4-8-2019	LL	48 LL
4-8-2019	BKP	48 BKP
4-8-2019	84	48 84
4-10-2019	I	410 I
4-10-2019	CH	410 CH
4-10-2019	CL	410 CL
4-10-2019	LL	410 LL
4-10-2019	BKP	410 BKP
4-10-2019	84	410 84

Results

Table 11 is the first run quantity, second run quantity and quantity mean per 2µl (targets) for each sample collection location for each of the collection dates obtained from running qPCR for all samples. The Days the samples where run are color coded. Green indicates samples run on 10-10-2019, purple indicates samples run on 11-4-2019 and blue indicates samples run on 11-6-2019. Each quantity mean per 2µl (targets) was divided by the extracted amount to get the Quantity per extracted amount (targets) then divided by the filtered volume and multiplied by 100ml to get the quantity per 100ml for the ½ filters that were used for the extraction, this is in Table 12. The quantity per 100ml for the ½ filters and the quantity for the full filter per 100ml (targets) is listed in Table 13.

Quantity per 100ml for ½ filter is plotted for each location over the collection sample date range in Figure 13. There is no clear pattern for any of the sample collection locations or for any of the dates the samples were taken.

Figures 14 through 17 show the quantity per 100ml for ½ filter plotted for each weather condition noted while each collection sample was taken. For the collection samples that had undetermined quantities, there is a blank for those dates and locations. There is no clear pattern indicating if weather, sunlight versus cloud cover, had any effect on the quantity of HF183 found at each location. There was not enough wet versus dry conditions to determine a pattern between samples taken during or after rain and during dry conditions.

Table 11: qPCR first run, second run and quantity mean per 2µl (targets) for all collection sites, green are samples run on 10-10-2019, purple are samples run on 11-4-2019 and blue are samples run on 11-6-2019

Table 11			
Date and Location	**First Run Quantity per 2uL (targets)**	**Second Run Quantity per 2uL (targets)**	**Quantity Mean for 2uL (targets)**
311 I	3.E+01	5.E+01	4.E+01
311 CH	8.E+00	6.E+01	3.E+01
311 CL	5.E+01	7.E+01	6.E+01
311 LL	1.E+02	5.E+01	8.E+01
311 BKP	6.E+01	7.E+01	7.E+01
311 84	5.E-01	3.E+01	1.E+01
313 I	1.E+01	2.E+01	2.E+01
313 CH	4.E+01	5.E+01	5.E+01
313 CL	3.E+01	3.E+01	3.E+01
313 LL	4.E+01	2.E+01	3.E+01

Table 11			
Date and Location	**First Run Quantity per 2uL (targets)**	**Second Run Quantity per 2uL (targets)**	**Quantity Mean for 2uL (targets)**
313 BKP	4.E+01	2.E+01	3.E+01
313 84	2.E+01	1.E+01	2.E+01
315 I	4.E+01	7.E+01	5.E+01
315 CH	2.E+01	2.E+01	2.E+01
315 CL	3.E+01	2.E+01	2.E+01
315LL	3.E+01	3.E+01	3.E+01
315 BKP	4.E+01	4.E+01	4.E+01
315 84	7.E-01	3.E+01	2.E+01
317 I	2.E+02	1.E+02	1.4E+02
317 CH	4.E+01	3.E+01	3.E+01
317 CL	2.E+01	3.E+01	2.E+01
317 LL	3.E+01	3.E+01	3.E+01
317 BKP	9.E+00	2.E+01	2.E+01
317 84	undetermined	undetermined	undetermined
319 I	3.E+01	6.E+01	5.E+01
319 CH	4.E+01	5.E+01	4.E+01
319 CL	3.E+01	7.E+01	5.E+01
319 LL	6.E+01	2.E+01	4.E+01

Table 11			
Date and Location	**First Run Quantity per 2uL (targets)**	**Second Run Quantity per 2uL (targets)**	**Quantity Mean for 2uL (targets)**
319 BKP	5.E+01	4.E+01	5.E+01
319 84	3.E+01	1.E+01	2.E+01
321 I	7.E+01	4.E+01	5.E+01
321 CH	4.E+01	4.E+01	4.E+01
321 CL	6.E+01	5.E+01	5.E+01
321 LL	7.E+01	3.E+01	5.E+01
321 BKP	6.E+01	6.E+01	6.E+01
321 84	undetermined	undetermined	undetermined
323 I	2.E+01	3.E+01	2.E+01
323 CH	4.E+01	4.E+01	4.E+01
323 CL	6.E+01	4.E+01	5.E+01
323 LL	1.E+02	5.E+01	8.E+01
323 BKP	8.E+01	5.E+01	7.E+01
323 84	undetermined	undetermined	undetermined
325 I	2.E+01	3.E+01	2.E+01
325 CH	2.E+01	3.E+01	3.E+01
325 CL	6.E+01	5.E+01	6.E+01
325 LL	7.E+01	7.E+01	7.E+01

Table 11			
Date and Location	**First Run Quantity per 2uL (targets)**	**Second Run Quantity per 2uL (targets)**	**Quantity Mean for 2uL (targets)**
325 BKP	1.E+02	6.E+01	8.E+01
325 84	undetermined	undetermined	undetermined
327 I	1.E+01	2.E+01	1.E+01
327 CH	2.E+01	3.E+01	2.E+01
327 CL	4.E+01	3.E+01	4.E+01
327 LL	5.E+01	5.E+01	5.E+01
327 BKP	4.E+01	5.E+01	5.E+01
327 84	undetermined	undetermined	undetermined
329 I	2.E+01	1.E+01	2.E+01
329 CH	5.E+01	3.E+01	4.E+01
329 CL	3.E+01	3.E+01	3.E+01
329 LL	6.E+01	3.E+01	4.E+01
329 BKP	1.E+02	9.E+01	1.1E+02
329 84	undetermined	undetermined	undetermined
331 I	2.E+01	2.E+01	2.E+01
331 CH	6.E+01	1.E+01	4.E+01
331 CL	3.E+01	7.E+01	5.E+01
331 LL	4.E+01	4.E+01	4.E+01

Table 11			
Date and Location	**First Run Quantity per 2uL (targets)**	**Second Run Quantity per 2uL (targets)**	**Quantity Mean for 2uL (targets)**
331 BKP	7.E+01	4.E+01	6.E+01
331 84	undetermined	undetermined	undetermined
42 I	2.E+01	4.E+00	1.E+01
42 CH	3.E+01	3.E+01	3.E+01
42 CL	4.E-01	1.E+00	8.E-01
42 LL	2.E+01	4.E+01	3.E+01
42 BKP	7.E+01	3.E+01	5.E+01
42 84	1.E+02	1.E+02	1.4E+02
44 I	3.E+01	3.E+01	3.E+01
44 CH	1.E+01	6.E+01	4.E+01
44 CL	4.E+01	8.E+01	6.E+01
44 LL	1.E+01	8.E+01	5.E+01
44 BKP	2.E+00	7.E+00	5.E+00
44 84	7.E-01	1.E-01	4.E-01
46 I	2.E+01	6.E+00	1.E+01
46 CH	5.E+01	4.E+01	4.E+01
46 CL	9.E+01	3.E+01	6.E+01
46 LL	5.E+01	1.E+02	9.E+01

Table 11			
Date and Location	**First Run Quantity per 2uL (targets)**	**Second Run Quantity per 2uL (targets)**	**Quantity Mean for 2uL (targets)**
46 BKP	3.E+01	4.E+01	3.E+01
46 84	9.E-02	6.E-01	3.E-01
48 I	5.E+00	3.E+00	4.E+00
48 CH	3.E+01	5.E+00	2.E+01
48 CL	1.E+01	8.E+00	1.E+01
48 LL	1.E-01	5.E-01	3.E-01
48 BKP	3.E+01	2.E+01	3.E+01
48 84	6.E+00	5.E+00	5.E+00
410 I	4.E+00	3.E+00	4.E+00
410 CH	3.E+00	2.E+00	2.E+00
410 CL	2.E+01	2.E+01	2.E+01
410 LL	2.E+00	8.E+00	5.E+00
410 BKP	2.E-01	2.E+00	1.E+00
410 84	5.E+01	7.E+01	6.E+01

Table 12: Each quantity mean per 2µl (targets) was divided by the extracted amount to get the Quantity per extracted amount (targets) then divided by the filtered volume and multiplied by 100ml to get the quantity per 100ml for the ½ filters

Table 12

Date and Location	Quantity Mean for 2uL (targets)	Extracted Amount (µl)	Quantity per Extracted Amount (targets)	Filter A Volume (ml)	Quantity per Filtered Amount from half filter (targets/(µl)	Quantity per 100mL half filter (targets)
311 I	4.E+01	101	2.E+03	470	4.E+00	4.E+02
311 CH	3.E+01	97	2.E+03	500	3.E+00	3.E+02
311 CL	6.E+01	104	3.E+03	470	7.E+00	7.E+02
311 LL	8.E+01	111	4.E+03	470	9.E+00	9.E+02
311 BKP	7.E+01	81	3.E+03	470	6.E+00	6.E+02
311 84	1.E+01	96	6.E+02	370	2.E+00	2.E+02
313 I	2.E+01	98	7.E+02	470	2.E+00	2.E+02
313 CH	5.E+01	97	2.E+03	470	5.E+00	5.E+02
313 CL	3.E+01	95	1.E+03	470	3.E+00	3.E+02
313 LL	3.E+01	95	1.E+03	470	3.E+00	3.E+02
313 BKP	3.E+01	98	2.E+03	470	3.E+00	3.E+02
313 84	2.E+01	99	8.E+02	500	2.E+00	2.E+02
315 I	5.E+01	73	2.E+03	470	4.E+00	4.E+02
315 CH	2.E+01	81	8.E+02	470	2.E+00	2.E+02
315 CL	2.E+01	91	1.E+03	500	2.E+00	2.E+02

Table 12						
Date and Location	**Quantity Mean for 2uL (targets)**	**Extracted Amount (µl)**	**Quantity per Extracted Amount (targets)**	**Filter A Volume (ml)**	**Quantity per Filtered Amount from half filter (targets/(µl)**	**Quantity per 100mL half filter (targets)**
315LL	3.E+01	95	2.E+03	470	3.E+00	3.E+02
315 BKP	4.E+01	108	2.E+03	500	4.E+00	4.E+02
315 84	2.E+01	99	8.E+02	500	2.E+00	2.E+02
317 I	1.4E+02	100	6.8E+03	470	1.5E+01	1.5E+03
317 CH	3.E+01	92	1.E+03	470	3.E+00	3.E+02
317 CL	2.E+01	97	1.E+03	470	3.E+00	3.E+02
317 LL	3.E+01	97	2.E+03	470	3.E+00	3.E+02
317 BKP	2.E+01	98	8.E+02	350	2.E+00	2.E+02
317 84	undetermined	96	undetermined	470	undetermined	undetermined
319 I	5.E+01	90	2.E+03	500	4.E+00	4.E+02
319 CH	4.E+01	78	2.E+03	500	3.E+00	3.E+02
319 CL	5.E+01	105	3.E+03	500	5.E+00	5.E+02
319 LL	4.E+01	91	2.E+03	500	3.E+00	3.E+02
319 BKP	5.E+01	113	3.E+03	500	5.E+00	5.E+02
319 84	2.E+01	96	8.E+02	500	2.E+00	2.E+02
321 I	5.E+01	96	3.E+03	500	5.E+00	5.E+02

Table 12

Date and Location	Quantity Mean for 2uL (targets)	Extracted Amount (µl)	Quantity per Extracted Amount (targets)	Filter A Volume (ml)	Quantity per Filtered Amount from half filter (targets/(µl)	Quantity per 100mL half filter (targets)
321 CH	4.E+01	96	2.E+03	500	4.E+00	4.E+02
321 CL	5.E+01	98	3.E+03	470	6.E+00	6.E+02
321 LL	5.E+01	92	2.E+03	470	5.E+00	5.E+02
321 BKP	6.E+01	92	3.E+03	470	6.E+00	6.E+02
321 84	undetermined	85	undetermined	470	undetermined	undetermined
323 I	2.E+01	86	1.E+03	500	2.E+00	2.E+02
323 CH	4.E+01	95	2.E+03	500	4.E+00	4.E+02
323 CL	5.E+01	95	2.E+03	500	5.E+00	5.E+02
323 LL	8.E+01	95	4.E+03	500	8.E+00	8.E+02
323 BKP	7.E+01	91	3.E+03	500	6.E+00	6.E+02
323 84	undetermined	90	undetermined	500	undetermined	undetermined
325 I	2.E+01	100	1.E+03	450	3.E+00	3.E+02
325 CH	3.E+01	104	1.E+03	450	3.E+00	3.E+02
325 CL	6.E+01	103	3.E+03	425	7.E+00	7.E+02
325 LL	7.E+01	102	3.E+03	500	7.E+00	7.E+02
325 BKP	8.E+01	102	4.E+03	500	8.E+00	8.E+02

Table 12

Date and Location	Quantity Mean for 2uL (targets)	Extracted Amount (µl)	Quantity per Extracted Amount (targets)	Filter A Volume (ml)	Quantity per Filtered Amount from half filter (targets/(µl)	Quantity per 100mL half filter (targets)
325 84	undetermined	88	undetermined	500	undetermined	undetermined
327 I	1.E+01	113	8.E+02	450	2.E+00	2.E+02
327 CH	2.E+01	85	1.E+03	500	2.E+00	2.E+02
327 CL	4.E+01	101	2.E+03	500	4.E+00	4.E+02
327 LL	5.E+01	115	3.E+03	500	6.E+00	6.E+02
327 BKP	5.E+01	108	3.E+03	500	5.E+00	5.E+02
327 84	undetermined	100	undetermined	500	undetermined	undetermined
329 I	2.E+01	97	8.E+02	500	2.E+00	2.E+02
329 CH	4.E+01	102	2.E+03	500	4.E+00	4.E+02
329 CL	3.E+01	103	2.E+03	500	3.E+00	3.E+02
329 LL	4.E+01	104	2.E+03	500	5.E+00	5.E+02
329 BKP	1.1E+02	95	5.2E+03	500	1.0E+01	1.0E+03
329 84	undetermined	96	undetermined	500	undetermined	undetermined
331 I	2.E+01	101	1.E+03	500	2.E+00	2.E+02
331 CH	4.E+01	95	2.E+03	500	3.E+00	3.E+02
331 CL	5.E+01	103	3.E+03	500	5.E+00	5.E+02

Table 12						
Date and Location	**Quantity Mean for 2uL (targets)**	**Extracted Amount (µl)**	**Quantity per Extracted Amount (targets)**	**Filter A Volume (ml)**	**Quantity per Filtered Amount from half filter (targets/(µl)**	**Quantity per 100mL half filter (targets)**
331 LL	4.E+01	97	2.E+03	500	4.E+00	4.E+02
331 BKP	6.E+01	94	3.E+03	500	5.E+00	5.E+02
331 84	undetermined	95	undetermined	500	undetermined	undetermined
42 I	1.E+01	102	5.E+02	500	1.E+00	1.E+02
42 CH	3.E+01	102	2.E+03	500	3.E+00	3.E+02
42 CL	8.E-01	110	4.E+01	500	8.E-02	8.E+00
42 LL	3.E+01	94	1.E+03	500	3.E+00	3.E+02
42 BKP	5.E+01	101	3.E+03	500	5.E+00	5.E+02
42 84	1.4E+02	88	6.3E+03	500	1.3E+01	1.3E+03
44 I	3.E+01	62	1.E+03	500	2.E+00	2.E+02
44 CH	4.E+01	58	1.E+03	500	2.E+00	2.E+02
44 CL	6.E+01	95	3.E+03	500	6.E+00	6.E+02
44 LL	5.E+01	98	2.E+03	500	4.E+00	4.E+02
44 BKP	5.E+00	91	2.E+02	500	4.E-01	4.E+01
44 84	4.E-01	151	3.E+01	500	6.E-02	6.E+00
46 I	1.E+01	90	6.E+02	500	1.E+00	1.E+02

Table 12						
Date and Location	**Quantity Mean for 2uL (targets)**	**Extracted Amount (µl)**	**Quantity per Extracted Amount (targets)**	**Filter A Volume (ml)**	**Quantity per Filtered Amount from half filter (targets/(µl)**	**Quantity per 100mL half filter (targets)**
46 CH	4.E+01	98	2.E+03	500	4.E+00	4.E+02
46 CL	6.E+01	99	3.E+03	500	6.E+00	6.E+02
46 LL	9.E+01	104	5.E+03	500	9.E+00	9.E+02
46 BKP	3.E+01	108	2.E+03	500	4.E+00	4.E+02
46 84	3.E-01	98	2.E+01	500	3.E-02	3.E+00
48 I	4.E+00	99	2.E+02	500	4.E-01	4.E+01
48 CH	2.E+01	94	9.E+02	500	2.E+00	2.E+02
48 CL	1.E+01	103	5.E+02	400	1.E+00	1.E+02
48 LL	3.E-01	93	2.E+01	500	3.E-02	3.E+00
48 BKP	3.E+01	88	1.E+03	500	2.E+00	2.E+02
48 84	5.E+00	89	2.E+02	500	5.E-01	5.E+01
410 I	4.E+00	114	2.E+02	500	4.E-01	4.E+01
410 CH	2.E+00	97	1.E+02	500	2.E-01	2.E+01
410 CL	2.E+01	89	9.E+02	500	2.E+00	2.E+02
410 LL	5.E+00	95	2.E+02	450	5.E-01	5.E+01
410 BKP	1.E+00	104	6.E+01	450	1.E-01	1.E+01

Table 12						
Date and Location	**Quantity Mean for 2uL (targets)**	**Extracted Amount (µl)**	**Quantity per Extracted Amount (targets)**	**Filter A Volume (ml)**	**Quantity per Filtered Amount from half filter (targets/(µl)**	**Quantity per 100mL half filter (targets)**
410 84	6.E+01	81	2.E+03	500	5.E+00	5.E+02

Table 13: quantity per 100ml for the ½ filters and the quantity for the full filter per 100ml (targets)

Table 13	
Quantity per 100mL half filter (targets)	**Quantity full filter per 100 mL (targets)**
4.E+02	9.E+02
3.E+02	6.E+02
7.E+02	1.E+03
9.E+02	2.E+03
6.E+02	1.E+03
2.E+02	3.E+02
2.E+02	3.E+02
5.E+02	9.E+02
3.E+02	5.E+02
3.E+02	6.E+02
3.E+02	7.E+02
2.E+02	3.E+02

Table 13	
Quantity per 100mL half filter (targets)	**Quantity full filter per 100 mL (targets)**
4.E+02	8.E+02
2.E+02	4.E+02
2.E+02	5.E+02
3.E+02	6.E+02
4.E+02	9.E+02
2.E+02	3.E+02
1.5E+03	3.E+03
3.E+02	6.E+02
3.E+02	5.E+02
3.E+02	7.E+02
2.E+02	4.E+02
undetermined	Undetermined
4.E+02	8.E+02
3.E+02	7.E+02
5.E+02	1.E+03
3.E+02	7.E+02
5.E+02	1.E+03
2.E+02	3.E+02

Table 13	
Quantity per 100mL half filter (targets)	**Quantity full filter per 100 mL (targets)**
5.E+02	1.E+03
4.E+02	8.E+02
6.E+02	1.E+03
5.E+02	9.E+02
6.E+02	1.E+03
undetermined	Undetermined
2.E+02	4.E+02
4.E+02	8.E+02
5.E+02	9.E+02
8.E+02	2.E+03
6.E+02	1.E+03
undetermined	Undetermined
3.E+02	5.E+02
3.E+02	6.E+02
7.E+02	1.E+03
7.E+02	1.E+03
8.E+02	2.E+03
undetermined	Undetermined

Table 13	
Quantity per 100mL half filter (targets)	**Quantity full filter per 100 mL (targets)**
2.E+02	4.E+02
2.E+02	4.E+02
4.E+02	8.E+02
6.E+02	1.E+03
5.E+02	1.E+03
undetermined	Undetermined
2.E+02	3.E+02
4.E+02	8.E+02
3.E+02	6.E+02
5.E+02	9.E+02
1.0E+03	2.E+03
undetermined	Undetermined
2.E+02	4.E+02
3.E+02	7.E+02
5.E+02	1.E+03
4.E+02	7.E+02
5.E+02	1.E+03
undetermined	Undetermined

Table 13	
Quantity per 100mL half filter (targets)	**Quantity full filter per 100 mL (targets)**
1.E+02	2.E+02
3.E+02	6.E+02
8.E+00	2.E+01
3.E+02	5.E+02
5.E+02	1.E+03
1.3E+03	3.E+03
2.E+02	4.E+02
2.E+02	4.E+02
6.E+02	1.E+03
4.E+02	9.E+02
4.E+01	8.E+01
6.E+00	1.E+01
1.E+02	2.E+02
4.E+02	9.E+02
6.E+02	1.E+03
9.E+02	2.E+03
4.E+02	7.E+02
3.E+00	7.E+00

Table 13	
Quantity per 100mL half filter (targets)	**Quantity full filter per 100 mL (targets)**
4.E+01	8.E+01
2.E+02	4.E+02
1.E+02	3.E+02
3.E+00	6.E+00
2.E+02	5.E+02
5.E+01	9.E+01
4.E+01	8.E+01
2.E+01	5.E+01
2.E+02	4.E+02
5.E+01	1.E+02
1.E+01	3.E+01
5.E+02	9.E+02

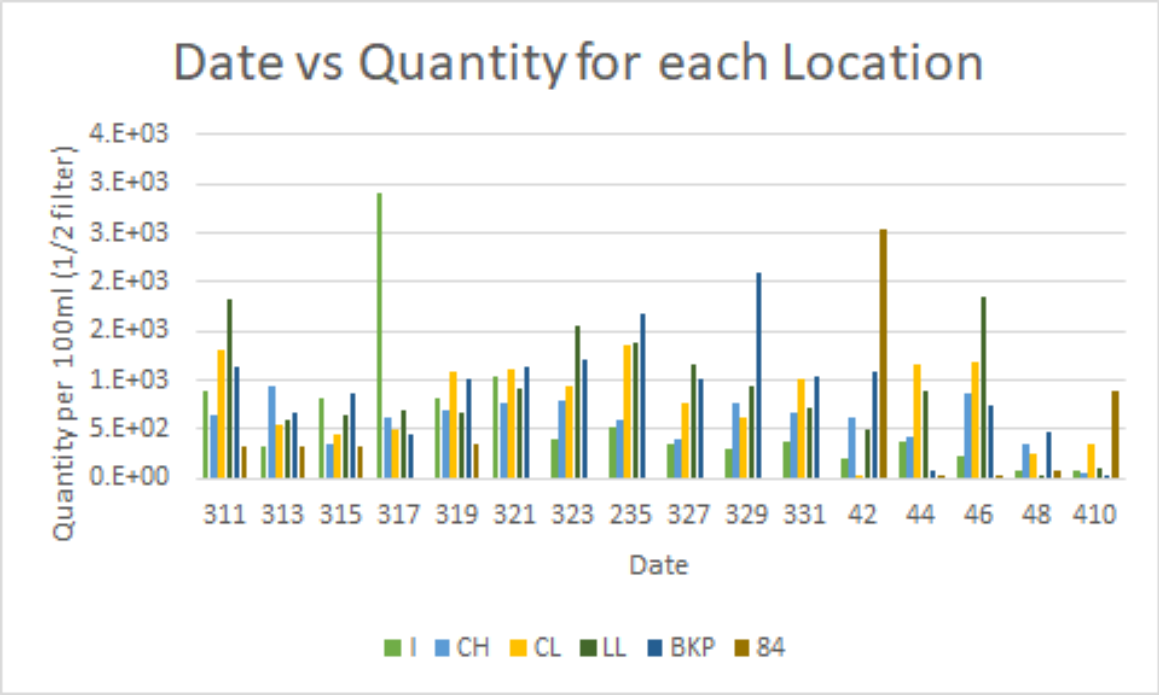

Figure 13: Date vs Quantity per 100ml (1/2 filter) for each sample location

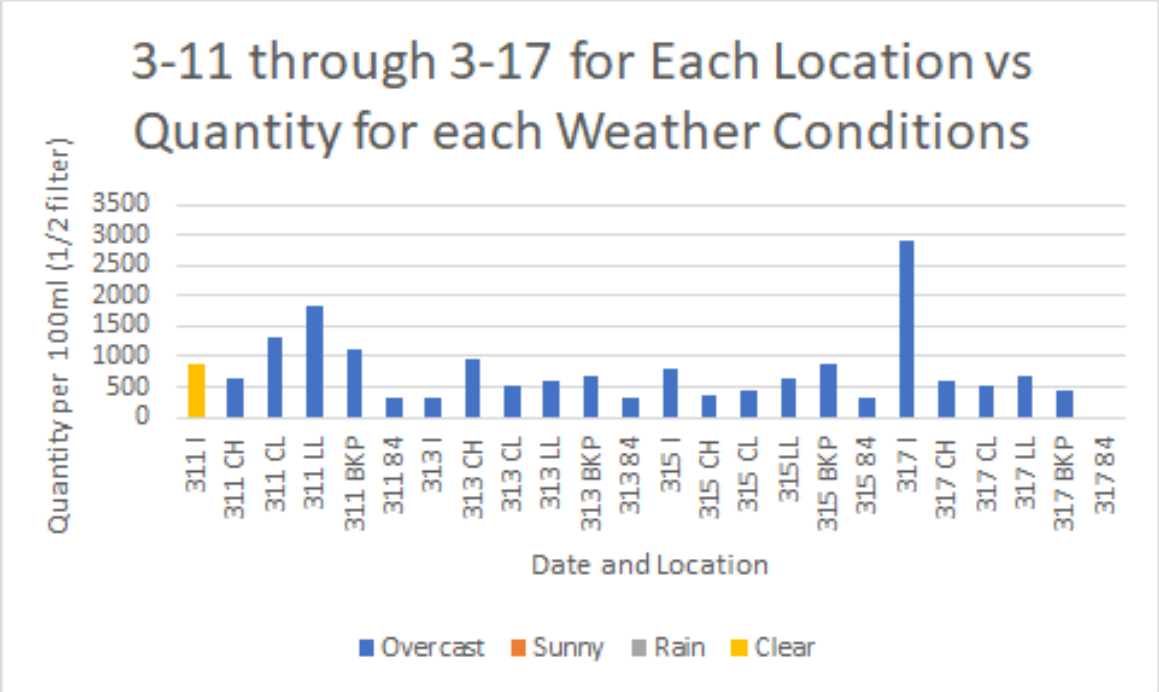

Figure 14: Dates 3-11 through 3-17 for each of the sample locations vs the Quantity per 100ml (1/2 filter) of each weather condition

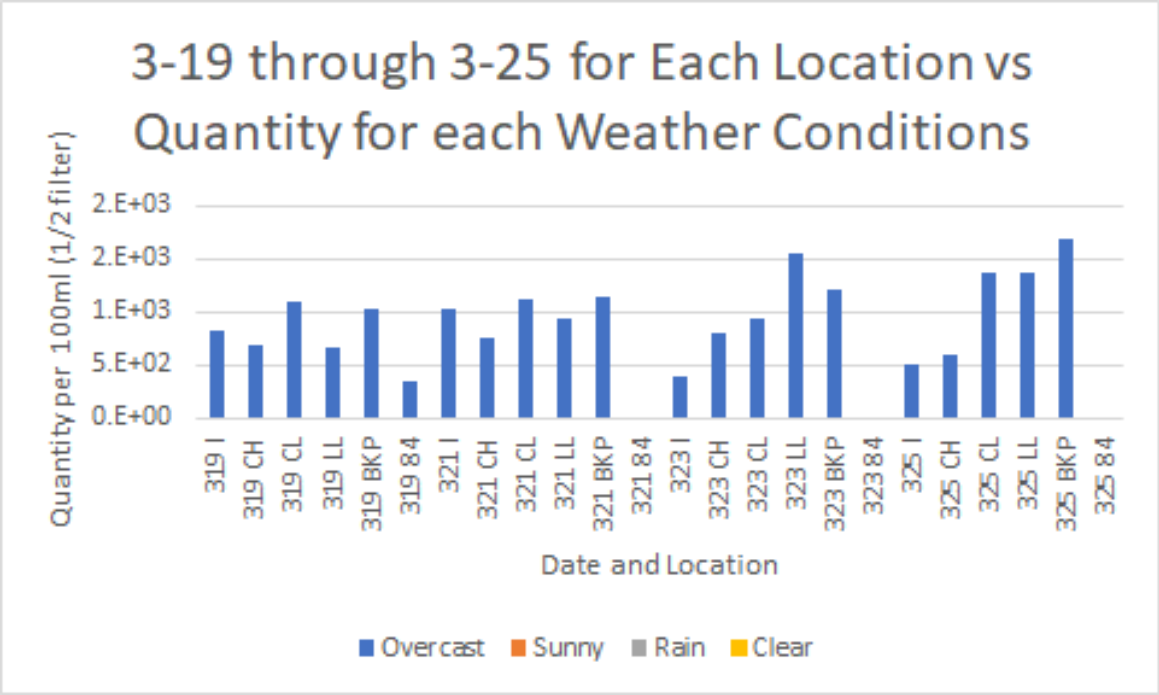

Figure 15: Dates 3-19 through 3-25 for each of the sample locations vs the Quantity per 100ml (1/2 filter) of each weather condition

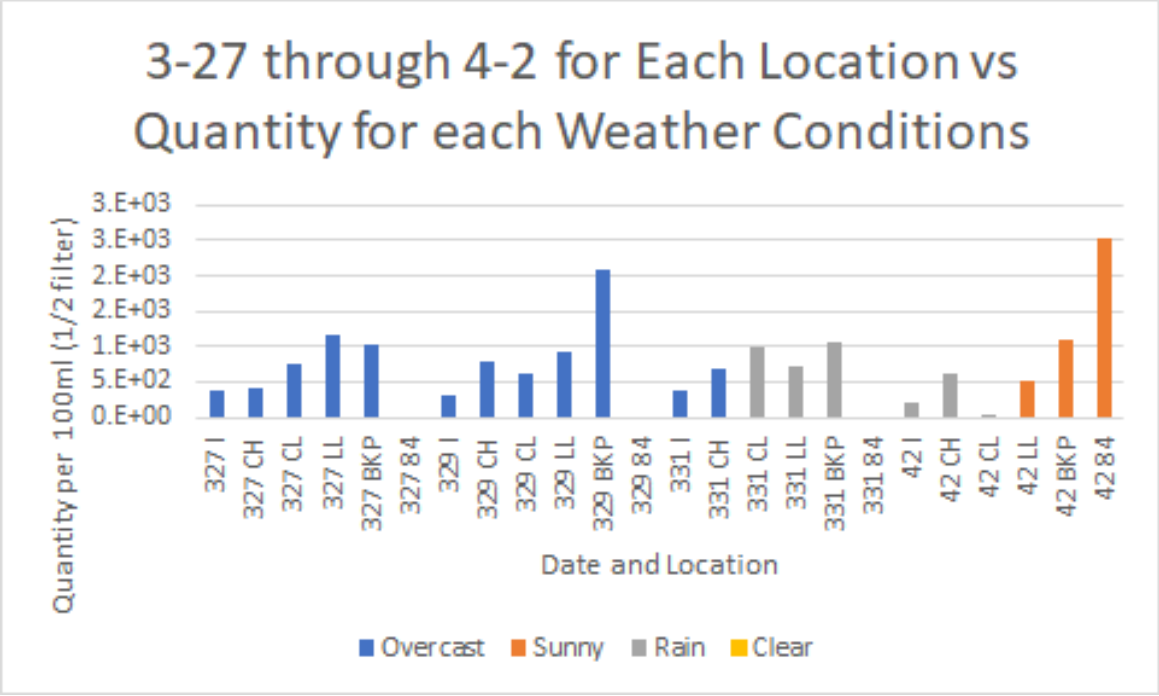

Figure 16: Dates 3-27 through 4-2 for each of the sample locations vs the Quantity per 100ml (1/2 filter) of each weather condition

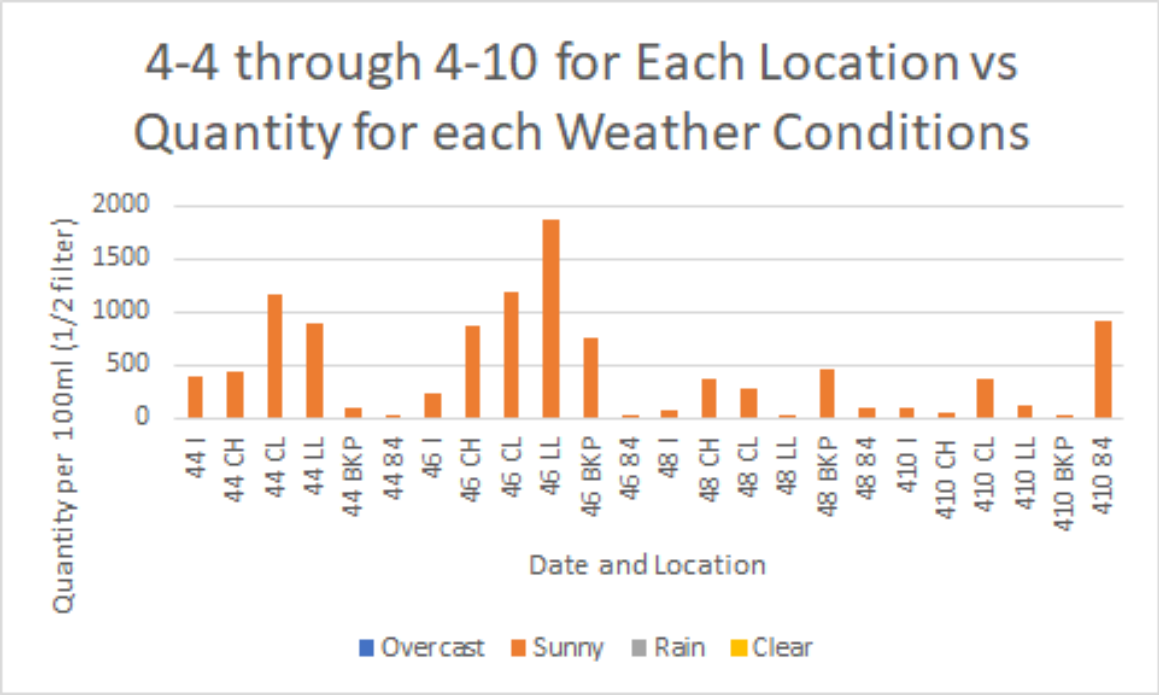

Figure 17: Dates 4-4 through 4-10 for each of the sample locations vs the Quantity per 100ml (1/2 filter) of each weather condition

Discussion

In this pilot study, water samples from six sites along the New River, South Fork New River and North New River Canal were tested for the presence of HF183. Samples were taken every other day for a 30-day period between March 11 through April 10th 2019. By comparing each water sample to a known HF183 sequence standard through amplification and quantification, the samples were compared with respect to one another and then calculated to form a mean value respecting significant figures throughout the procedure. Because this study represented nonpoint fecal sources there was no clear pattern of concentrations of HF183 for any of the sample collection locations or for any of the collection dates. It is important to identify the presence of HF183 in such a prominent area of Broward County, Florida. A risk factor could be calculated by comparing to a general number from Boehm to ascertain the possibility of human fecal pollution in the New River (Boehm, Soller and Shanks, 2015). The value that was used as a human risk threshold was 100 targets/100mL of water collected. As this was a non-validated study the values obtained were only an estimate and ONLY to be used for further study in a fully validated procedure as per EPA method 1696 (Shanks, et. al 2019).

There are pitfalls of this pilot study and one included that all data should have been conducted using the same PCR instrument rather than having two different instruments and therefore a different Mastermix and PCR conditions for different runs. It would have been beneficial to have all the samples run in duplicate using the same instrument. Another option would have been to have all the samples run in triplicate through the Broward County Environmental Lab and in triplicate through the lab at the Oceanographic Center. This would ensure that the data is accurate and add another layer of comparison to qPCR methods applied. Running data in triplicate is standard practice and would ensure more accuracy in the technique being used over running data in duplicate. Analyzing data from both filters would have been another way to confirm accuracy of the data. While these would have been a better way to run this study, funds were a factor and the reasoning behind the limitation of this study.

Understanding the fecal contamination source influencing the water quality is important for risk assessment (Scott et al, 2002). In order to develop an appropriate control, prevention and risk management practice, it is critical to distinguish the original source of the fecal pollution (Jiang et al, 2018). By identifying the difference between human and other animal sources of fecal pollution, steps to remediate pollution and protect human health can be achieved by using a more strategic approach to mitigate pollution problems and preserve a healthy ecosystem (Ahmed et al, 2018a;; Zimmer-Faust et al, 2018; Hughes et al, 2017; Waso, Khan and Kahn 2018). Contamination originating from human sources carries a greater risk to human health than contamination originating from other animal sources. (Scott et al, 2002). It is critical to identify the type of fecal pollution existing in an area.

Identifying the source of the human fecal pollution found in the New River, South Fork New River and North New River Canal should be the next phase of this study. Given the number of recent bursting sewage pipes in Broward County, several in Fort Lauderdale near the beginning of the New River, a before and after comparison should be conducted to determine if sewage pipe leakage could account for the quantity of HF183 at each location. It would be beneficial in future studies to determine where any old sewage pipes might have fed into the New River in relation to the six collection sites in this study. At the time of this study that information was unavailable. New collection locations near old sewage pipes and near boat basins may aid in determining the source of human fecal pollution.

In the 2018 study conducted by Boehm, Graham and Jennings, it was stated that additional experiments under diverse conditions of various surface water would allow them to consider how the risk-based thresholds change under different environmental conditions, the example given was marine water versus fresh water. Human qPCR markers endurance in water can vary depending on several factors, water type, cell state, predation, oxygen, temperature, sunlight, salinity and sediment (Boehm, Soller and Shanks, 2015). Korajkic et al. (2014) found that decay dynamics for HF183 was significantly impacted by sunlight and Green et al. (2011) lists temperature, particulate concentration, particulate size, predation, salinity and sunlight as marker decay factors. Taking samples along the New River, South Fork New River and North New River Canal under various conditions may give insight into the HF183 threshold in Broward County waterways. Suggested collection information is turbidity, oxygen levels and salinity. Taking samples in light vs dark, low vs high tide and during different times of the year to capture different water temperatures is recommended for future studies.

In several studies there was a higher level of HF183 when rain water was present. McGinnis et al found that rainfall along with combined sewer overflows were positively correlated with *Bacteroides*. In similar study done by Ahmed et al (2018b), where concentrations of fecal indicator bacteria and eleven potential bacterial pathogens in stormwater drain outfalls were determined during dry and wet weather periods, it was found that concentrations of microbial parameters were greater in samples collected in wet weather periods compared to samples collected during dry weather periods. Ahmed et al (2018b) suggests age sewage infrastructure and other nonpoint pollution sources may be contributing to fecal pollution load of surface water through stormwater drain outfalls. This study did not have enough wet versus dry conditions to make a comparison, further research should be conducted to determine if this is a factor in the quantity of HF183 found in the New River water.

This is a useful preliminary study in identifying the presence of *Bacteroides* in the New River, South Fork New River and New River canal. While levels of HF183 were not significant in any one particular area based on the sample site, this is a jumping off point for further studies. There are many factors to consider and study to learn more about the source and factors affecting the levels of HF183 in the New River.

Since this is a model for the next phase of the study. Filter membranes of extracts have been kept at -80 degrees centigrade for further study. Important directions were learned from this pilot study.

In the next phase Shanks and Korajkic suggest in their chapter the following benchmarks (Shanks, 2020);

Define the water quality challenge: In the case of the New River there are many challenges including sampling, statistical analysis, and of course the formulation of a definitional hypothesis which answers a specific series of questions.

Identify key influencing factors: The New River gives the researcher a special set of influencing factors which could affect the final results. These include the turbidity, salinity, and weather conditions when the samples are collected. The New River is also subject to low and high tides which in fact may bring in pollutants from the intracoastal waterway and as well wash pollutants into the intracoastal waterway and finally out to the ocean.

Organize a team of students and officials to implement this endeavor: probably the biggest hurdle to overcome is the coordination of public health officials in Broward County. The Broward County Environmental Monitoring Laboratory is beginning to use HF183 in their monitoring operations which is a great sign of progress along with the main analysis vectors E.coli and Enterococcus (Broward County Environmental Monitoring Lab, 2020).

Include appropriate controls: very small quantities of HF183 must be analysed by the qPCR process from 2×10^6 targets (0.07pg) down to 2×10^2 targets (0.00007pg). For a positive finding which may impact and increase the risk factor for swimmers and recreational users of a body of water the finding has to be over 100 targets/100mL of water collected (Boehm, Soller and Shanks, 2015). In Table 11 one can see that many of the collection sites exhibited values at or above this level. Since there was a large relative standard deviation between the two qPCR runs from the same sample the significant figure was set to one. Again, since this is a pilot run this would allow us to estimate if in fact there was HF183 present in the water and further analysis would be worth performing.

Document all procedures in detail; careful analysis should be taken to follow the MIQE guidelines recommended by (Bustin et al, 2009). This would assure the analysis was performed in accordance with scientific transparency, consistency, and integrity.

Employ a standardized procedure; because of the small sample size that is being tested, a rigorous procedure must be validated which is reproducible and robust. One should always conduct blind proficiency testing before reporting results as well (Shanks, 2019). The following found in Shanks, et al (2019) should be applied in ALL cases from an Analytical Chemistry standpoint to assure that the correct answer is given at the end of the analysis:

1. Sample processing control (SPC) sequence: this control is used to measure the efficiency of DNA extraction. Since the quantity of the sample may be extremely small this is used for to monitor and detect substances that may interfere with DNA purification and/or amplification. These errors are generally called stochastic phenomena especially when using very small quantities of target analyte. SPC sequences, sometimes known as extraction and amplification controls are added as part of a total reference DNA solution in equal quantities to all environmental water and method blank (MB) sample filters prior to extracting DNA. Most times Salmon DNA is used for the above purpose.
2. Reference DNA material: a purified, RNA-free and pre-quantified DNA preparation must be used as a standard reference material and an internal amplification control (IAC) as well.
3. Internal amplification control (IAC): this refers to a reference DNA material consisting of a purified, RNA-free and pre-quantified DNA which can be added into the qPCR assay mix to access any amplification interference.
4. No template control (NTC): this control alerts the analyst that reagents and/or the laboratory environment did not introduce amplification or extraction contaminants that could result in false positives. A false positive is known as a type I error which is the most serious error rather than a type II error which may be a false negative. These contaminating target sequences may be introduced during preparation of the reagents and/or plasticware used in the amplification (PCR) process.
5. Method Blanks (MB): These are controls (PCR-grade water samples) are used to guarantee that measurable levels of contaminating target sequences are not present during filtration, DNA extraction, and preparation of the reagents. These controls form the basis of quality assurance (QA) tests/controls.
6. Amplification efficiency (E): E values should range from 0.90 to 1.10 and are calculated from the calibration curve slope as previously mentioned.

7. r^2 is a measure of the variability in the qPCR assay calibration curve as previously discussed. r^2 values should be ≥ 0.980.
8. Lower limit of quantification (LLOQ) also known as the detection limit or lower limit of detection: This is the smallest quantity of analyte that is significantly different from the blank. The goal is to achieve a 99% chance of this value being greater than a blank. This is accomplished by repeating seven to 10 samples (replicates) with a concentration one to 5 times the detection limit calculated from experience of the analyst or documented data (Harris, 2003).

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