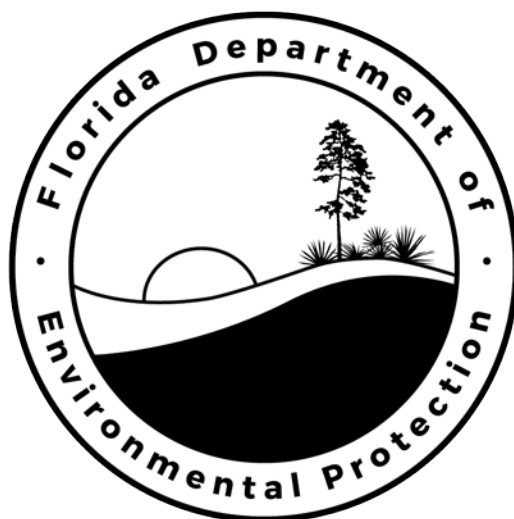


FINAL

*South Fork St. Lucie Estuary and River
Microbial Source Tracking Study*

**Division of Environmental Assessment and Restoration
Florida Department of Environmental Protection**



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Transmittal Letter

This report is intended to provide an evaluation of results from three sampling events that occurred during the wet season in June, July, and August 2014 and the three sampling events that occurred during the dry season in February, April, and early May 2015. The sampling events were performed in coordination with Martin County Utilities in an effort to identify potential bacterial sources contributing to the St. Lucie River and Estuary. The report discusses the main elements of the study design, results, and recommendations.

For additional information concerning laboratory results, contact:

Loretta Wolfe
Florida Department of Environmental Protection
Biology Program
2600 Blair Stone Rd, Mail Station 6515
Tallahassee, Florida 32399-2400
Email: Loretta.Wolfe@dep.state.fl.us
Phone: 850-245-8191

For additional information concerning study design, please contact:

Julie Espy
Florida Department of Environmental Assessment and Restoration
Water Quality Assessment Program
2600 Blair Stone Rd, Mail Station 6515
Tallahassee, Florida 32399-2400
Email: Julie.Espy@dep.state.fl.us
Phone: 850-245-8416

Executive Summary

Martin County requested technical expertise from the Florida Department of Environmental Protection Division of Environmental Assessment and Restoration (DEAR) in a microbial source tracking study on the St. Lucie River and tributaries. Martin County identified 14 tributary and river sites suspected as “hot spots” that may contribute bacterial pollution to the St. Lucie River and Estuary. DEAR provided study design, water quality sampling support, and analytical services to assist Martin County in the identification of bacterial sources to the St. Lucie River and Estuary.

Three separate monitoring events were performed in June, July and August of 2014 for the wet season evaluation. In order to capture possible seasonal influence, three separate monitoring events during the dry season were also performed in February, April, and May 2015. The same chemical and biological markers and bacterial analyses were conducted for all wet and dry season events. The results were evaluated for each season and compared. Environmental factors, such as rainfall and tide that may influence the results were evaluated.

Wet Season Results:

- 12 of the 14 sites had at least one sampling event with elevated bacteria counts.
- 13 of the 14 sites had sucralose, which is an indicator for treated wastewater, detected below treated effluent concentrations.
- All American Ditch was the only site with a sucralose detection $\geq 1.0\mu\text{g/L}$ – a screening level that may be indicative of human-derived wastewater.
- Acetaminophen, which is an indicator for untreated wastewater, was detected at the Marina at Haney Creek and All American Ditch sites.
- The presence of a human fecal source marker (qPCR Bacteroidales HF183) was detected at the Golden Gate, Sandsprit, and All American Ditch sampling locations.

Dry Season Results:

- 5 of the 14 sites had at least one sampling event with elevated bacteria counts.
- All 14 sites had sucralose detections.
- 3 of the 14 sites had sucralose detections $\geq 1.0\mu\text{g/L}$.
- Acetaminophen was detected at 2 of the 14 sites, Sandsprit, and Danforth Creek West.
- The presence of a human fecal source marker (qPCR Bacteroidales HF183) was detected at Golden Gate, Sandsprit, Marina at Haney Creek, Danforth Creek West, and C23 sampling locations.

Fecal coliform counts for this study followed the monthly average seasonal rainfall. In this study, peak fecal coliform counts coincided with, or followed periods of cumulative increased rainfall. This rainfall provides volumes of water that flush through surface soils and stormwater ponds to surface waters. To the contrary, human marker and acetaminophen detections were lower during the wet season. Overall, acetaminophen and human fecal source marker were detected at the same sites.

The differences in the results found between the wet and dry seasons suggest a dilution effect on the chemical markers in the wet season. Acetaminophen was detected more often in the dry

season. However, the detection of the human fecal source marker and fecal coliforms at higher concentrations during the wet season may indicate that the higher water table allows for increased leaching from onsite disposal systems to the waterways.

Introduction

Fecal coliforms, *E. coli*, and *Enterococci spp.* (also known as fecal indicators) persist throughout our environment (Davenport, et. al 1976; Anderson, et al. 2005). They are found in soil (Desmarais et. al 2002) and sediment (Davies, et. al 1995), water, periphyton, and biofilms (Byappanahalli et.al 2003) as well as in human and animal wastes. Ubiquitous persistence in subtropical climates, habitat expansion, and riparian sediments along with human and animal waste are important factors to consider when interpreting consistent fecal indicator occurrences (Anderson, et. al 1997; Byappanahalli, et. al 2003). Fecal indicators, as the name suggests, are not the direct pathogens that cause illnesses in humans. Moreover, bacteria is not a discriminative tool by itself, but can be used in combination with other tools collectively known as microbial source tracking.

Microbial source tracking can be a resourceful water quality assessment tool used when investigating potential sources of pollution. Standard culture-based methods cannot discriminate enteric (from the gut of a host animal) from environmental bacteria (free living bacteria not associated with fecal waste or elevated health risks) (Deng, et.al 2014 and Byappanahalli, et.al 2012). It is in this case where microbial source tracking studies can be most useful because the analytical methods available can discriminate between animal sources, such as deer, birds, dogs, and human sources from environmental sources. Identifying bacterial source helps agencies identify potential problem areas more quickly and better understand and communicate any risks associated with human exposure. For the tools to be useful, the investigator prioritizes sampling sites based upon human activities in the area (i.e. humans recreating in or otherwise exposed to the water) and results of standard culture-based methods. To fully evaluate how the natural elements impact a microbial source tracking study design and, thus, the presence and/or absence of the target analytes, multiple sampling events are required and should occur under a variety of conditions (e.g., wet/dry season, recent rain, high/low water table, seasonal use).

Microbial source tracking tools include the use of chemical indicators or “tracers”. Chemical indicators are fundamental to any source tracking study and serve as a valuable screening tool for potential anthropogenic sources. In the aquatic environment, tracers are compounds that can be tied back to a specific type of source. Of relevance to this study, sucralose and acetaminophen can be used together to trace the degree of influence untreated wastewater may have in a waterbody. In addition, the tracers can be used to differentiate between treated and untreated wastewater. Sucralose can indicate a source of wastewater, but cannot discriminate between treated versus untreated sources. However, the presence of acetaminophen in surface waters suggests the source is much more likely to be untreated wastewater. If sucralose or acetaminophen are detected, further analyses can be conducted to determine if other compounds associated with wastewater are present.

Study Design

Martin County requested assistance from the Florida Department of Environmental Protection's (FDEP) Division of Environmental Assessment and Restoration (DEAR) to support Martin County Utilities and Engineering staff in a microbial source tracking study for the St. Lucie River and tributaries. DEAR provided water quality sampling and analysis to Martin County for the identification of potential fecal pollution sources in St. Lucie River and Estuary using wet season and dry season sampling regime conditions.

Martin County selected 14 tributary and river sites with suspected or confirmed elevated fecal indicator bacteria (**Figure 1**) for sampling during the wet and dry seasons on the following dates:

Wet Season Sampling Dates – June 19, July 22, and August 21, 2014

Dry Season Sampling Dates – February 25, April 9, May 12, 2015

Water samples were collected from all 14 sites during the sampling events for the following parameters:

- Bacterial indicators
 - *E. coli*
 - Enterococci
 - Fecal coliform
- Human fecal waste indicator
 - Bacteroidales – HF183
- Chemical wastewater indicators
 - Acetaminophen
 - Sucralose

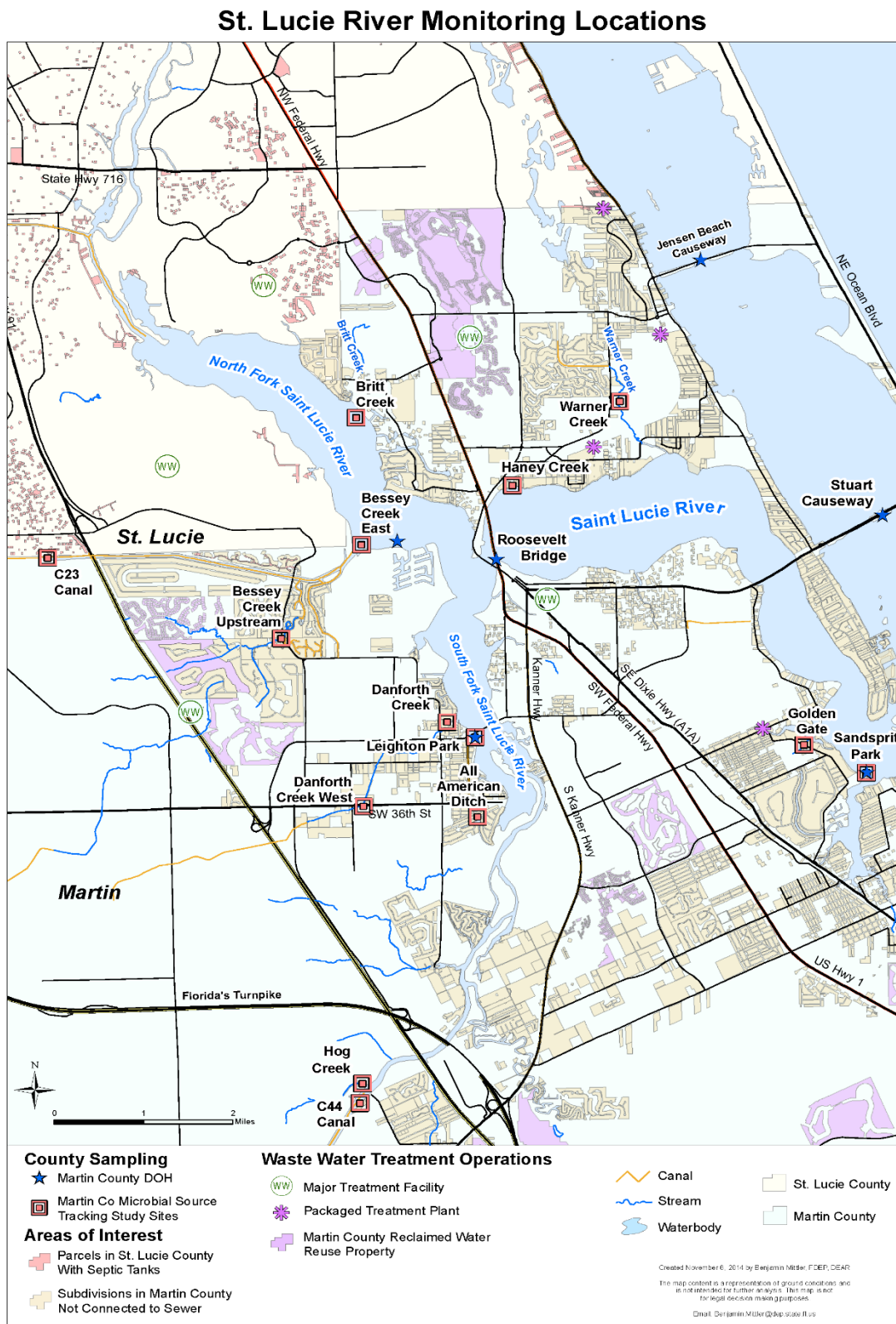
Additionally, the following field parameters were measured at each site:

- Temperature
- Dissolved Oxygen
- pH
- Conductivity
- Salinity

E. coli bacteria were sampled and analyzed for at predominantly freshwater sites and Enterococci were sampled and analyzed for at predominantly saline sites as recommended by EPA's 1986 ambient water recreational bacteria criteria.

Information that is assessed in the evaluation of microbial source tracking results include ancillary, synoptic data such as antecedent rainfall, tides (if appropriate), site photographs, observations of wildlife, homeless camps, or other potential fecal sources, water depth, sampling depth, and the amount of periphyton present. To evaluate human marker results, it is recommended that the sampling design include samples collected and analyzed during periods with and without rainfall preceding the events.

Figure 1. The Map depicts locations of 14 sampling sites along St. Lucie River and tributaries during the wet and dry season events.



When sampling in tidal conditions for source tracking studies, it is recommended that the sampling event occur at the lowest possible tide. Sampling at low tide reduces the likelihood that the analytical results will be confounded by downstream sources. By sampling at low tide, the potential sources contributing to that sample location are most likely upstream. Sampling during incoming or high tides could introduce fecal indicators from downstream sources due to tidal pumping. Sampling at low tide also allows whatever groundwater head pressure there may be to push groundwater into tributaries, further helping to identify what might otherwise be a hidden source. The logistics of sampling at the lowest tide can be complicated given the number of sites, the extent of the area covered, and the distance from the coast. The sampling timeframes relative to the tidal period were taken into account at sites that are considered predominantly marine by Chapter 62-302.200 F.A.C. and tidally influenced. Although the tide was considered for the sampling events, the exact tidal periods were not calculated for each site. Tidal predictions for Stuart, FL (USGS Station Id: 8722357) were considered for the wet season sampling times for all sites. The tidal predictions for Port Salerno, Manatee Pocket, FL (USGS Station Id: 8722383) and Stuart, FL (USGS Station Id: 8722357) were considered for the dry season sampling times. The sampling locations and the corresponding tide station for the dry season events are as follows:

Port Salerno, Manatee Pocket, FL USGS Station Id: 8722383

Golden Gate
Sandsprit Park

Stuart, FL USGS Station Id: 8722357

Britt Creek
Marina at Haney Creek
Bessey Creek East
Danforth Creek
Leighton Park
All American Ditch

Results and Discussion

Microbial source tracking is relatively new to environmental monitoring and future method development studies will enhance the ability of resource managers to better evaluate the results. DEAR's Laboratory analyzed the samples for the human fecal source marker, Bacteroidales HF183, using a qPCR method. The evaluation of the presence of the human marker in this section of the report is based on a wide range of values found in literature and DEAR's recent experience with other microbial source tracking studies.

Fecal coliform, *E. coli*, and Enterococci bacteria were collected and analyzed to determine the pathogen of concern coupled with the targeted human fecal source marker Bacteroidales HF183. The criteria used in this study for Enterococci and *E. coli* are not a part of the State's water quality standards. It should be noted that the results for Enterococci and *E. coli* highlighted in this document are not recommendations for water quality criteria or water quality criteria exceedances that DEP recognizes at this time. Martin County Health Department uses the EPA's

1986 criteria for marine waters to set advisories at the recreational beaches and monitoring sites located on the St. Lucie River. These criteria, however, are not appropriate for the tributary sites used in this study to set advisories. For this study, the criteria for marine and freshwater are used, depending on site conditions, as guidance values to recognize possible contributions to the St. Lucie River relative to those seen at the Martin County Health Department's river monitoring sites. Florida Department of Health Healthy Beaches Program Categories for Enterococci are used for marine waters and are as follows:

Enterococci (cfu /100 ml)

- Good: 0 – 35
- Moderate: 36 – 104
- Poor: 105 or more

E. coli is not a FDOH Healthy Beaches Monitoring Program parameter; however, EPA has criteria for *E. coli* and the same method for using the Healthy Beaches Program descriptive categories were applied for this study. The categories are as follows:

E. coli (cfu /100 ml)

- Good: 0 to 125
- Moderate: 126 to 235
- Poor: 235 or more

The criteria for fecal coliform used is from the State's surface water quality criteria Chapter 62-302.530 F.A.C. A site is defined as having an exceedance or listed as "poor" if it exceeds the daily criteria of >800 cfu /100 ml.

The chemical markers, sucralose, and acetaminophen were collected and analyzed from the 14 selected sites. These markers are used to assist in the determination of the presence of treated and untreated human wastewater. Acetaminophen (brand name Tylenol™) breaks down in wastewater treatment processes and therefore, rarely passes through to the waterbody via discharges in detectable concentrations. The detection of acetaminophen indicates a potential untreated sewage source such as sanitary and combined sewer overflows, illicit discharges, malfunctioning septic systems, and/or boating waste (USGS 2002, Phillips, et al. 2009). On the other hand, sucralose, an artificial sweetener commercially marketed as Splenda™, passes through treatment systems without degrading or mixing with the biosolids (Labare et al., 1993). Research has demonstrated that biodegradation of sucralose is a slow process with limited microbial action (Labare et al., 1993); therefore, it can be found in ambient waters that are influenced by reuse water. In a recent statewide study, DEP found sucralose in the 10 to 40 parts per billion (µg/L) range in the final effluent of 50 wastewater treatment plants with the results correlating with concentrations found in re-use/reclaimed waters. A sucralose detection ≥ 1.0 µg/L indicates a high likelihood that greater than 1% of the volume of sample water is composed of human-derived wastewater. DEP has not conducted studies on levels of sucralose concentrations expected in septic tank leachate. For reporting the results for this study, sucralose concentrations ≥ 1.0 µg/L will be referred to as significant detections. The 0.1 – 1.0 µg/L range is referred to as moderate and < 0.1 µg/L is referred to as low concentrations. This nomenclature is for descriptive purposes and does not dictate a threshold for sucralose values.

Sampling was conducted by DEAR and analyzed for chemical markers along with bacterial and fecal indicators. Results from all 14 study sites can be found in Appendix A and B for the wet season and dry season, respectively. The result qualifier codes and the bacterial exceedance thresholds used to evaluate the results can be found in Appendix C and D respectively. The detections that occurred in the dry and wet seasons are shown in **Table 1**. Overall, the human fecal source marker and acetaminophen were detected at Marina at Haney Creek, Golden Gate, Sandsprit, Danforth Creek West, and All American Ditch. Finding both the human fecal source marker and acetaminophen at the same site indicates a high likelihood of untreated human wastewater contamination at the sampling sites.

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Table 1. The table below shows how many bacteria exceedances and human and chemical marker detections occurred at each location during the wet and dry season. A number included with the “wet” or “dry” text (ex. Wet (1)) indicates the number of detections or exceedances. Sites that are in bold had human fecal source marker detection(s).

Site Name	Exceedance		Detection		
	E. coli or enterococci	Fecal coliform	Bacteroidales HF 183	Acetaminophen	Sucralose $\geq 1\text{ppb}$
Britt Creek	wet(1)*	none	none	none	none
Marina at Haney Creek	none*	wet(1)	dry(1)	wet(1)	none
Warner Creek	none	Wet(1)	none	none	dry(2)
Golden Gate	dry(3)*	dry(2)	wet(2)/dry(3)	none	dry(2)
Sandsprit	none	none	wet(1)/dry(2)	dry(3)	none
C23	none	none	dry(1)	none	none
Bessey Creek Upstream	wet(2)* / dry(3)	none	none	none	none
Bessey Creek East	wet(1)	none	none	none	none
Danforth Creek	wet(3)* / dry(3)	wet(1)/dry(1)	none	none	none
Leighton Park	wet(2)	wet(1)	none	none	none
Danforth Creek West	wet(1)/dry(2)	dry(1)	dry(1)	dry(1)	none
All American Ditch	wet(3)	wet(3)	wet(1)	wet(1)	dry(1)
C44	none	wet(1)	none	none	none
Hog Creek	wet(1)	wet(1)	none	none	none

*DEP was unable to obtain test results for enterococci for these sites for the July sampling event.

Rainfall was obtained from Martin County Waste Water Treatment Plants located on the northern and southern region of the sampling area. Monthly averages for years 2000 – 2014 were used to compare the wet and dry season results (**Figures 2-3**). The wet season is considered May – October and the dry season is considered November – April. The rainfall charts show discreet transitional periods in May and October. Using the rainfall data from the two waste water facilities, North and Tropical Farms, it was possible to compare average monthly rainfall over the years 2000-2014 with fecal coliform, enterococci, and *E. coli* exceedance rates for this study (**Figures 4 and 5**). In this study, peak bacteria counts coincided with or followed periods of increasing rainfall. Rainfall provides water volumes that flush through surface soils and stormwater ponds to surface waters.

Figure 2. Average Monthly Rainfall from Tropical Farms Waste Water Treatment Plant 2000 – 2014.

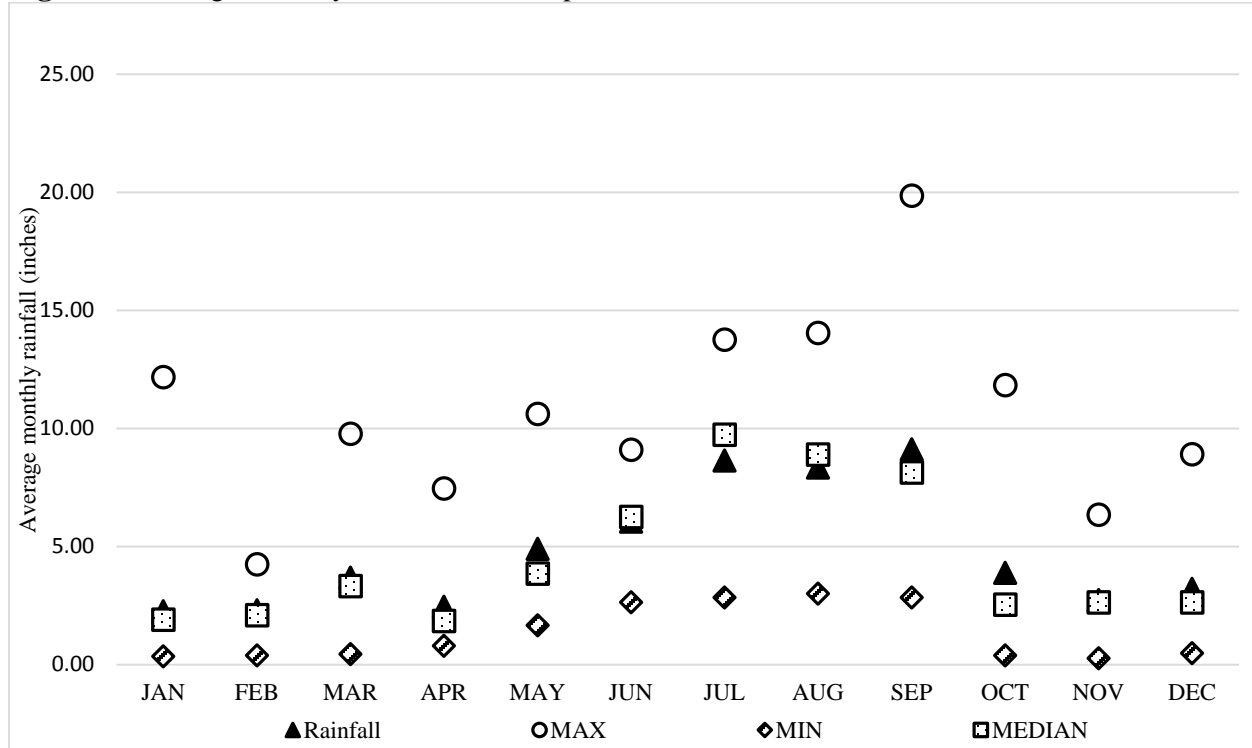


Figure 3. Average Monthly Rainfall from North County Waste Water Treatment Plant 2000 – 2014.

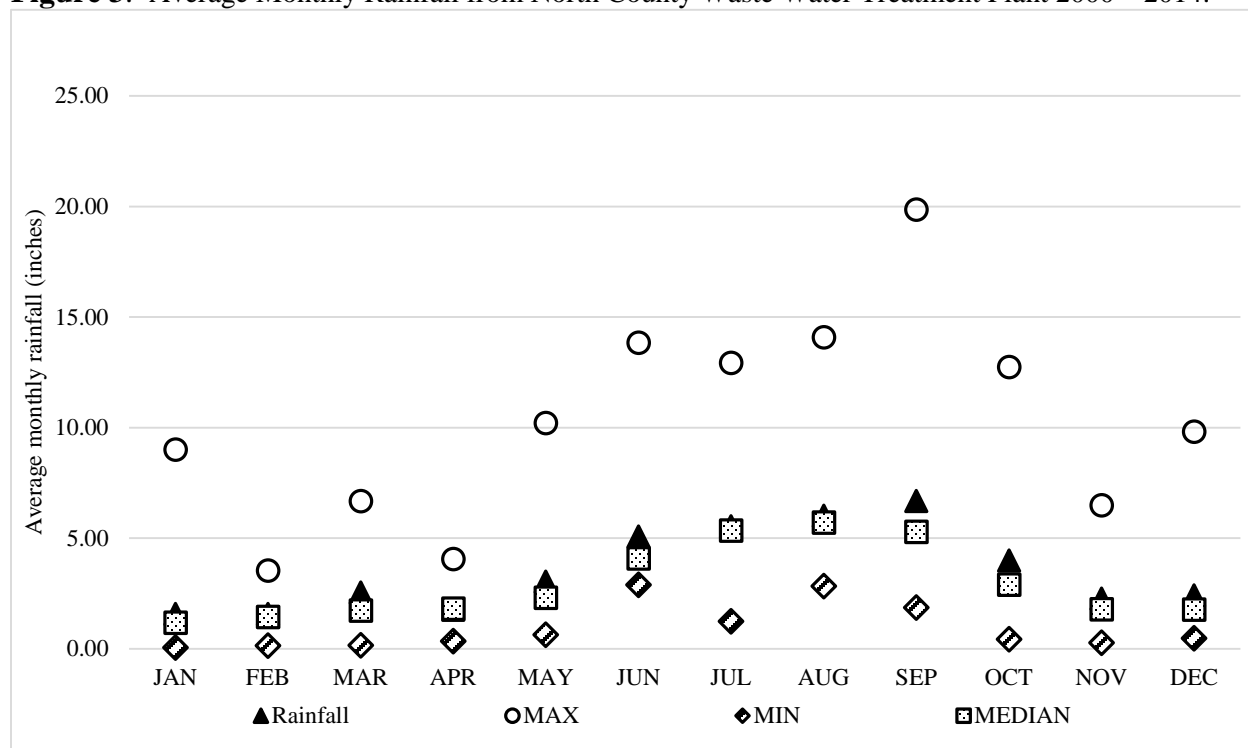


Figure 4. Fecal coliform Exceedances at Martin County Microbial Source Tracking Sites and Average Monthly Rainfall (2000 – 2014) from Tropical Farms and North County Waste Water Plants.

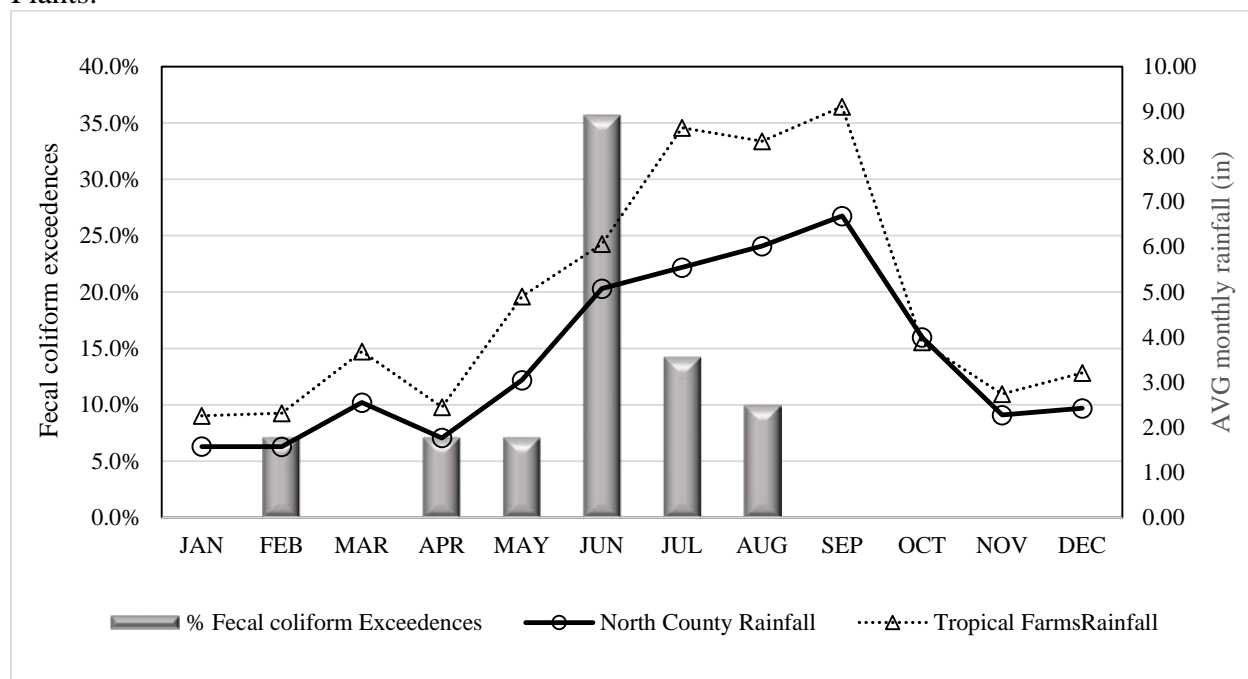
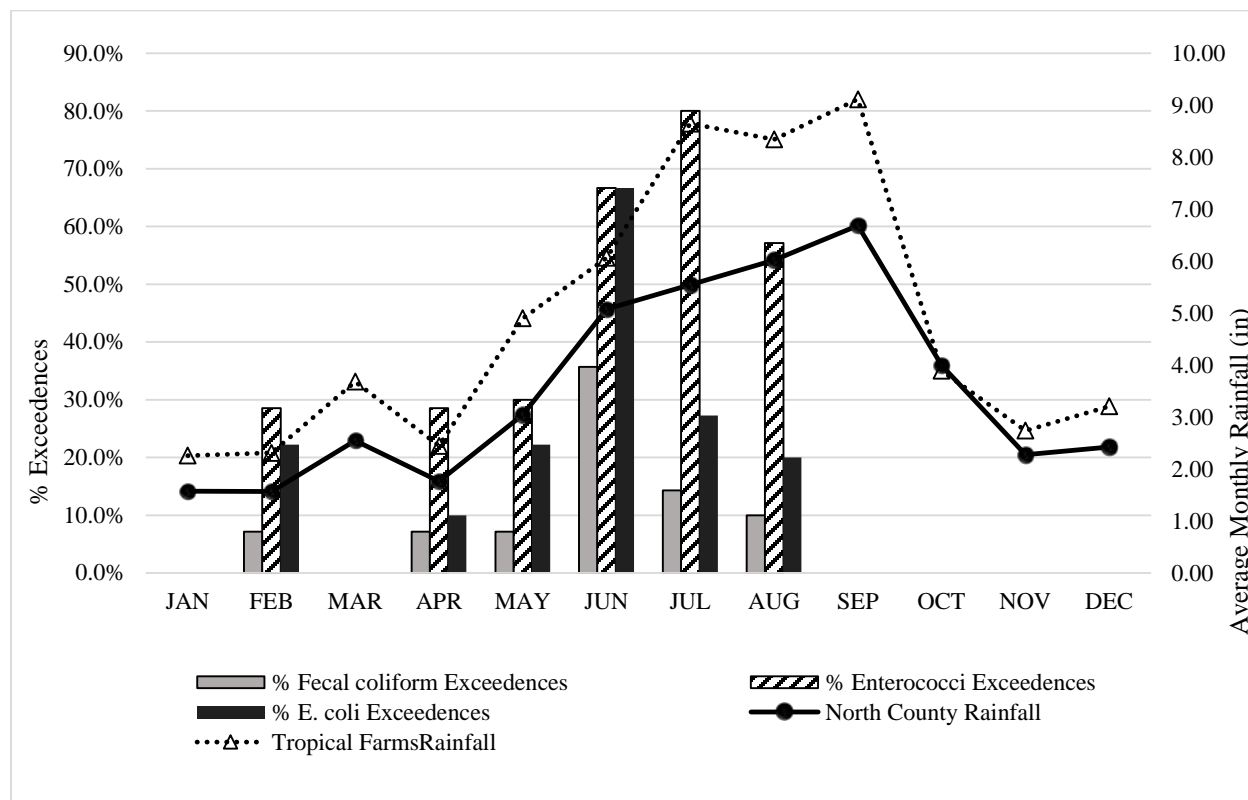


Figure 5. Bacteria Exceedances at Martin County Microbial Source Tracking Sites and Average Monthly Rainfall (2000 – 2014).



Acetaminophen, sucralose, and human fecal source marker detections were more frequent during the dry season (**Figure 6 - 7**). However, the amount of human marker found in 3 samples was much higher in the wet season whereas very low levels were detected in the dry season. Acetaminophen detection occurred with the human marker at 4 sites in the wet and dry seasons (**Table 1**). Finding fewer detections of acetaminophen may be due to a dilution by stormwater in the wet season. It is also worth mentioning that though the reported value of acetaminophen for most samples is associated with the I-qualifier, the detection is of importance. Significant sucralose detections ($\geq 1.0 \mu\text{g/L}$) occurred at 3 out of 14 sites; at one site in April (dry season) and at two sites during the May event (**Table 1; Appendix B**). The sucralose concentrations appear to increase noticeably during the dry season. It was detected at levels $> 1.0 \mu\text{g/L}$ at least once per site during the wet season.

Figure 6. Human and Chemical Marker and Average Monthly Rainfall (2000 – 2014)

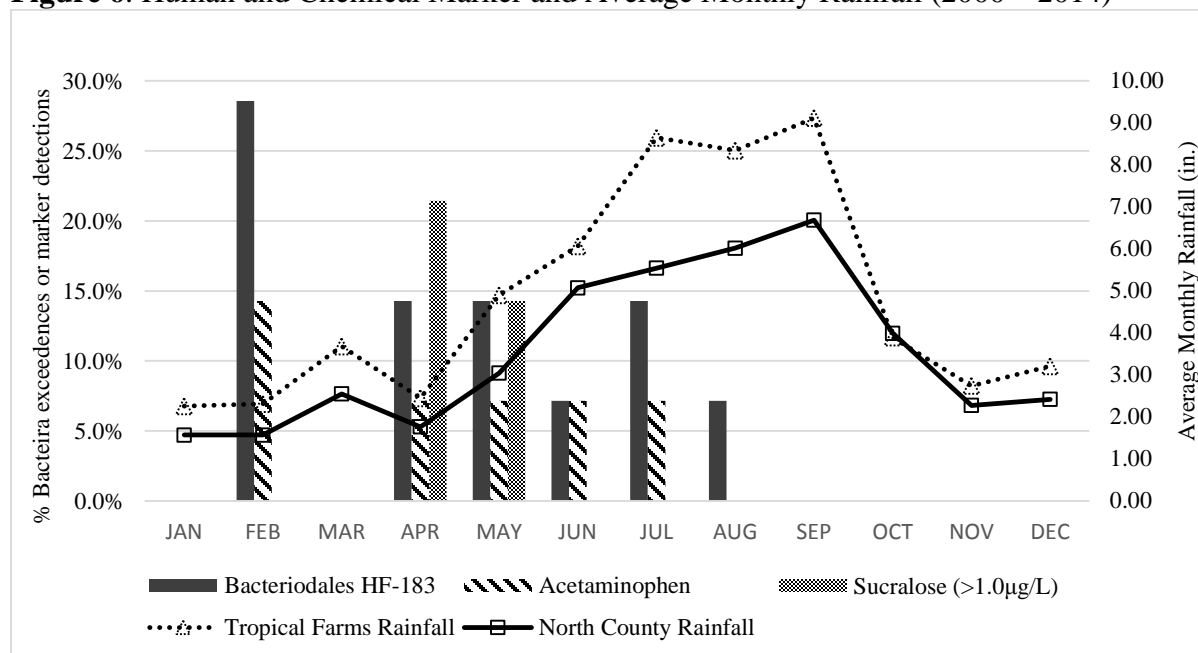
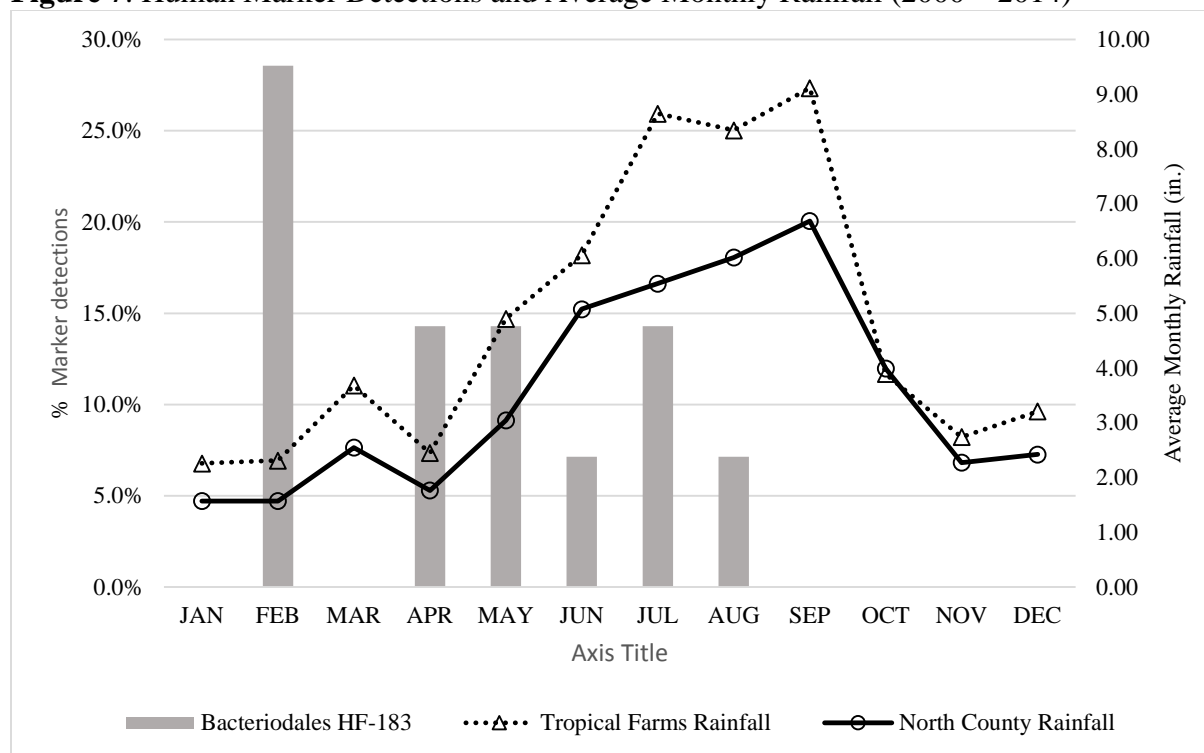


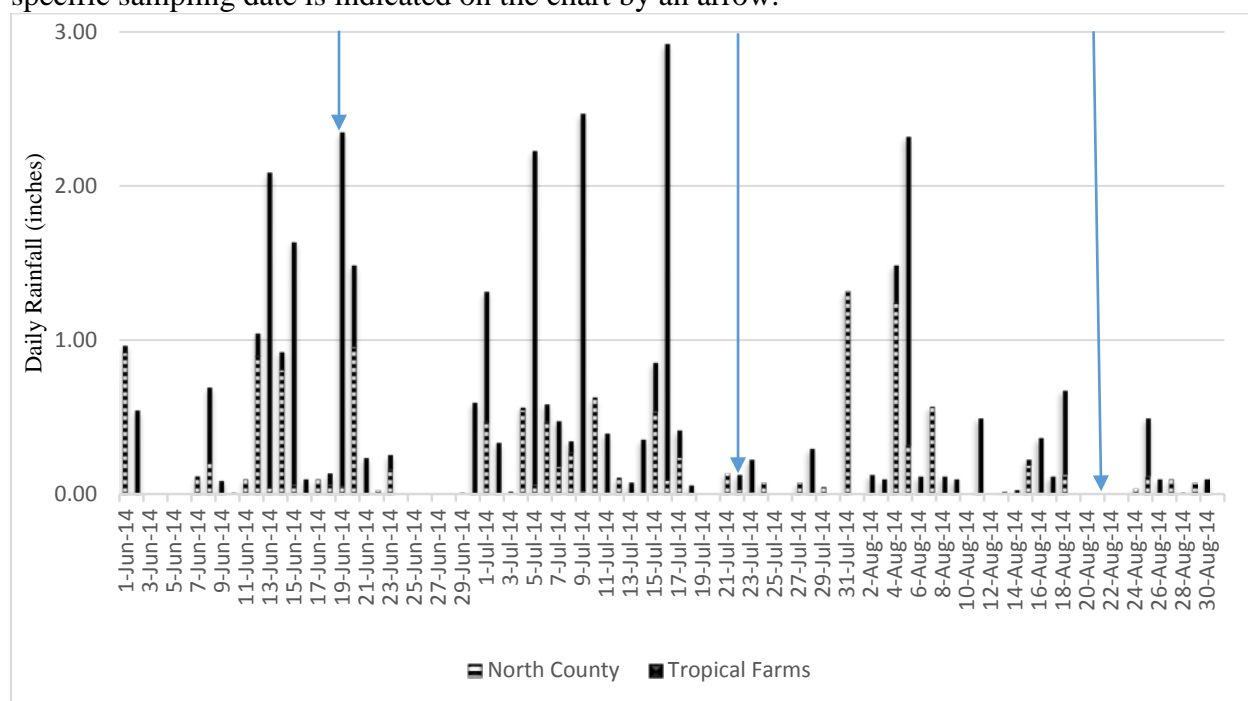
Figure 7. Human Marker Detections and Average Monthly Rainfall (2000 – 2014)



Wet Season - Site Specific Results

The average rainfall for the period of record of 2000 – 2014 June – August wet season sampling months is 5.07, 5.54, and 6.02 inches, respectively, as recorded at North County and 6.06, 8.65, and 8.34 inches, respectively, as recorded at Tropical Farms. Rain fell a couple of inches less than average during June – August 2014. Sample dates occurred when the rainfall happened to be at the lowest (**Figure 8**) tide for that month. High fecal indicator levels following rainfall may indicate surface runoff as a significant source of fecal contamination, whereas elevated fecal indicator levels during low rainfall periods may indicate direct discharges to a waterbody (*e.g.*, illicit drain pipes, septic drain field leachate).

Figure 8. Daily rainfall reported from two Martin County wastewater treatment facilities. The North County facility is located in the northeast sampling area. The Tropical Farm facility is somewhat centrally located in the sampling area. Facility locations are depicted on **Figure 1** relative to the monitoring sites. Unit measurement for rainfall is inches. Rainfall record for the specific sampling date is indicated on the chart by an arrow.



Figures 9a – c. Tide charts for the St. Lucie River at Stuart, Florida for each wet season sampling date. The timeframe of each sampling event is identified in each chart within the 2 vertical lines. Charts were obtained from <http://tidesandcurrents.noaa.gov>. The June event was taken closest to the lowest tide (**Figure 9a**). The July and August events were sampled at the early phases of the falling tide (**Figures 9b-c**).

Figure 9a. Tide chart for sampling event occurring on June 19, 2014. Sampling timeframe 8:35am – 12:20pm.

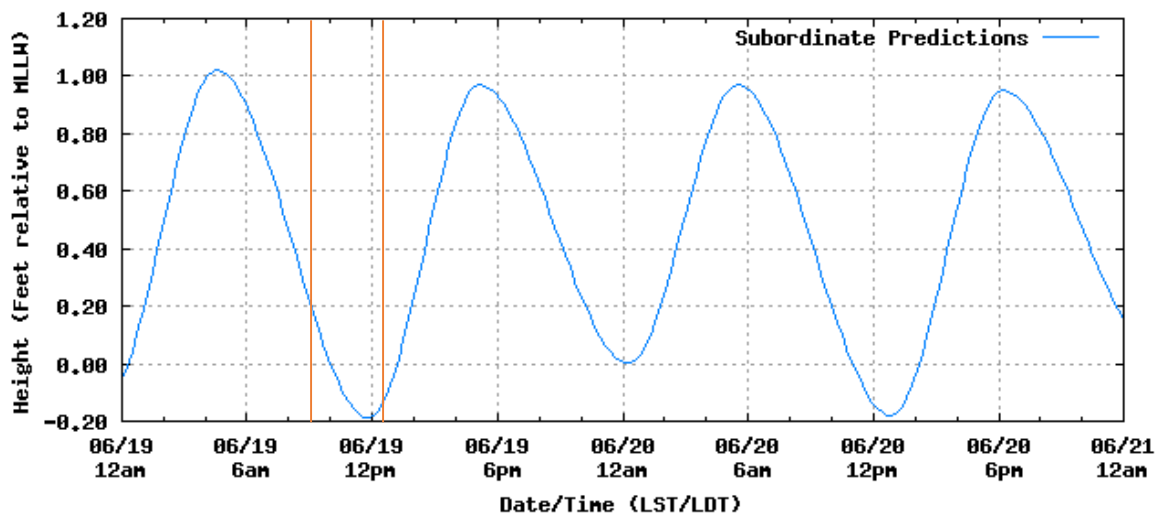


Figure 9b. Tide chart for sampling event occurring on July 22, 2014. Sampling timeframe 9:10am – 10:59am.

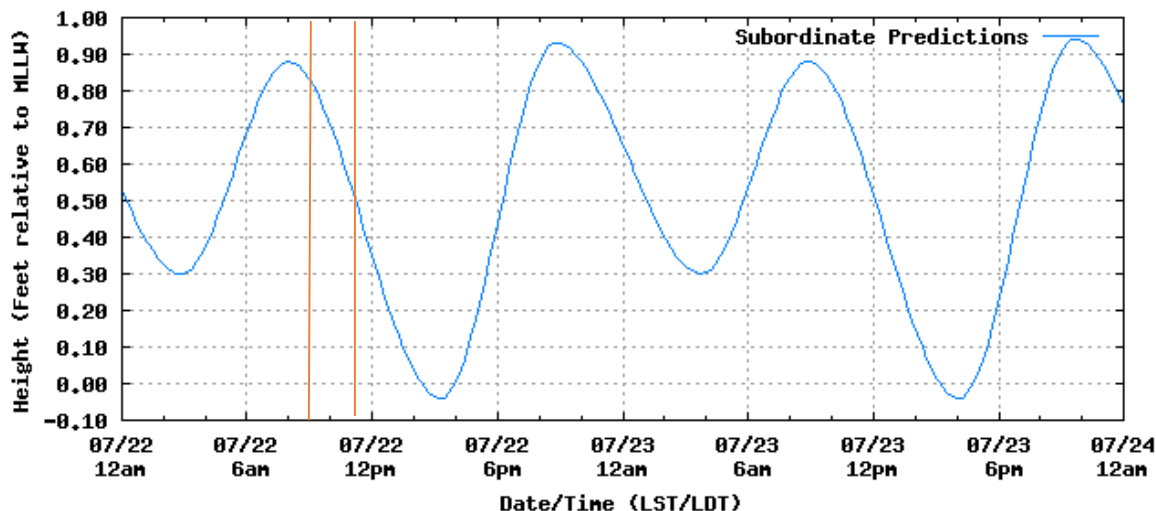
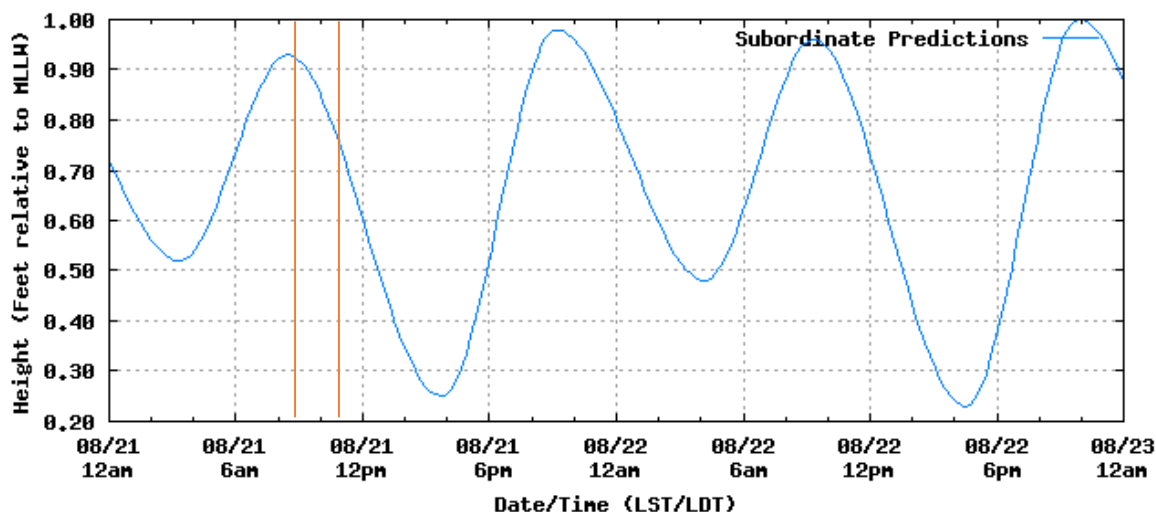


Figure 9c. Tide chart for sampling event occurring on August 21, 2014. Sampling timeframe 8:45am – 10:50am.



Golden Gate (Tables 1 and 2; Appendix A)

The Golden Gate site is considered predominantly marine. The samples were taken during almost slack tide in August and falling tide in June and July. The wet season samples collected at the Golden Gate site indicated moderate levels of sucralose detections between 0.1 – 1.0 µg/L with the presence of elevated bacteria counts from each June, July, and August sampling events. The presence of the human fecal source marker was detected at a moderate range along with significant sucralose detections in the samples collected during the July and August events. The combination of consistently elevated counts of fecal coliform bacteria and the presence of the human marker is indicative of a source of some level of untreated human wastewater.

Marina at Haney Creek (Tables 1 and 2; Appendix A)

The Marina at Haney Creek site is a predominantly marine system. Sucralose was detected at a moderate level in July and very low levels in June and August. Fecal coliforms exceeded the State's maximum daily threshold for a single sample of >800 cfu /100ml (62.302.530 F.A.C.) in August, but were lower in June and July. Enterococci counts were in the moderate range (36 – 104 cfu /100ml) in June and August. Acetaminophen was detected in July at this site, but there were neither bacteria exceedances nor a human marker detection. The human marker was not detected during any of the wet season events at this site.

All American Ditch (Tables 1 and 2; Appendix A)

The All American Ditch site is a predominantly freshwater system, therefore tide was not a factor in the analysis of the results. Sucralose was detected in the moderate range in June and July and at the low range in the sample collected during the August event at this site. Fecal coliforms and *E. coli* were elevated in samples from all sampling events at this site. Acetaminophen and the human marker were detected in the sample collected in June. The

human marker was not detected at the All American Ditch site in July or August. The combination of consistently elevated counts of bacteria, the presence of the human marker, and detections of acetaminophen is indicative of a source of some level of untreated human wastewater.

Sandsprit Park (Tables 1 and 2; Appendix A)

Sandsprit Park is considered predominantly marine. Sucralose was detected at very low levels. Neither fecal coliform nor enterococci bacteria exceeded thresholds, nor was acetaminophen detected during any of the sampling events. The presence of the human marker had a moderate detection in the sample collected during the July event.

Table 2. Descriptive results for the wet season sites with human marker detections. Results with a dash (-) indicate the analysis was not performed.

Sample Date	Parameter	Golden Gate	Marina at Haney	All American	Sandsprit
6/19/2014	<i>E. coli</i> ¹	-	-	Poor	-
6/19/2014	Enterococci ²	-	Moderate	Poor	Good
6/19/2014	Fecal coliform ³	-	Good	Poor	Good
6/19/2014	Bacteroidales HF183	-	Undetected	Detected	Detected
6/19/2014	Acetaminophen	-	Undetected	Detected	Undetected
6/19/2014	Sucralose ⁴	-	Low	Significant	Low
7/22/2014	<i>E. coli</i>	Poor	Good	Poor	-
7/22/2014	Enterococci	-	Good	Poor	Good
7/22/2014	Fecal coliform	Poor	Good	Poor	Good
7/22/2014	Bacteroidales HF183	Detected	Undetected	Undetected	Detected
7/22/2014	Acetaminophen	Undetected	Detected	Undetected	Undetected
7/22/2014	Sucralose	Moderate	Moderate	Moderate	Low
8/21/2014	<i>E. coli</i>	-	-	Poor	-
8/21/2014	Enterococci	Poor	Moderate	-	Moderate
8/21/2014	Fecal coliform	Poor	Poor	Poor	Good
8/21/2014	Bacteroidales HF183	Detected	Undetected	Undetected	Undetected
8/21/2014	Acetaminophen	Undetected	Undetected	Undetected	Undetected
8/21/2014	Sucralose	Moderate	Low	Low	Low

¹*E. coli* (cfu/100ml): good (0 – 125), moderate (126 – 235), poor (236 or more)

²Enterococci (cfu/100ml): (0 – 35), moderate (36 – 104), poor (105 or more)

³Fecal coliform (cfu/100ml): good (≤800), poor (>800)

⁴Sucralose (µg/L): Low (<0.1), moderate (0.1 – 1.0), significant (>1.0)

Warner Creek (Table 1; Appendix A)

The Warner Creek site is predominantly freshwater. Detections of sucralose occurred in the moderate range in the samples collected in June and July and low in August. *E. coli* counts remained low during the wet season. Fecal coliform counts were also low in June and July, but raised in August. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Bessey Creek Upstream (Table 1; Appendix A)

Bessey Creek Upstream is considered a predominantly freshwater site. Sucralose was detected in the moderate range in the samples collected during each event of the wet season. *E. coli* counts were elevated during June and lower in July and August. Fecal coliform counts exceeded the maximum single sample daily threshold (>800 cfu /100ml) in August. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Bessey Creek East (Table 1; Appendix A)

Bessey Creek East is considered predominantly marine. Low level detections of sucralose occurred in each of the samples collected during the wet season. Enterococci counts were elevated and fecal coliform counts were below the threshold for all the samples collected during the wet season. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Danforth Creek (Table 1; Appendix A)

The Danforth Creek site is considered predominantly freshwater. Sucralose was detected in the moderate range in samples collected at this site in June and July and in the low range in August. *E. coli* counts were above the threshold in each of the samples collected during the wet season. Fecal coliform counts exceeded the maximum single sample daily threshold (>800 cfu /100ml) in July. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Danforth Creek West (Table 1; Appendix A)

The Danforth Creek West site is considered predominantly freshwater. Sucralose was detected in the moderate range in July at Danforth Creek West and at a low concentration in June and August. *E. coli* counts were above the threshold in June and lower in July and August. Fecal coliform counts did not exceed the threshold in June, July, and August. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Leighton Park (Table 1; Appendix A)

Leighton Park is considered a predominantly marine site. Sucralose was detected at low concentrations throughout the wet season at this site. Leighton Park had very high enterococci and fecal coliform bacteria results in June (13900 and 39000 cfu /100 ml, respectively). Enterococci counts dropped in July and were even lower in August. The fecal coliform bacteria counts dropped significantly in July and August. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

C44 Canal (Table 1; Appendix A)

C44 Canal is considered a predominantly freshwater site. Sucralose was detected at a low concentration in June and August was not detected in July. *E. coli* counts were elevated in the sample collected in June, but lower in the samples collected in July and August. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

C23 Canal (Table 1; Appendix A)

C23 Canal is considered a predominantly freshwater site. Sucralose was detected at a low level in July and undetected in August and June. *E. coli* and fecal coliform counts were low during all sampling events of the wet season. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Britt Creek (Table 1; Appendix A)

Britt Creek is considered a predominantly marine site. Sucralose was detected at low levels in all of the samples collected during the wet season. Enterococci counts were low in the sample collected in June and increased in August. Fecal coliform counts remained below the threshold. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

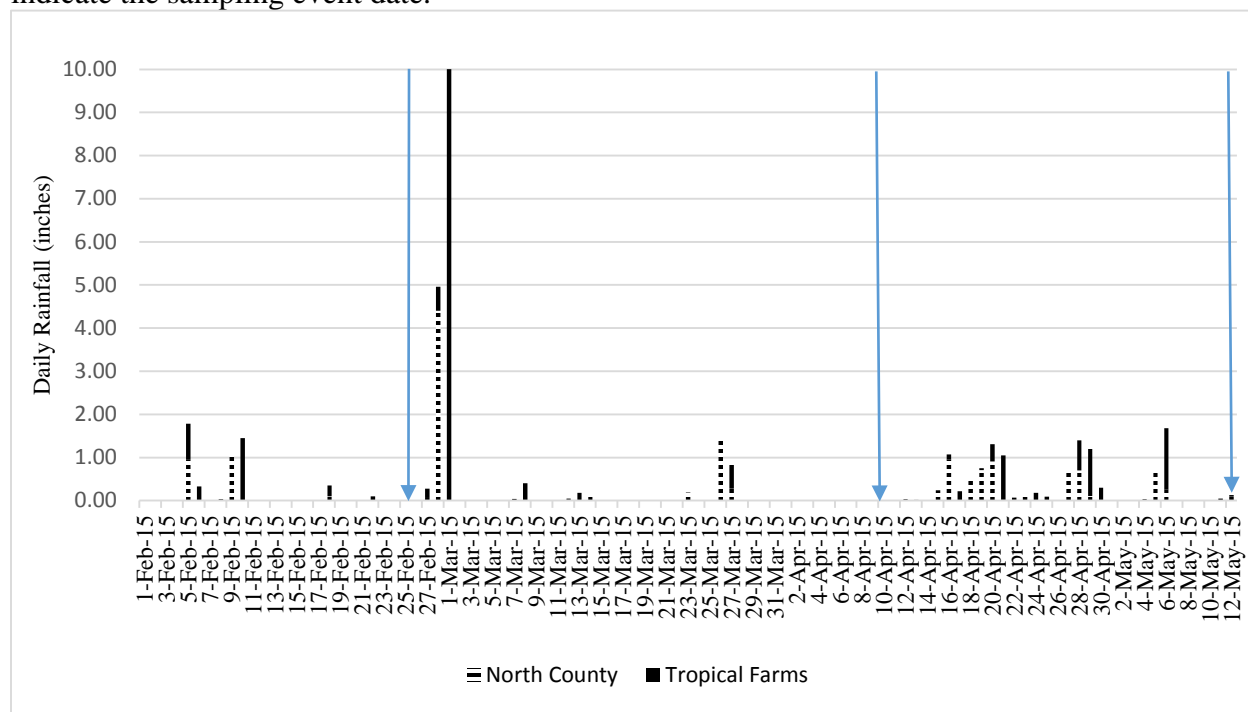
Hog Creek (Table 1; Appendix A)

Hog Creek is considered a predominantly freshwater site. Sucralose was not detected in the sample collected in June and was detected in low levels in the samples collected in July and August. *E. coli* counts were elevated in the sample collected in June and decreased in the samples collected in July and August. Fecal coliform counts varied throughout the wet season. The fecal coliform counts were above the threshold in the sample collected in June, but below the threshold in July and in the moderate range in August. Neither acetaminophen nor the human fecal source marker were detected in the wet season at this site.

Dry Season

The rainfall activity was as expected of the dry season except for the storm activity on February 28, the North County facility reported 4.40 inches and Tropical Farms reported 10.08 inches on March 1, 2015. The 10.65 inch rainfall at Tropical Farms occurred on March 1, 2015 was the highest recorded for that month in the record from 2000 – 2015. The sample design prescribed that samples be taken at the lowest rainfall; therefore the planned sampling event for early March was delayed to April 9, 2014. The March heavy rain event is not expected to influence any results since the next sampling event did not occur until April. No rain occurred the week preceding the February 21, 2015 or April 9, 2015 sampling events (**Figure 10**). There was a very small amount of rain in the monitored area (0.21 inches) in the preceding week and day of the May sampling event.

Figure 10. Martin County Utilities Daily Rainfall Report February – May 12, 2015. The arrows indicate the sampling event date.



Tide charts for the St. Lucie River at Stuart, Florida (Station Id 8722357) for each dry season sampling date at each of the corresponding sites are shown in **Figures 11a – c**. The timeframe of each sampling event is identified in each chart within the 2 vertical lines. During the dry season, the samples were taken at states of falling near low tide and early phase of a rising tide. Charts were obtained from <http://tidesandcurrents.noaa.gov>.

Figure 11a. Tide chart for Stuart, FL Station Id 8722357 for corresponding sites sampled on February 25, 2015. Sampling timeframe 10:50am – 12:35pm.

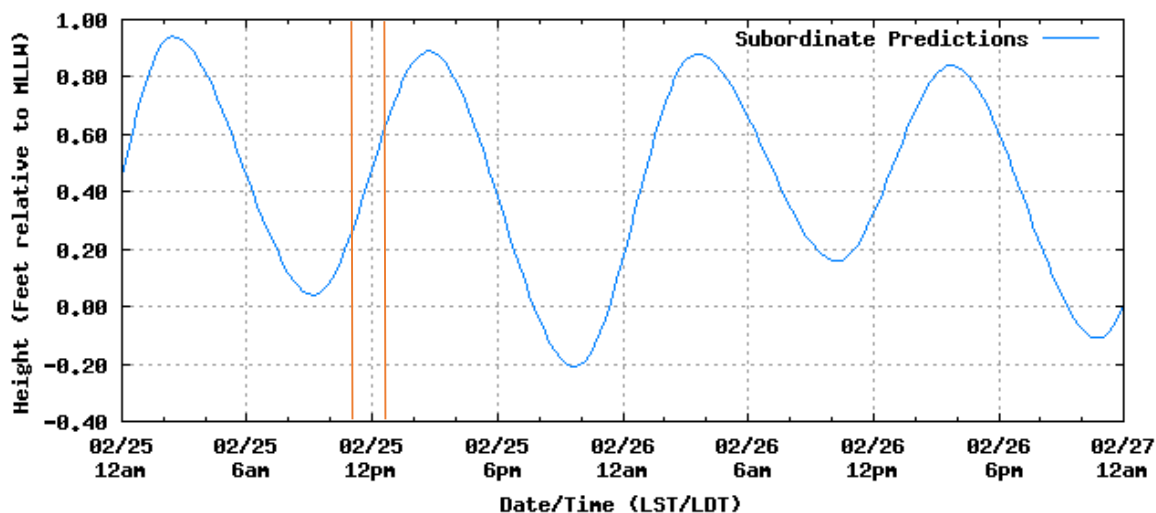


Figure 11b. Tide chart for Stuart, FL Station Id 8722357 for the corresponding sites sampled on April 9, 2015. Sampling timeframe 8:50am – 10:45am.

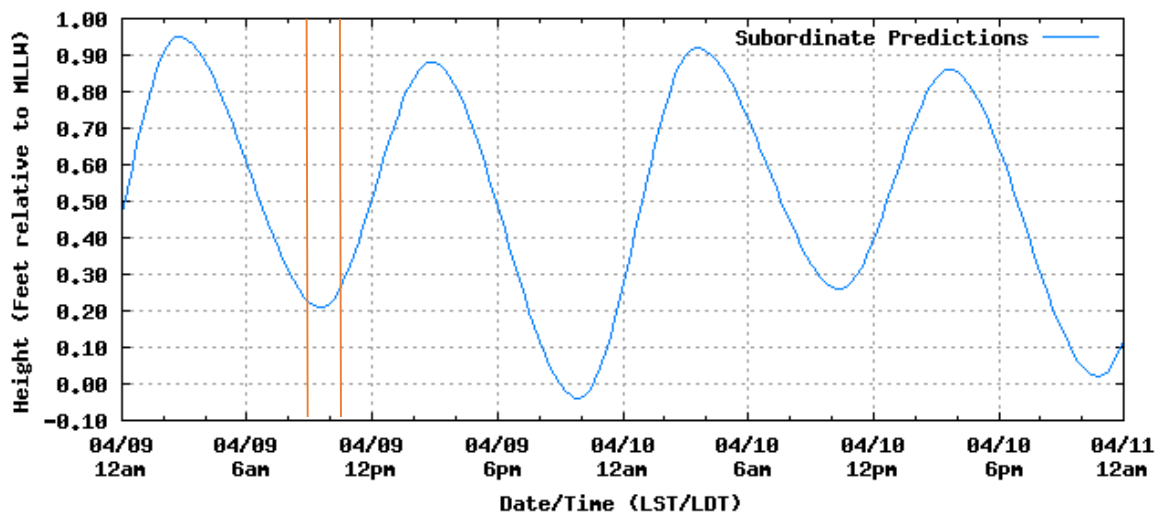
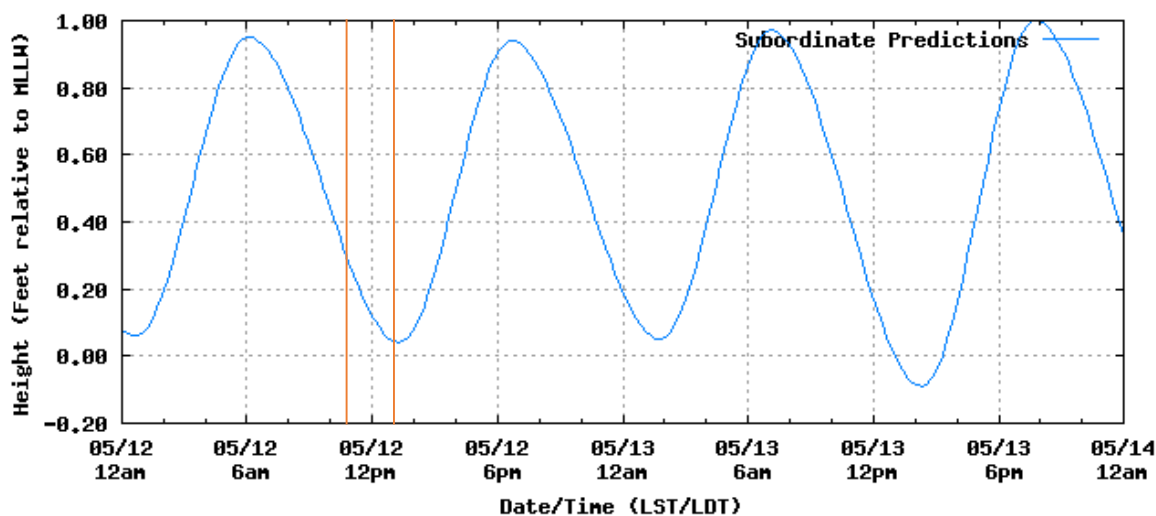


Figure 11c. Tide chart for Stuart, FL Station Id 8722357 for the corresponding sites sampled on May 12, 2015. Sampling timeframe 10:40am – 12:45pm.



Tide charts for Port Salerno, Manatee Pocket, FL Station Id: 8722383 for each dry season sampling date at the corresponding sites are shown in **Figures 12a – c**. The timeframe of each sampling event is identified in each chart within the 2 vertical lines. During the dry season, the samples were taken at states of falling near low tide and early phase of a rising tide. Charts were obtained from <http://tidesandcurrents.noaa.gov>

Figure 12a. Tide chart for Port Salerno, Manatee Pocket, FL Station Id: 8722383 for corresponding sites sampled on February 25, 2015. Sampling timeframe: 9:40am – 10:10am

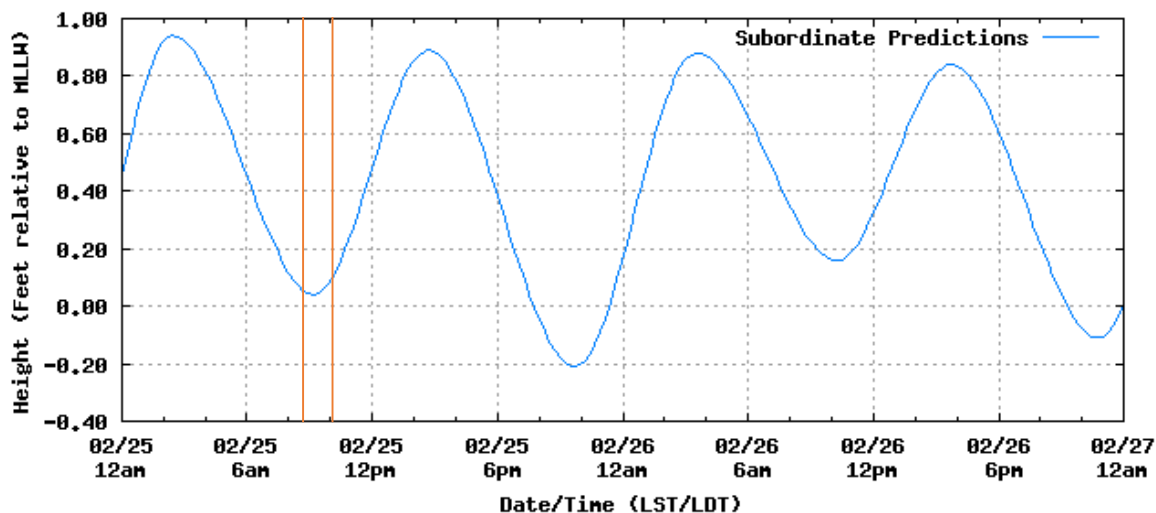


Figure 12b. Tide chart for Port Salerno, Manatee Pocket, FL Station Id: 8722383 for corresponding sites sampled on February 25, 2015. Sampling timeframe 7:35am – 7:50am.

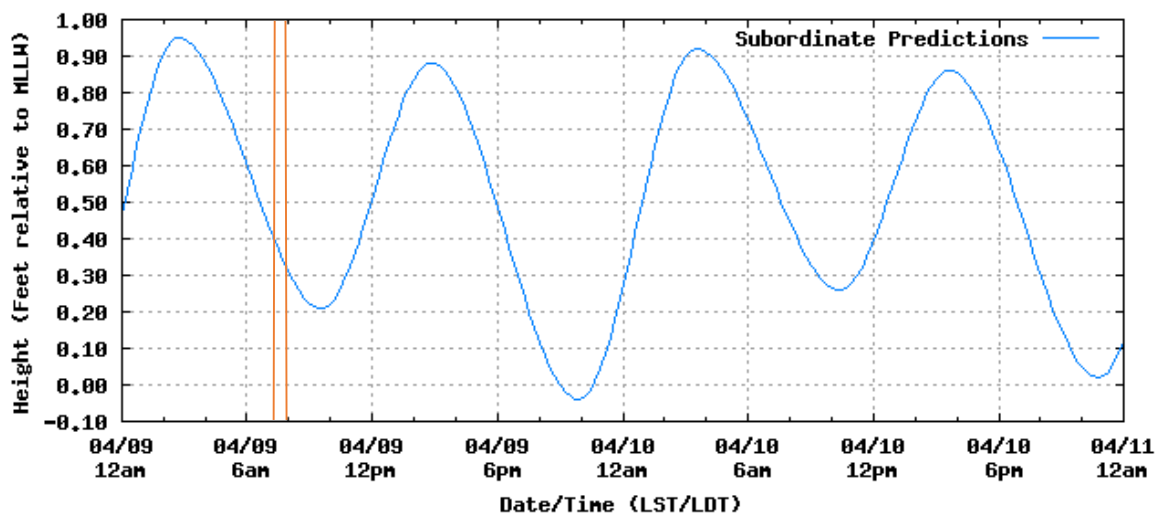
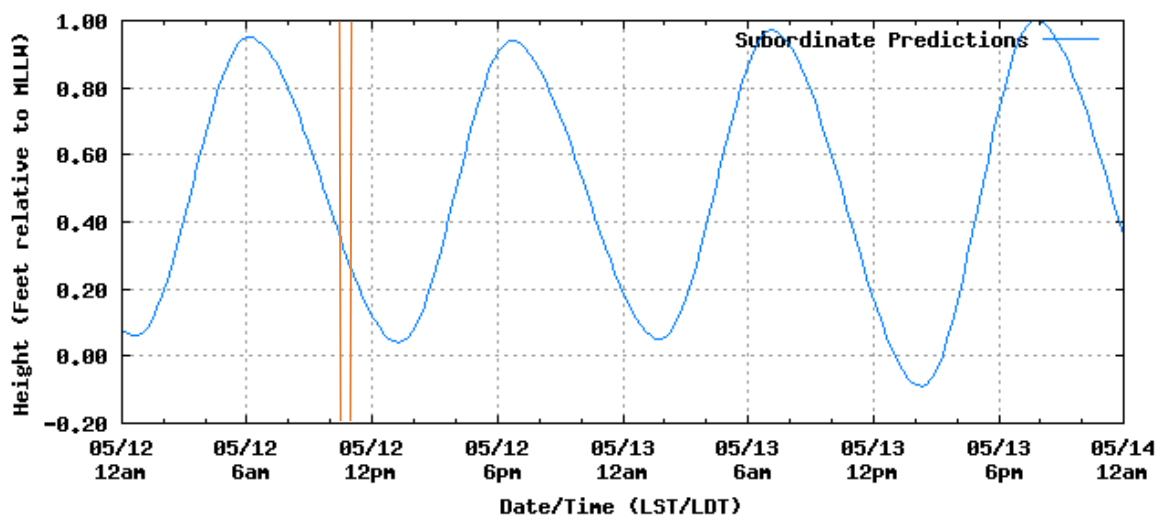


Figure 12c. Tide chart for Port Salerno, Manatee Pocket, FL Station Id: 8722383 for corresponding sites sampled on February 25, 2015. Sampling timeframe 10:35am – 11:00am.



Golden Gate (Tables 1 and 3; Appendix B)

The Golden Gate site is predominantly marine. Sucralose was detected at significant levels ($> 1.0 \mu\text{g/L}$) in the samples collected during the April and May events. Enterococci counts were elevated for all three sampling events (**Table 1**). Fecal coliform bacteria counts exceeded the threshold in samples collected during the April and May events. The human fecal source marker was detected at very low levels in the samples collected in all three events.

Marina at Haney Creek (Tables 1 and 3; Appendix B)

The Marina at Haney Creek site is predominantly marine. Sucralose was detected in the low range in February and in the moderate range during the April and May events. Enterococci counts were elevated in the sample collected in April but lower in the samples collected in February and May. The human fecal source marker was detected at a low level in the sample collected in February. The human marker was not detected in April or May. Acetaminophen was not detected in any of the dry season samples.

Danforth Creek West (Tables 1 and 3; Appendix B)

Danforth Creek West site is predominantly freshwater. Sucralose was detected in the moderate range in each dry season sampling event. *E. coli* and fecal coliform counts were elevated in the samples during each dry season sampling event. The human marker and acetaminophen were detected at a low range in the samples collected during the February sampling event.

Sandsprit Park (Tables 1 and 3; Appendix B)

The Sandsprit Park site is predominantly marine. Sucralose was detected at low levels in the samples collected in the February and April events and found in the moderate range in the sample collected during the May event. No bacteria exceedances occurred during the dry season at Sandsprit Park. The human fecal source marker was detected at low levels during the February and April sampling events. Acetaminophen was detected in all of the dry season sampling events.

C23 Canal (Tables 1 and 3; Appendix B)

C23 is considered a predominantly freshwater site. Sucralose was detected at low levels in the samples collected during all dry season events. Bacteria counts remained low and acetaminophen was never detected. The human fecal source marker was detected at a very low level in May.

Table 3. Descriptive results for sites with human marker detections. Results with a dash (-) indicate the analysis was not performed.

Sample Date	Parameter	Golden Gate	Marina at Haney	Danforth Creek West	Sandsprit	C23
2/25/2015	<i>E. coli</i> ¹	-	-	Poor	-	Good
2/25/2015	Enterococci ²	Poor	Good	-	Good	-
2/25/2015	Fecal coliform ³	Moderate	Good	Good	Good	Good
2/25/2015	Bacteroidales HF183	Detected	Detected	Detected	Detected	Undetected
2/25/2015	Acetaminophen	Undetected	Undetected	Detected	Detected	Undetected
2/25/2015	Sucralose ⁴	Moderate	Low	Moderate	Low	Low
4/9/2015	<i>E. coli</i>	-	-	Poor	-	Good
4/9/2015	Enterococci	Poor	Poor	-	Good	-
4/9/2015	Fecal coliform	Poor	Good	Moderate	Good	Good
4/9/2015	Bacteroidales HF183	Detected	Undetected	Undetected	Detected	Undetected
4/9/2015	Acetaminophen	Undetected	Undetected	Undetected	Detected	Undetected
4/9/2015	Sucralose	Significant	Moderate	Moderate	Low	Low
5/12/2015	<i>E. coli</i>	-	-	Poor	-	Good
5/12/2015	Enterococci	Moderate	Poor	-	Good	Good
5/12/2015	Fecal coliform	Poor	Good	Poor	Good	Good
5/12/2015	Bacteroidales HF183	Detected	Undetected	Undetected	Detected	Undetected
5/12/2015	Acetaminophen	Undetected	Undetected	Undetected	Detected	Undetected
5/12/2015	Sucralose	Significant	Moderate	Moderate	Moderate	Low

¹*E. coli* (cfu/100mL): good (0 – 125), moderate (126 – 235), poor (236 or more)

²Enterococci (cfu/100mL): (0 – 35), moderate (36 – 104), poor (105 or more)

³Fecal coliform (cfu/100mL): good (≤800), poor (>800)

⁴Sucralose (µg/L): Low (<0.1), moderate (0.1 – 1.0), significant (>1.0)

All American Ditch (Table 1; Appendix B)

All American Ditch is a predominantly freshwater site. Sucralose had a significant detection in the sample collected in April at this site. The bacteria counts were normal and well below the threshold. Neither detections of the human marker or acetaminophen occurred during the dry season.

Warner Creek (Table 1; Appendix B)

Warner Creek is a predominantly freshwater site. Sucralose had a significant detection in the sample collected in April and May at Warner Creek. *E. coli* and fecal coliform counts were low in all of the samples collected during the dry season. Neither the human fecal source marker nor acetaminophen was detected.

Bessey Creek Upstream (Table 1; Appendix B)

Bessey Creek Upstream is considered to be a predominantly freshwater site. Sucralose was detected in the moderate range in each dry season sampling event. *E. coli* and fecal coliform counts were acceptable in all the samples collected in the dry season. Neither the human fecal source marker nor acetaminophen was detected.

Bessey Creek East (Table 1; Appendix B)

The Bessey Creek East site is considered predominantly marine. Sucralose was detected in the moderate range in each dry season sampling event. Enterococci counts were low in February and May samples and slightly higher in the April sample. Neither the human fecal source marker nor acetaminophen was detected.

Danforth Creek (Table 1; Appendix B)

Danforth Creek is considered to be a predominantly freshwater site. Sucralose was detected in the moderate range in each dry season sampling event. *E. coli* remained elevated during all dry season sampling events at Danforth Creek. Neither the human fecal source marker nor acetaminophen was detected.

Leighton Park (Table 1; Appendix B)

Leighton Park is considered predominantly marine. Sucralose was detected at a low level in February and in the moderate range in the April and May samples. Enterococci counts at the Leighton Park site did not fall into the poor range during the dry season. Fecal coliform counts were below the threshold in all the samples collected during the dry season. Neither the human fecal source marker nor acetaminophen was detected.

C44 Canal (Table 1; Appendix B)

C44 Canal site is considered to be predominantly freshwater. Sucralose was detected at low levels in the samples collected in February and April and in the moderate range in May. Both *E. coli* and fecal coliform counts were low in all the samples collected in the dry season. Neither the human fecal source marker nor acetaminophen was not detected.

Britt Creek (Table 1; Appendix B)

Britt Creek is considered predominantly marine. Sucralose was detected in the moderate range in samples collected from all dry season events. Both Enterococci and fecal coliform counts were low in all the samples collected in the dry season. Neither the human fecal source marker nor acetaminophen was detected.

Hog Creek (Table 1; Appendix B)

Hog Creek is considered to be a predominantly freshwater site. Sucralose was detected at low levels throughout the dry season. *E. coli* counts were acceptable in February and decreased in April and May. Fecal coliform counts were below the threshold in February, April and May. Neither the human fecal source marker nor acetaminophen was detected.

Conclusion

The differences in the results found between the wet and dry seasons reveal a possible dilution effect on acetaminophen and sucralose in the wet season when higher rainfall occurred. Sucralose was found at higher concentrations in the dry season; and although acetaminophen was not detected at higher concentrations it was detected more often during the dry season than wet. The elevated sucralose concentrations at the majority of the sites cannot be attributed to reuse irrigation because the sites are not located in re-use areas. Acetaminophen is not expected in re-use since it is easily broken down in the wastewater treatment process. Britt Creek, Marina at Haney Creek, Bessey Creek Upstream and Bessey Creek East are the only sites that have the potential to be impacted by reuse. Bessey Creek may also be impacted by City of Port St. Lucie reclaimed water irrigation from a golf courses in Tesoro (just north of C-23 but east of our C-23 sampling point) and the Floridian communities which are adjacent to the North Fork of the St. Lucie Estuary. Septic tanks are other possible sources that may contribute to the human marker and acetaminophen detections in particular. The detection of the human fecal source marker and fecal coliforms at higher concentrations during the wet season may indicate that the higher water table allows for increased leaching from the onsite disposal systems to the waterways. Unsaturated soils are necessary for treatment of bacteria.

The sites at Golden Gate, Sandsprit Park, All American Ditch, Danforth Creek West, and Marina at Haney Creek indicate that there are areas of onsite disposal systems with possible leaching into the waterway. The combination of consistently elevated counts of fecal coliform bacteria, the presence of the human marker, and detections of sucralose and/or acetaminophen is indicative of a source of some level of untreated human wastewater. Since there were no indications of bacteria exceedances, human marker, or acetaminophen at the All American Ditch site in the dry season, another round of sampling in the wet season is recommended to confirm any issues with that area are still occurring.

References Cited

Anderson, Kimberly L. Whitlock, John E., Harwood, Valerie J., 2005. Persistence and Differential Survival of Fecal Indicator Bacteria in Subtropical Waters and Sediments. *Applied and Environmental Microbiology*, Vol. 71(6), pp 3041 – 3048.

Byappanahalli, Muruleedhara N., Shively, Dawn A., Nevers, Meredith B., Sadowsky, Michael J., Whitman, Richard L., 2003. Growth and survival of *Escherichia coli* and enterococci populations in the macro-alga *Cladophora* (Chlorophyta). *FEMS Microbiology Ecology*, Vol. 46, pp 203 – 211.

Byappanahalli, Muruleedhara N., Fowler, Malanie, Shively, Dawn, Whitman, Richard, 2003. Ubiquity and persistence of *Escherichia coli* in a Midwestern Coastal Stream. *Applied and Environmental Microbiology*, Vol. 69, pp 4549 – 4555.

Byappanahalli, Muruleedhara N., Nevers, Meredith B., Korajkic, Asja, Staley, Zacher R., harwood, Valeri J., 2012. Enterococci in the Environment. *Microbiology and Molecular Biology Reviews*, Vol. 76(4), pp 685. DOI: 10.1128/MMBR.00023-12.

Davies, Cheryl M., Long, Julian A.H., Donald, Margaret, and Ashbolt, Nicholas J., 1995. Survival of Fecal Microorganisms in Marine and Freshwater Sediments. *Applied and Environmental Microbiology*, Vol. 61(5), pp 1888 – 1896.

Labare, M. P. and M. Alexander, 1993. Biodegradation of Sucralose, a Chlorinated Carbohydrate, in Samples of Natural Environments. *Environmental Chemistry*, Vol. 12, pp 797 – 804.

Phillips, P. and A. Chalmers, 2009. Wastewater Effluent, Combined Sewer Overflows, and Other Sources of Organic Compounds to Lake Champlain. *Journal of the American Water Resources Association*, Vol. 45, pp 45 – 57.

Soh, L. K. A., Connors, B. W. Brooks, and J. Zimmerman, 2011. Fate of Sucralose through Environmental and Water Treatment Processes and Impact on Plant Indicator Species. *Environmental Science & Technology*, Vol. 45, pp 1365 – 1369.

Appendix A

Table 1. Wet season results from the 14 study sites for the bacteria E.coli, Enterococci, and Fecal coliform, human marker Bacteroidales HF 183, and chemical indicators acetaminophen and sucralose from samples collected and analyzed through DEAR. Definitions to qualifier codes can be found in **Appendix A, Table 1**.

Sample Date	Parameter	Method	Units	Britt Creek (MCWQ-6)	Marina at Haney Creek (MCWQ-5)	Warner Creek (MCWQ-12)	Golden Gate (MCWQ-11)	Sandsprit Park (MCWQ-4)	C23 Canal (MCWQ-1)	Bessey Creek Upstream (MCWQ-10)	Bessey Creek East (MCWQ-3)	Danforth Creek (MCWQ-9)	Leighton Park (MCWQ-2)	Danforth Creek West (MCWQ-13)	All American Ditch (MCWQ-14)	C44 Canal (MCWQ-7)	Hog Creek (MCWQ-8)
6/19/2014	E. coli	Quanti-tray	MPN/100 mL	-	-	8.6 AQ	579.4 Q	-	3.1 AJQ	648.8 Q	34.8 AQ	648.8 Q	-	248.1 Q	2419.6 LQ	178.5 Q	1986.3 Q
6/19/2014	Enterococci	Membrane filtration	CFU/100 mL	8.00 B	80.0	-	1250 B	3.00 B	-	-	250.0	-	13900 B	882 B	4900.0	2900 Field Dup	-
6/19/2014	Fecal coliform	Membrane filtration	CFU/100 mL	21.0 B	30.0 B	154 B	1010 B	2.00 B	66 Q	580 Q	29.0 B	727 B	39000 B	490.0	5500.0	6900 B	6000 Z
6/19/2014	Bacteroidales HF183	Q-PCR	GEU/100 mL	1020 U	2040 U	1630 U	3600 U	2040 U	1020 U	1360 U	2040 U	2720 U	4080 U	2720 U	23700.0	1360 U	4080 U
6/19/2014	Acetaminophen	EPA Method 8321B	µg/L	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 UJ	0.0005 U	0.0005 U	0.0005 UJ	0.0005 U	0.0005 U	0.0005 UJ	0.00073 I	0.0005 U	0.0005 UJ
6/19/2014	Sucralose	EPA Method 8321B	µg/L	0.073 I	0.039 I	0.52	0.1	0.040 I	0.020 U	0.57	0.022 I	0.11	0.037 I	0.044 I	0.99	0.032 I	0.010 U
7/22/2014	E. coli	Quanti-tray	MPN/100 mL	4 Q	-	36.4 Q	1732.9	-	3 Q	131.4 Q	4.1 Q	1119.9 Q		23.3 Q	1769.7 AQ	3.1 Q	66.3 Q
7/22/2014	Enterococci	Membrane filtration	CFU/100 mL	-	-	-	-	-	-	-	300	-	350	64	420	-	173.0
7/22/2014	Fecal coliform	Membrane filtration	CFU/100 mL	5 B	250.0	310.0	4400.0	3.0 B	8 B	300.0	21 B	809.0 B	56.0	350.0	1290 B	20 B	350.0
7/22/2014	Bacteroidales HF183	Q-PCR	GEU/100 mL	2040 U	2040 U	2040 U	32100.0	17600	1360 U	2040 U	2040 U	340 U	2040 U	2040 U	2040 U	1360 U	227 U
7/22/2014	Acetaminophen	EPA Method 8321B	µg/L	0.0005 U	0.0011 I	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U
7/22/2014	Sucralose	EPA Method 8321B	µg/L	0.091	0.1	0.47	0.54	0.083 I	0.04 I	0.44	.042 I	0.16	0.063	0.1	0.44	0.010 U	0.025 I
8/21/2014	E. coli	Quanti-tray	MPN/100 mL	-	-	35 Q	-	-	2 Q	190.4 Q	15.8 Q	285.1 Q	8.4 Q	93.3 Q	579.4 Q	2.0 Q	112.4 Q
8/21/2014	Enterococci	Membrane filtration	CFU/100 mL	510.0	104	-	3600	50	-	845 B	134 B	-	80	-	-	-	-
8/21/2014	Fecal coliform	Membrane filtration	CFU/100 mL	11 B	4600.0	4300.0	2900	7 B	360	827 B	152 B	540	39 B	440	3000	8 B	655 B
8/21/2014	Bacteroidales HF183	Q-PCR	GEU/100 mL	2400 U	2400 U	2400 U	11500	1800 U	600 U	3600 U	2400 U	600 U	600 U	600 U	3600 U	3600 U	3600 U
8/21/2014	Acetaminophen	EPA Method 8321B	µg/L	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U	0.0005 U
8/21/2014	Sucralose	EPA Method 8321B	µg/L	0.049 IJ	0.036 IJ	0.31 J	0.34	0.021 I	0.01 UJ	0.18 J	0.062 I	0.096 J	0.044 IJ	0.074 J	0.046	0.023 I	0.02 I

Appendix A, continued

Table 2. Wet season results for field measurements recorded at each site by DEAR.

Sample Date	Parameter	Units	Britt Creek (MCWQ-6)	Marina at Haney Creek (MCWQ-5)	Warner Creek (MCWQ-12)	Golden Gate (MCWQ-11)	Sandsprit Park (MCWQ-4)	C23 Canal (MCWQ-1)	Bessey Creek Upstream (MCWQ-10)	Bessey Creek East (MCWQ-3)	Danforth Creek (MCWQ-9)	Leighton Park (MCWQ-2)	Danforth Creek West (MCWQ-13)	All American Ditch (MCWQ-14)	C44 Canal (MCWQ-7)	Hog Creek (MCWQ-8)
6/19/2014	Conductivity	umhos/cm	10895.0	15255.0	357.0	-	32158.0	1039.0	3152.0	5948.0	527.0	550.0	450.0	249.0	537.0	408.0
6/19/2014	Temperature	Celsius	27.65	28.40	27.58	-	27.98	27.67	25.31	27.93	25.54	27.09	25.66	25.06	27.45	24.53
6/19/2014	Dissolved Oxygen	mg/L	6.52	6.00	3.25	-	6.20	3.73	2.46	6.13	3.97	5.68	4.15	5.02	3.68	2.44
6/19/2014	Dissolved Oxygen	%	85.80	81.70	41.20	-	88.50	47.50	30.20	79.70	49.00	71.50	50.90	60.70	46.70	29.30
6/19/2014	pH	SU	7.80	7.80	6.89	-	8.04	7.24	7.11	7.31	6.84	7.10	7.22	6.46	7.27	7.04
6/19/2014	Salinity	mg/L	6.14	8.84	0.17	-	20.04	0.51	1.61	3.22	0.25	0.26	0.22	0.12	0.26	0.19
7/22/2014	Conductivity	umhos/cm	3078	12836	382	30798	36113	690	625	3572	490	2364	451	265	471	631
7/22/2014	Temperature	Celsius	29.9	30.2	29.4	30.3	30.1	29.5	27.6	29.6	27.5	29.7	27.9	27.1	30.1	25.8
7/22/2014	Dissolved Oxygen	mg/L	6.83	4.04	2.96	1.60	8.41	2.95	1.78	4.89		3.26	5.63	6.40	4.78	3.69
7/22/2014	Dissolved Oxygen	%	91.2	55.2	38.7	23.6	127.7	38.6	22.7	64.8	49.8	43.5	71.8	79.8	63.0	45.4
7/22/2014	pH	SU	7.7 J	7.3 J	6.5 J	7 J	7.7 J	7.0 J	7.1 J	7.1 J	6.8 J	7.1 J	7.1 J	6.4 J	7.2 J	7.1 J
7/22/2014	Salinity	mg/L	1.6	6.9	0.2	19.0	22.6	0.3	0.3	1.9	0.2	1.2	0.2	0.1	0.2	0.3
8/21/2014	Conductivity	umhos/cm	9630	24104	390	13401	42345	619	1499	11644	3063	11444	482	334	535	669
8/21/2014	Temperature	Celsius	29.6	30.4	29.3	28.6	28.7	30.3	28.5	31.2	28.6	31.3	27.3	26.3	30.6	26.8
8/21/2014	Dissolved Oxygen	mg/L	5.66	4.44	4.34	1.27	7.04	1.79	2.05	3.92	3.65	2.86	3.57	2.79	3.65	2.07
8/21/2014	Dissolved Oxygen	%	76.2	64.0	54.6	16.9	106.0	23.7	26.7	55.6	47.5	43.0	44.6	34.6 J	48.8 J	26.0 J
8/21/2014	pH	SU	7.6 J	7.4 J	6.3 J	6.8 J	7.8 J	7.2 J	7.2 J	7.4 J	7.0 J	7.4 J	7.0 J	6.3 J	7.4 J	7.2 J
8/21/2014	Salinity	mg/L	5.38	14.56	0.18	7.69	27.10	30	0.73	6.46	1.50	6.50	0.23	0.16	0.26	0.32

Appendix B

Table 1. Dry season results from the 14 study sites for the bacteria E.coli, Enterococci, and Fecal coliform, human maker Bacteroidales HF 183, and chemical indicators acetaminophen and sucralose from samples collected and analyzed through DEAR. Definitions to qualifier codes can be found in **Appendix A, Table 1**.

Sample Date	Parameter	Method	Units	Britt Creek (MCWQ-6)	Marina at Haney Creek (MCWQ-5)	Warner Creek (MCWQ-12)	Golden Gate (MCWQ-11)	Sandsprit Park (MCWQ-4)	C23 Canal (MCWQ-1)	Bessey Creek Upstream (MCWQ-10)	Bessey Creek East (MCWQ-3)	Danforth Creek (MCWQ-9)	Leighton Park (MCWQ-2)	Danforth Creek West (MCWQ-13)	All American Ditch (MCWQ-14)	C44 Canal (MCWQ-7)	Hog Creek (MCWQ-8)
2/25/2015	E. Coli	Quanti-tray	MPN/100 mL	-	-	26.1 AQ	-	-	1.0 Q	55.6 Q	151.5 Q	1083.0 AQ	37.3 Q	248.1 Q	95.7 Q	69.1 Q	161.6 Q
2/25/2015	Enterococci	Membrane filtration	CFU/100 mL	10 B	7 B	-	1050 B	16 B	-	300.0	28 B	-	50	-	-	-	-
2/25/2015	Fecal Coliform	Membrane Filtration	CFU/100 mL	13 B	8 B	34 B	645 B	16 B	1.6 U	130 B	60.0	845 B	31 B	210.0	80.0	90.0	210
2/25/2015	QPCR - Bacteroidales HF183	qPCR	GEU/100 mL	1100 U	209 T	1100 U	85.9 T	165 T	1100 U	1100 U	1100 U	1100 U	1100 U	678 T	1100 U	1600 U	1100 U
2/25/2015	Acetaminophen	EPA Method 8321B	ug/L	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0039 I	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.016	0.0020 U	0.0020 U	0.0020 U
2/25/2015	Sucralose	EPA Method 8321B	ug/L	0.075	0.072	0.78	0.67	0.083	0.016 I	0.36	0.12	0.23	0.049 I	0.25	0.860	0.032 I	0.022 I
4/9/2015	E. Coli	Quanti-Tray	MPN/100 mL	-	-	10.0 Q	-	-	1.0 UQ	110 Q	128.4 Q	672.2 AQ	10 Q	173.1 Q	86.0 Q	20.1 Q	107.8 Q
4/9/2015	Enterococci	Membrane filtration	CFU/100 mL	74	157 B	-	1810 B	23 B	-	390.0	76	-	92	-	-	-	-
4/9/2015	Fecal Coliform	Membrane Filtration	CFU/100 mL	7 B	42.0	52.0	1810 B	16 B	7 B	128 B	28.0	727 B	13 B	151 B	94.0	5 B	70.0
4/9/2015	QPCR - Bacteroidales HF183	qPCR	GEU/100 mL	1100 U	1100 U	1100 U	476.7 T	170.7 T	1100 U	1100 U	1600 U	1100 U	1100 U	270 U	180 U	1100 U	1100 U
4/9/2015	Acetaminophen	EPA Method 8321B	ug/L	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0034 I	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U
4/9/2015	Sucralose	EPA Method 8321B	ug/L	0.20	0.12	1.1	1.8	0.072	0.059	0.68	0.27	0.44	0.16	0.36	1.3	0.077	0.071
5/12/2015	E. Coli	Quanti-Tray	MPN/100 mL	-	-	10.0 Q	-	-	10.0 UQ	108.9 Q	10 Q	528.4 Q	10 Q	1926.4 AQ	40.9 Q	30.4 Q	110 Q
5/12/2015	Enterococci	Membrane filtration	CFU/100 mL	20 B	66.0	48 B	1150 B	5 B	23 B	280.0	18 B	243	44	-	-	-	-
5/12/2015	Fecal Coliform	Membrane Filtration	CFU/100 mL	1.6 U	240.0	267.0	2500.0	11 B	3.2 U	280.0	5 B	250	15 B	3100.0	106.0	30 B	420.0
5/12/2015	QPCR - bacteroidales HF183	qPCR	GEU/100 mL	800 U	180 UJ	1600 U	219 IJ	1100 U	68.6 TJ	1100 U	1100 U	1100U	1600 U	1600 U	1600 U	1100 U	1100 U
5/12/2015	Acetaminophen	EPA Method 8321B	ug/L	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0049 I	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U	0.0020 U
5/12/2015	Sucralose	EPA Method 8321B	ug/L	0.15	0.2	1.0	2.4	0.13	0.023 I	0.72 A	0.15	0.52	0.1	0.3	0.59	0.10	0.089

Appendix B, continued

Table 2. Dry season results for field measurements recorded at each site by DEAR.

Sample Date	Parameter	Units	Britt Creek (MCWQ-6)	Marina at Haney Creek (MCWQ-5)	Warner Creek (MCWQ-12)	Golden Gate (MCWQ-11)	Sandsprit Park (MCWQ-4)	C23 Canal (MCWQ-1)	Bessey Creek Upstream (MCWQ-10)	Bessey Creek East (MCWQ-3)	Danforth Creek (MCWQ-9)	Leighton Park (MCWQ-2)	Danforth Creek West (MCWQ-13)	All American Ditch (MCWQ-14)	C44 Canal (MCWQ-7)	Hog Creek (MCWQ-8)
2/25/2015	Conductivity	umhos/cm	21708	30233	390	43316	46630	1334	13039	22877	17255	21339	609	9835	502	609
2/25/2015	Temperature	Celsius	20.9	22.0	22.1	21.7	21.3	22.9	21.1	20.7	22.0	20.9	21.0	21.3	22.9	22.1
2/25/2015	Dissolved Oxygen	mg/L	8.50	8.74	10.81	6.91	7.27	9.91	7.25	8.91	7.38	8.07	8.39	6.00	10.61	6.86
2/25/2015	Dissolved Oxygen	%	102.8	109.1	123.9	92.1	97.8	15.7	85.1	107.8	90.1	96.8	94.3	70.5	124.0	78.7
2/25/2015	pH	SU	7.7	7.8	7.3	7.8	7.8	7.8	7.4	7.7	7.5	7.8	7.3	7.3	8.1	7.4
2/25/2015	Salinity	mg/L	13.06	18.80	0.19	27.93	30.31	0.67	7.58	13.83	10.19	12.86	0.30	5.55	0.24	0.30
4/9/2015	Conductivity	umhos/cm	17115	22631	381	27589	46607	1279	6440	18066	2913	12462	704	7332	565	626
4/9/2015	Temperature	Celsius	26.3	25.4	26.0	25.9	25.6	23.8	25.2	26.4	23.4	25.6	22.8	26.9	25.3	24.1
4/9/2015	Dissolved Oxygen	mg/L	5.21 J	38.0 J	4.61 J	1.85 J	5.91 J	6.84 J	4.33 J	17.53 J	21.69 J	10.23 J	3.66	2.84	6.84	6.22
4/9/2015	Dissolved Oxygen	%	68.0 J	56.1 J	56.6 J	25.8 J	86.1 J	101.4	54.0 J	254.0 J	266.0 J	130.0	42.2	36.0	83.1	73.4
4/9/2015	pH	SU	7.7	7.3	7.3	7.1	7.7	7.3	7.1	7.5	6.9	7.6	6.8	7.1	7.7	7.3
4/9/2015	Salinity	mg/L	10.04	13.62	0.18	23.26	30.25	-	-	-	-	-	0.34	4.02	0.27	0.30
5/12/2015	Conductivity	umhos/cm	16697	15768	481	21300	42560	1084	3568	14784	776	1306	578	1830	477	603
5/12/2015	Temperature	Celsius	28.9	28.8	28.6	27.7	28.3	27.0	25.9	28.3	26.2	27.9	26.2	28.4	27.9	26.8
5/12/2015	Dissolved Oxygen	mg/L	6.68	26.90	6.21	0.36	6.37	8.10	6.78	8.69	6.40	8.44	5.6	6.80	6.60	5.90
5/12/2015	Dissolved Oxygen	%	91.4	36.0	80.1	5.4	95.0	101.3	85.0	116.7	77.1	110.1	68.0	88.0	85.0	74.0
5/12/2015	pH	SU	8.1 J	7.5 J	7.2 J	7.3 J	8.2 J	7.8	7.2	7.7	7.2	7.8	7.2	7.2	7.7	7.1
5/12/2015	Salinity	mg/L	9.75	9.15	0.23	10.03	27.27	0.53	1.90	8.62	0.37	0.65	0.28	0.92	0.23	0.29

Appendix C

Bacteriological Quality (Fecal Coliform Bacteria) F.A.C. 62.302.530 Surface Water Quality Criteria

Class III predominantly freshwater. MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.

Class III predominantly marine water. MPN or MF counts shall not exceed a monthly average of 200, nor exceed 400 in 10% of the samples, nor exceed 800 on any one day. Monthly averages shall be expressed as geometric means based on a minimum of 10 samples taken over a 30 day period.

EPA's Ambient Water Quality Criteria for Bacteria (Enterococci and *E. coli*) - 1986

The criteria for Enterococci and *E. coli* are not a part of the State's water quality standards. It should be noted that the results for Enterococci and *E. coli* highlighted in this document are not recommendations for water quality criteria or water quality criteria exceedances that DEP recognizes at this time. The County Health Departments use EPA's 1986 criteria to set health advisories at recreational beaches and at monitored sites on the St. Lucie River. Martin County Health Department uses the EPA's criteria for marine waters to set advisories at the recreational beaches and monitoring sites located on the St. Lucie River. These criteria, however, are not appropriate for the tributary sites used in this study to set advisories. For this study, the criteria for marine and freshwater are used, depending on site conditions, as guidance values to recognize possible contributions to the St. Lucie River relative to those seen at the Martin County Health Department's river monitoring sites.

Designated Beach Area Single Sample Maximum Allowable Density

Marine Water

Enterococci – 104 per 100 mL

Fresh Water

E.coli – 235 per 100 mL

Appendix C, continued

Table 1. Florida Department of Health Healthy Beaches Program Categories for Enterococci. *E. coli* is not a DOH Healthy Beaches Program parameter. EPA has criteria for *E. coli* and the same method for using the Healthy Beaches Program descriptive categories were applied for this study.

Parameter	Freshwater (cfu/100 ml)	Saltwater (cfu/100 ml)
Enterococci - Good		0 to 35
Enterococci - Moderate		36 to 104
Enterococci - Poor		105 or more
<i>E. coli</i> - Good	0 to 125	
<i>E. coli</i> - Moderate	126 to 235	
<i>E. coli</i> - Poor	235 or more	

Appendix D

From 2008 DEP QA Rule 62-160.700

Symbol	Meaning
U	Indicates that the compound was analyzed for but not detected. The reported value shall be the method detection limit.
A	Value reported is the average of two or more determinations.
B	Colony counts were outside acceptable range. The value reported is an estimated count (This code applies to microbiological tests and specifically to membrane filter colony counts.)
I	The reported value is greater than or equal to the laboratory method detection limit but less than the laboratory practical quantification limit.
T	Value reported is less than the laboratory method detection limit.
K	Off-scale low. The actual value is known to be less than the value given.
N	Presumptive evidence of presence of material; component tentatively identified based on mass spectral library search or there is an indication that the analyte is present, but quality control requirements for the confirmation were not met.
O	Sampled but analysis lost or not performed.
Q	Sample held beyond the accepted holding time.
L	Off-scale high. The actual value is known to be greater than the value given.
J	Estimate value. Shall be accompanied by a detailed explanation to justify the reason(s) for designating the value as estimated. Examples of situations in which a “J” code must be reported include: instances where a quality control item associated with the reported value failed to meet the established quality control criteria (the specific failure must be identified); instances when the sample matrix interfered with the ability to make any accurate determination; instances when data are questionable because of improper laboratory or field protocols (e.g., composite sample was collected instead of a grab sample); instances when the analyte was detected at or above the method detection limit in a blank other than the method blank (such as calibration blank or field-generated blanks and the value of 10 times the blank value was equal to or greater than the associated sample value); or instances when the field or laboratory calibrations or calibration verifications did not meet calibration acceptance criteria.
V	Indicates that the analyte was detected at or above the method detection limit in both the sample and the associated method blank and the value of 10 times the blank value was equal to or greater than the associated sample value.
X	Indicates, when reporting results from a Stream Condition Index Analysis (LT 7200 and FS 7420), that insufficient individuals were present in the sample to achieve a minimum of 280 organisms for identification (the method calls for two aliquots of 140-160 organisms), suggesting either extreme environmental stress or a sampling error.
Y	The laboratory analysis was from an unpreserved or improperly preserved sample. The data may not be accurate.
Z	Too many colonies were present for accurate counting. Historically, this condition has been reported as “too numerous to count” (TNTC). The “Z” qualifier code shall be reported when the total number of colonies of all types is more than 200 in all dilutions of the sample. When applicable to the observed test results, a numeric value for the colony count for the microorganism tested shall be estimated from the highest dilution factor (smallest sample volume) used for the test and reported with the qualifier code
!	Indicates that the reported value deviates from historically established concentration ranges.
?	Data are rejected and should not be used. Some or all of the quality control data for the analyte were outside criteria, and the presence or absence of the analyte cannot be determined from the data.

