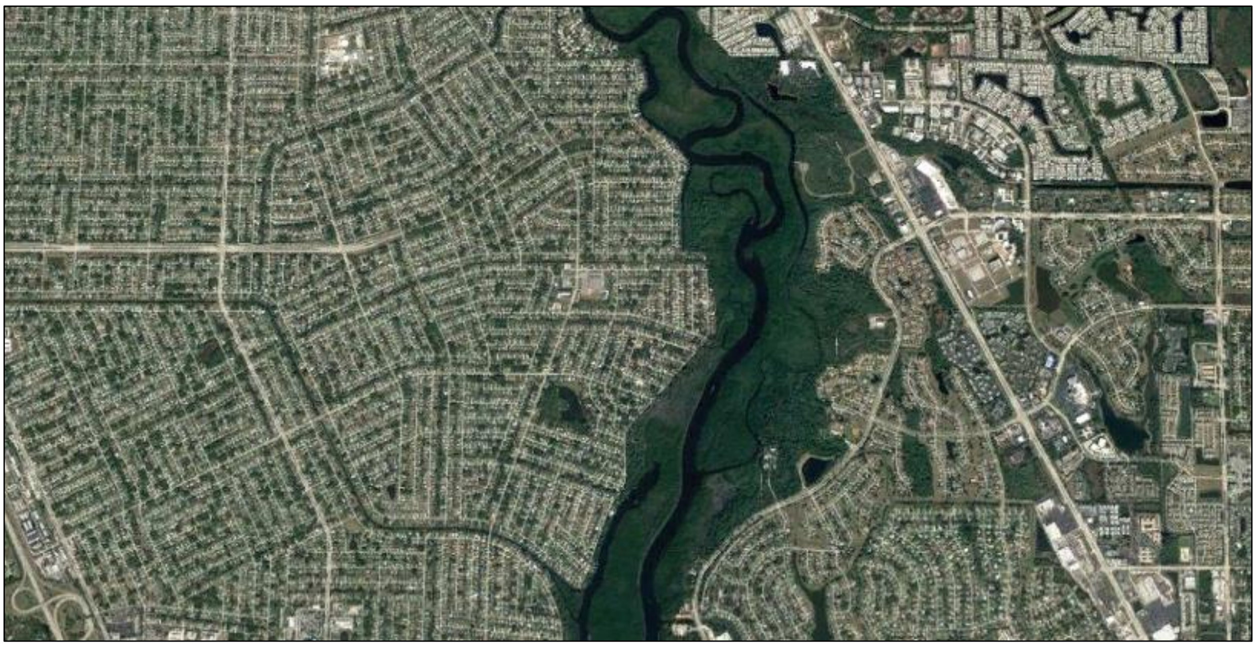
Microbial Source Tracking of Bacterial Pollution in the North Fork of the St. Lucie River



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Executive Summary

Nationally, a trend of steady development in coastal areas has led to rising population densities and degraded water quality. Accordingly, as the City of Port St. Lucie (CPSL) and surrounding areas have grown, the North Fork of the St. Lucie Estuary (North Fork) has experienced increasingly polluted waters. In particular, bacterial concentrations have exceeded public health standards, leading to multiple closures of the system for recreational use. Furthermore, nutrient concentrations (total nitrogen and total phosphorus) have repeatedly exceeded surface water quality standards, leading to the classification of the North Fork as "impaired" on the United States Environmental Protection Agency's 303d list and the subsequent development of a Basin Management Action Plan. To address these issues, a microbial source tracking study was conducted through a partnership between Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU), CPSL, St. Lucie County, St. Lucie County Department of Health, and Florida Department of Environmental Protection (FDEP). The purpose of the study was to improve understanding of the sources contributing to this recurring bacterial and nutrient pollution.

This study involved collection of surface water samples at tributaries of the North Fork (Tenmile Creek and Fivemile Creek), within the North Fork main stem from the confluence of the tributaries to the termination in the St. Lucie estuary, and within canals draining into the North Fork (C-107, Sagamore, Hogpen Slough, Veterans Memorial, Elkcam, Monterrey, C-24, E-8, Horseshoe A-18, and Southbend/Horseshoe A-22). Samples were collected during the 2016 wet season (3 events), 2017 dry season (2 events), and after a 2017 rain event. These samples were analyzed for molecular markers and chemical tracers to help identify fecal and wastewater sources, as well as bacterial concentrations. Additional samples for dissolved reactive nutrient analyses (ammonium, nitrate + nitrite, and phosphate) were collected to help further identify pollution sources. Dissolved nutrient samples were collected once during the 2017 dry season, the 2017 rain event, and during an additional 2017 wet season sampling. These combined data were used to assess sources of pollution contributing to bacterial impairment and eutrophication in the North Fork.

Results of the MST analyses confirmed that elevated fecal indicator bacteria concentrations occurred at many sites in the North Fork. The greatest concentrations consistently occurred in dense urbanized areas, including White City Park and canals in CPSL (Sagamore, Hogpen Slough, Veterans Memorial, Elkcam, and Southbend/Horseshoe A-22). Sucralose, an indicator of domestic wastewater, was detected at all sites and increased from upstream near White City Park to the more urbanized areas downstream. Canal and river sites near septic systems had the highest sucralose concentrations. The pharmaceuticals, acetaminophen and carbamazepine, were also found at many sites. Based on the prevalence of these chemical tracers, there is a strong indication that wastewater is widely influencing water quality in the North Fork via tributary and canal inputs. At some sites, including Fivemile Creek, Tenmile Creek, and Sagamore, these data suggest that septic systems are likely contributing to the bacterial pollution. At other sites, specifically Hogpen Slough and Veterans Memorial, these data indicate that wastewater from nearby communities and stormwater runoff may be critical issues. Furthermore, the widespread presence of herbicides and pesticides also suggests that stormwater runoff and chemical macrophyte control may also affect water quality.

Elevated dissolved nutrient concentrations were also found in densely populated urban areas, especially during the wet season. For example, the highest concentrations of ammonium were found in the North Fork at White City Park, while the highest nitrate concentrations were found at Sagamore; both these areas have high densities of old septic systems. The two greatest phosphate concentrations were found upstream in Fivemile Creek and in the C-24 canal. Ratios of dissolved nutrients are ecologically relevant and useful in understanding the occurrence of harmful algal blooms. The North Fork, its tributaries, and C-24 had very low (<5) dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP ratios) in the wet and dry seasons, indicating strong nitrogen-limitation for primary production (e.g. algae blooms and plant growth). This contrasts with the other residential canal sites, that generally had much higher DIN:SRP ratios, ranging from 8 to 103, indicating phosphorus-limitation. These high DIN:SRP ratios suggest the influence of septic tank effluent via groundwater flow where phosphorus is selectively removed by adsorption in soils relative to nitrogen. More detailed information on the relationships of dissolved nutrients and MST tracers would be useful for understanding pollution loading to the North Fork.

The results indicate there is a suite of issues within the North Fork that contribute to the poor water quality and that continued investigations will be required to accurately identify exact sources. Future studies should seek to understand the interactions of groundwater, soil, and surface waters in the North Fork. These couplings should be investigated using stable nitrogen isotopes to identify pollution sources contributing to water quality degradation. Furthermore, biological markers, such as bacteriophages, could be introduced into septic systems and the rate of transport through groundwaters and surface waters determined. This would provide much needed data regarding the interactions of septic tank effluent and surface water quality in the North Fork. Finally, a long-term sampling program that encompasses both MST markers and dissolved nutrient concentrations would provide greater insight into what is driving the poor water quality in the North Fork. These data would also be useful for observing recovery of the system as efforts to abate water quality issues are implemented.

Multiple lines of evidence in this study provide compelling evidence that wastewater is a major contributor impacting water quality in the North Fork. The data suggest that focusing septic-to-sewer programs on waterfront areas and investing in wastewater infrastructure will help moderate the water quality issues in the North Fork. Stormwater management projects and increased monitoring of macrophyte control programs would also help improve water quality within the system. The results of this project will be useful to resource managers, policy makers, and the citizens of CPSL in making decisions for improved infrastructure. CPSL has been proactive in the development of modern sewer systems in an effort to protect and improve water quality. This study is another important step towards achieving that goal. As local populations continue to grow, effective use of these data and continued water quality research in the North Fork will be essential for the health of the community and a sustainable economic future.

Summary Table. Compilation of all data collected during a microbial source tracking study within the North Fork of the St. Lucie Estuary, showing relative levels of analytes; green shading indicates a low value relative to applicable standards (not all analytes have numerical standards) or a non-detection, yellow shading indicates a value above background levels or approaching the standard, and red shading indicates exceedance of surface quality water standards or a significant presence. A dash (-) indicates the substance was below detection limits or did not amplify, "NA" indicates the substance was not analyzed for at that site, see table legend for details regarding individual parameter classifications. There are no numerical standards for reactive nutrients, so classifications were based on an estimated percent contribution: dissolved inorganic nitrogen (DIN) was considered elevated at 20% of the FDEP surface water standard for total nitrogen (TN; 0.72 mg/L), ammonium and nitrate were considered elevated at 10% of the TN standard, and phosphate was considered elevated at 20% of the total phosphorus standard (0.081 mg/L).

Type	Site	GULL2	HF183	Fecal coliforms	*Escherichia coli*	Enterococci	Sucralose	Acetaminophen	Carbamazepine	Diuron	Fenuron	Fluridone	Imidacloprid	Ammonium	Nitrate + Nitrite	DIN	SRP
Tributary	Tenmile 2 - upstream	-				NA			-								
	Tenmile 1 - downstream	-	-			NA					-						
	Fivemile 2 - upstream	-	-			NA					-						
	Fivemile 1 - downstream	-	-			NA					-						
River	R1 - upstream				NA						-						
	R2	-	-		NA						-						
	R3	-	-		NA			-			-						
	R4	-	-		NA						-						
	R5	-	-		NA						-						
	R6	-	-		NA			-									
	R7 - downstream	-	-		NA												
Canal	C-107	-	-			NA			-								
	Sagamore	-				NA											
	Hogpen Slough	-	-			NA					-						
	Vet Memorial	-	-			NA			-								
	Elkcam	-	-			NA											
	Monterrey	-	-			NA		-									
	C-24	-	-			NA		-	-		-						
	E-8	-	-			NA		-									
	Horseshoe A-18	-	-			NA		-									
	Southbend/Horseshoe A-22	-	-			NA											
Legend	Low	1,700-9,999	71-9,999	<400	<126	<35	0.01-0.09	0.004-0.009	0.0004-0.0099	0.0008-0.0099	0.008-0.0099	0.0004-0.009	0.0008-0.009	<0.02	<0.02	<0.04	<0.005
	Moderate	10,000-99,999	10,000-99,999	400-799	126-409	35-129	0.1-0.99	0.01-0.049	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.02-0.06	0.02-0.06	0.04-0.13	0.005-0.01
	Signifigant	≥ 100,000	≥ 100,000	≥ 800	≥ 410	≥ 130	≥1.0	≥0.05	≥1.0	≥1	≥1	≥1	≥1	≥0.07	≥0.07	≥0.14	≥0.02
	Parameter Units	TSC/100mL	GEU/100mL	cfu/100mL	MPN/100mL	cfu/100mL & MPN/100mL	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L

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Supplemental Information

Acronyms Used in the Text

BDL	below detection limit
BMAP	Basin Management Action Plan
CPSL	the City of Port St. Lucie
cfu	colony forming units
DIN	dissolved inorganic nitrogen (nitrate + nitrite + ammonium)
DIN:SRP	ratio of dissolved inorganic nitrogen to soluble reactive phosphorus
DO	dissolved oxygen
FAC	Florida Administrative Code
FDEP	Florida Department of Environmental Protection
FIB	fecal indicator bacteria
ft^3/sec	cubic feet per second
GDC	General Development Corporation
GEU	genomic equivalent units
HAB	harmful algal bloom
HBOI-FAU	Harbor Branch Oceanographic Institute-Florida Atlantic University
MDL	minimum detection limit
mg/L	milligrams per liter
MGM	Monthly Geometric Mean
MPN	most probable number
MST	microbial source tracking
NH_4	ammonium
NNC	Numeric Nutrient Criteria
NO_3	nitrate + nitrite
ppt	part per thousand
PQL	practical quantitation limit
SE	standard error
SM	standard method
SLC DOH	St. Lucie County Department of Health
SRP	soluble reactive phosphorus
TMDL	Total Maximum Daily Load
TN	total nitrogen
TP	total phosphorus
TPTV	Ten Percent Threshold Value
TSC	target sequence copies
μg/L	micrograms per liter
USEPA	United States Environmental Protection Agency

1. Introduction

1.1 Problem Statement and Project Objective

Similar to other urbanized areas in the United States (NRC 2000), the North Fork of the St. Lucie Estuary (North Fork hereafter) has experienced degraded water quality (Lapointe et al. 2012) leading to recurring closures of the water body for recreational use (**Fig. 1a**). To determine the sources of this recurring impairment, a microbial source tracking (MST) study was conducted through a collaborative effort with the City of Port St. Lucie (CPSL), St. Lucie County, Florida Department of Health in St. Lucie County (SLC DOH), and Florida Department of Environmental Protection (FDEP) with the analytical assistance of Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU). MST studies target source-specific gene fragments and chemicals as indicators to determine the source of microbial pollution. **The objective of this study was to gain a better understanding of bacterial prevalence in the North Fork in relation to source tracking parameters, water quality, and land-use in this watershed in order to determine the sources of this persistent pollution to the North Fork.** Furthermore, the North Fork drains into the middle and lower St. Lucie Estuary and ultimately terminates into the Indian River Lagoon (**Fig. 2c**). Widespread algae blooms of *Microcystis sp.* in the St. Lucie River and Estuary in 2016 (**Fig. 1b**) made national news headlines, resulted in negative financial impacts for the local economy, and created potential health issues for exposed individuals (Zhang et al. 2015). Therefore, there is great value in minimizing pollutants flowing downriver and into the estuary.

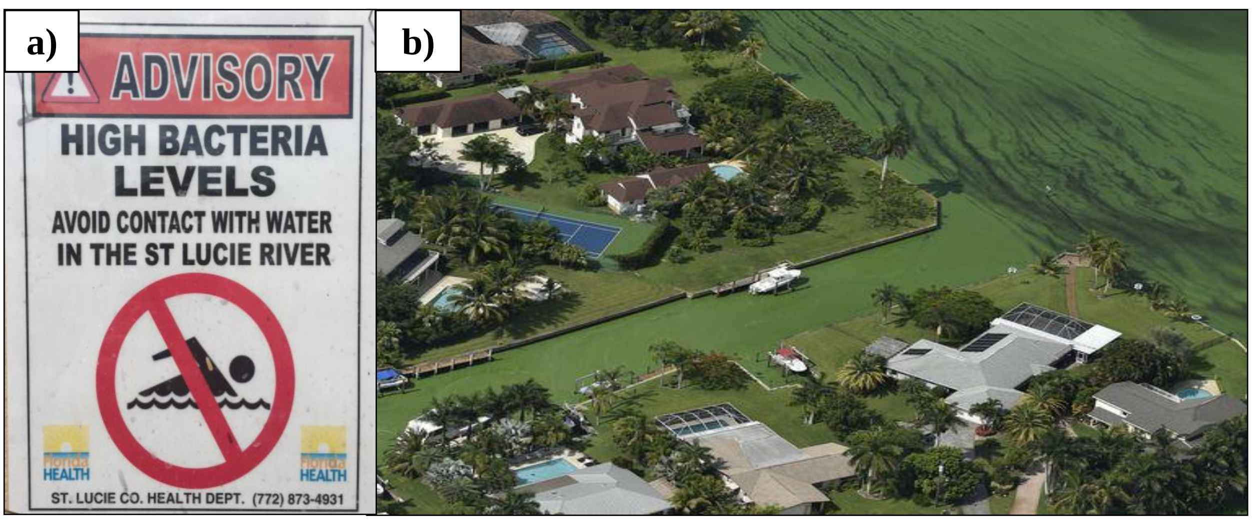

Figure 1. Easily visible indicators of a degraded watershed, including a) a posted sign indicating the closure of the St. Lucie River for recreational usage by Florida Department of Health and b) an aerial image of a *Microcystis* algae bloom during 2016 in the St. Lucie River in St. Lucie County (image from TCPalm).

1.2 Study Site

St. Lucie County encompasses 572 sq. miles and was one of the fastest growing counties in the United States (US) between 2000 and 2010. As of 2016 the US Census estimated the population to be over 306,000 (**Fig. 3**). Covering 120 square miles, the most populous municipality in St. Lucie County is CPSL, which is Florida's 8^{th} largest city having 179,413 residents in 2015.

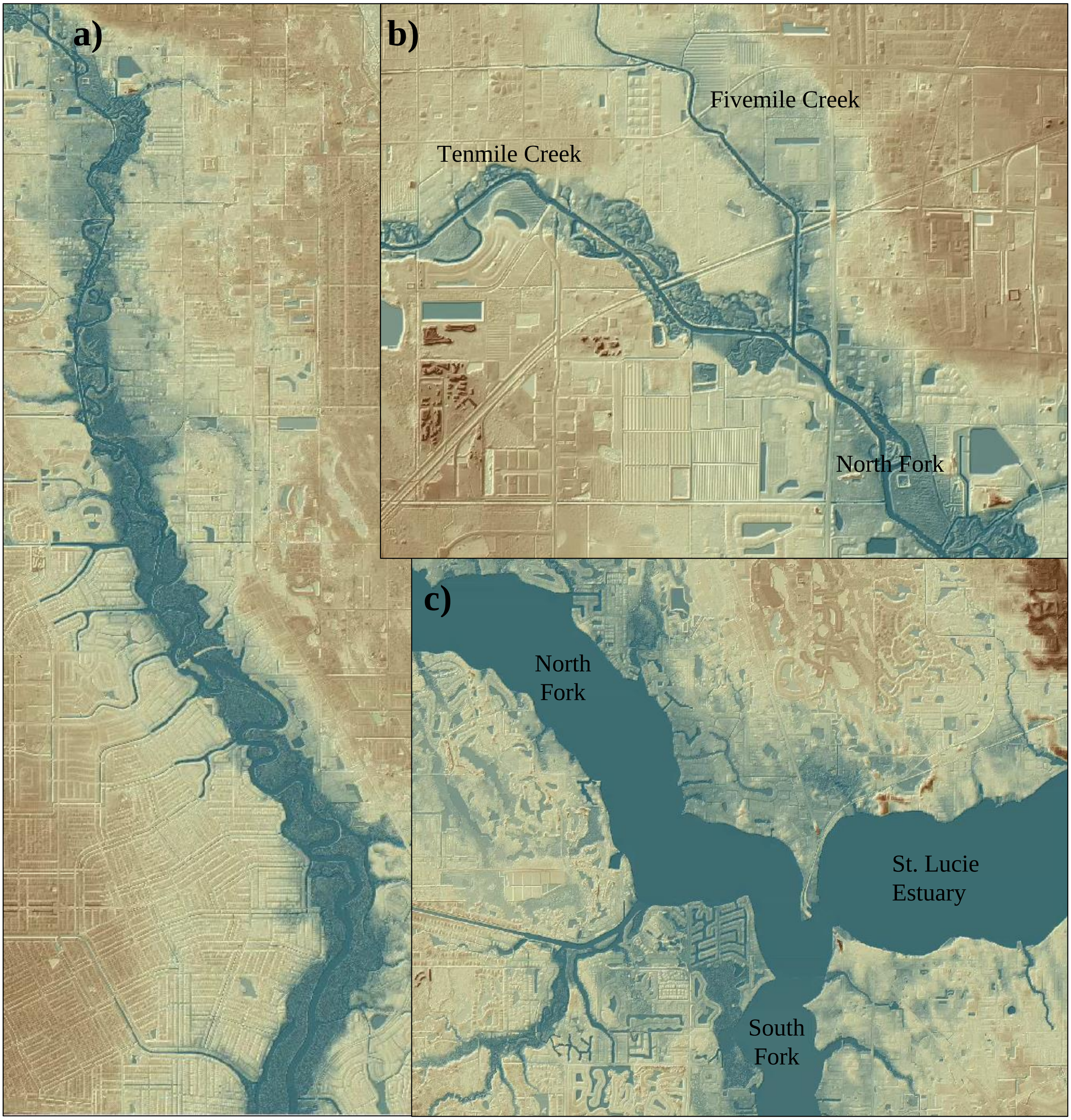

Figure 2. Digital elevation model (DEM) of the (a) North Fork of the St. Lucie Estuary in St. Lucie County showing (b) the origin at the confluence of Tenmile Creek and Fivemile Creek, and (c) the termination into the middle St. Lucie Estuary, digitized from the 2007 Florida Division of Emergency Management (FDEM) Statewide Coastal LiDAR project (Delivery Block 8, flown between Aug. and Oct. 2007) by South Florida Water Management District.

CPSL was founded in 1961 by the General Development Corporation (GDC), which also founded Port Charlotte, Port St. John, and Port Malabar (now Palm Bay), among other South Florida communities. Unfortunately, GDC communities were not planned with sufficient infrastructure to allow for sustainable development, as evidenced by degraded water quality in cities such as Palm Bay (Arnade 1999) and Port Charlotte (Lapointe et al. 2016). Based on 2009 survey data, the population of the areas in St. Lucie and Indian River counties surrounding the North Fork is primarily medium-high density in the range of 196,238 – 389,320 individuals. As the population in these areas continues to grow it becomes increasingly important to address local environmental issues, such as water quality, to maintain the high quality of life that initially attracted new residents.

In an effort to grow sustainably and to alleviate subsequent water quality issues, CPSL developed a septic-to-sewer program beginning in 1999. To date, 7,527 septic systems have been converted to a low-pressure, centralized sewer system. This program demonstrates CPSL's commitment to maintaining and improving local watersheds. CPSL remains one of the only coastal cities in Florida to implement such an ambitious wastewater infrastructure plan, which is critical for achieving long-term development and water quality goals. Additionally, 22,152 new homes have been built that were connected to the sewer system immediately. This demonstrates that CPSL has been proactive in increasing the availability of sewer connections to its residents and decreasing the reliance on septic systems, which are not appropriate in such high-density areas.

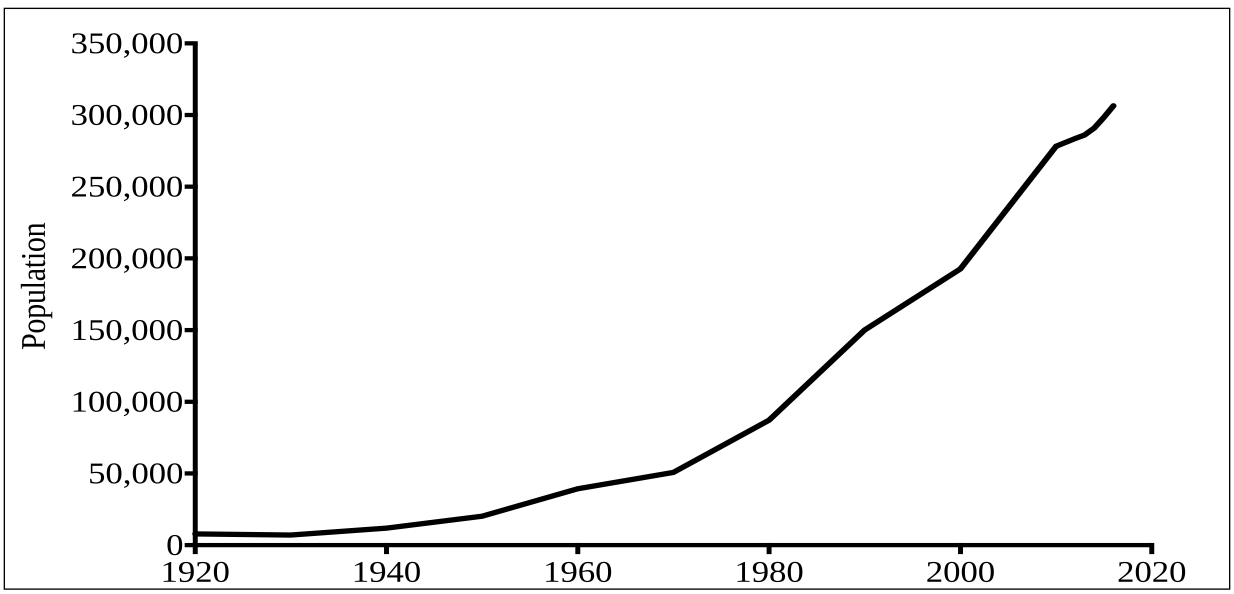

Figure 3. Population growth in St. Lucie County from 1920 – 2016, showing the rapid increase in population size beginning in the 1980s. (source U.S. Census).

The North Fork is a Class III water body with an area of approximately 58 sq. miles and the designated uses of recreation, propagation, and maintenance of a healthy, well-balanced population of fish and wildlife (White and Turner 2012). The North Fork originates from the confluence of Tenmile Creek and Fivemile Creek in eastern St. Lucie County (**Fig. 2a,b**) and flows south for about 15 miles before joining the South Fork in the Middle Estuary (**Fig. 2c**). The area is low, poorly drained, flat land with sandy soils (FDEP 2003; **Fig. 2**). Furthermore, in this area soils remain wet for most of the year because the flatness reduces water runoff (White and Turner 2012) and the unconfined, surficial aquifer is at or near the surface (Miller 1990).

Beginning in 2005, SLC DOH monitored 14 sites within the North Fork for enterococci and from 2005-2011 fecal coliforms were monitored as well. Fecal coliform levels, in colony forming units (cfu) per 100 mL, were high in 2005-2006, with many exceedances for both "moderate" (200-399 cfu/100mL) and "poor" (400+ cfu/100mL) criteria based on Florida Department of Health (FL DOH) standards, however from 2007-2011 the number of exceedances declined (**Fig. 4a**). Enterococci levels have been consistently high, exceeding the FL DOH criteria for "moderate" (36-70 cfu/100mL) and "poor" (71 or more cfu/100mL) multiple times every year since 2005 (**Fig. 4b**); the United States Environmental Protection Agency (USEPA) recommends a swimming advisory at the "poor" criteria. The lowest number of exceedances for enterococci were observed during 2012 and 2013, unfortunately these increased again in 2014 (**Fig. 4b**). Furthermore, in 2014, 2016 and 2017 the number of exceedances for "poor" criteria was greater than the number of exceedances for "moderate" criteria for each year, indicating water quality conditions may be worsening in the North Fork (**Fig. 4b**).

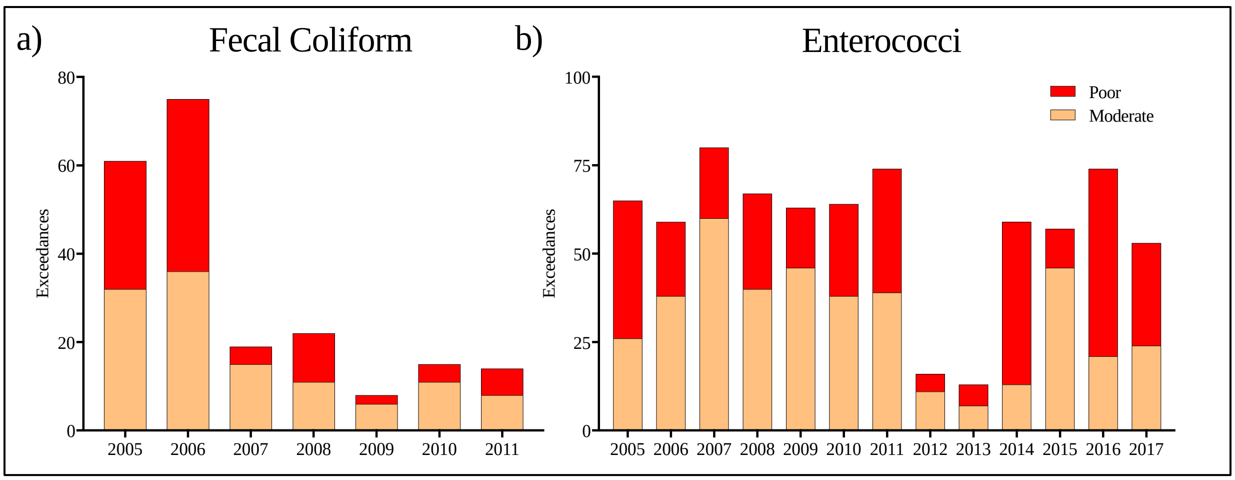

Figure 4. Number of bacteria water quality standard exceedances per year within the North Fork of the St. Lucie Estuary, classified as "poor" or "moderate," based on Florida Department of Health standards for a) fecal coliforms and b) enterococci; all "poor" classifications result in the issuance of a suggested closure of the waterbody for swimming and other uses that involve contact with water.

The North Fork has been verified by FDEP to be impaired for fecal coliform bacteria since 2009 and has been intermittently deemed unsafe for contact since 2005 (Lapointe et al. 2012; **Fig. 4**). For a waterbody to be considered impaired for fecal coliforms under the previous water quality standards (new standards adopted in 2017) it must have exceeded the standard of 800 cfu/100mL in a single sample or 400 cfu/100mL in more than 10% of the samples collected. During the verification period from 2001-2008, the North Fork exceeded the standard for 10 of 55 samples (18%; **Table 1**). Based on USEPA recommended values, current FDEP standards now reference criteria based on the Monthly Geometric Mean (MGM) using a minimum of ten samples or the

Ten Percent Threshold Value (TPTV) with no minimum sample size. The new FDEP standards are based on *Escherichia coli* (*E. coli*) for freshwater samples (126 MGM and 410 TPTV) and enterococci in marine waters (35 MGM and 130 TPTV).

Table 1. Florida Department of Environmental Protection fecal coliform bacteria data used in the impaired classification for the North Fork of the St. Lucie Estuary (White and Turner 2012).

Parameter	Count
Total number of samples	55
Number of exceedances	10
Maximum count	2,200
Minimum count	8
Mean count	275

Accurately quantifying the fecal coliform loadings from nonpoint source pollution requires an understanding of land-use (White and Turner 2012), and the relationship between land-use and water quality has been well documented in other locations (Bu et al. 2014; Kibena et al. 2014). Similarly, anthropogenic alterations to land-use tend to negatively affect downstream water quality (Bolstad and Swank 1997). In fact, as little as 10% alteration in land-use can result in significant deviations in surface water quality (Zampella et al. 2007). As such, differences in land-use are often related to bacterial and dissolved nutrient concentrations in surface waters (Larned et al. 2004; Bu et al. 2014, 2016). While surveys of land-use have been made along the North Fork (**Table 2, Fig. 5)**, no investigations have been made to correlate land-use with respective water quality.

Table 2. Land-use in the North Fork of the St. Lucie Estuary showing total acres and percent (%) acreage (adapted from White and Turner 2012).

Land Use Classification	Acres	Percent Acreage
Medium Density Residential	15,735	42.47
Upland Forest	4,135	11.16
Urban and Built up	3,993	10.78
Low Density Residential	3,722	10.05
Wetlands	3,018	8.14
High Density Residential	1,678	4.53
Water	1,624	4.38
Transportation, Communication, & Utilities	1,436	3.87
Agriculture	1,099	2.97
Rangeland	534	1.44
Barren Land	79	0.21

In the North Fork, there are no permitted outfalls or direct waste discharges (point sources) that would be expected to contribute to fecal coliform impairment (White and Turner 2012). The three major wastewater treatment facilities within the basin (Westport, Glades, and St. Lucie West Wastewater Treatment Facilities) discharge effluent via deep well injection at depths ranging from approximately 3,200 – 3,500' below surface. Furthermore, the use of reclaimed water, or wastewater that has been converted into a useable form for irrigation, is primarily limited to the southern end of the North Fork. Therefore, wastewater treatment is likely not a major source of contamination to the North Fork.

In the North Fork watershed, medium density residential makes up the highest percentage of land-use, followed by upland forest, urban and built up, and low density residential (**Table 2, Fig. 5**). Some of these neighborhoods are primarily serviced by septic systems, which have been shown to degrade adjacent water bodies in similar soil conditions (Harden et al. 2008; Levett et al. 2010; Lapointe et al. 2015, 2016, 2017). In areas with low elevations and high water tables, such as much of South Florida, septic systems often do not function properly (Arnade 1999; Bicki and Brown 1990; Lipp et al. 2001; Lapointe et al. 2016). For proper functioning, septic systems must have at minimum 24-inches of unsaturated soil, however laboratory studies demonstrate that even this depth is not always sufficient (Bicki and Brown 1990). Groundwater sampling in Charlotte County, FL, which also has porous, sandy, poorly drained soils similar to Port St. Lucie, revealed the high water table varies seasonally and often does not meet the required depth to water for proper septic system functionality (Lapointe et al. 2016). When septic systems do not function as intended, increased contaminant loading to groundwater and adjacent surface water can occur even without complete failure (Lipp et al. 2001; Lapointe et al. 2017). Nitrification, the conversion of ammonium to nitrate, requires well drained, aerated soils unlike those in the North Fork watershed. Without aerated soils to promote nitrification, this can result in increased ammonium concentrations near septic systems (Lapointe et al. 1990, 2017), due to leaching prior to complete nitrification in the holding tank or drainfield. Increased concentrations of dissolved reactive nutrients can have adverse effects to water bodies, including decreased clarity due to high phytoplankton biomass and harmful algal blooms (HABs; Lapointe et al. 2015, 2017). The ratios of dissolved nutrients (total nitrogen:total phosphorus, dissolved inorganic nitrogen:soluble reactive phosphorus) are particularly important to ecosystem health. For example, in the North Fork, increased nutrient loading to the St. Lucie Estuary can lead to eutrophication and create downstream conditions favorable for blue-green algae blooms (low nitrogen to phosphorus ratios), such as the *Microcystis aeruginosa* bloom of 2016 (Lapointe et al. 2017).

Eutrophication has many negative effects for aquatic ecosystems, such as increased algal and plant growth, as well as low dissolved oxygen (DO; Carpenter el al. 1998). Nutrient loading is a recognized issue in the North Fork, and the system has been classified as impaired on the USEPA 303(d) list for total phosphorus (TP), total nitrogen (TN), and DO since 1998 (verified in 2004). High dissolved inorganic nitrogen (DIN) has been recorded in the North Fork and attributed to the influence of residential land-use (Yang et al. 2008) and septic systems (Lapointe et al. 2012). In 2009, site specific surface water Total Maximum Daily Loads (TMDLs) were developed for the St. Lucie Basin (amended in 2012), including the North Fork (Rule 62-304-705, FAC). The St. Lucie Basin TMDL goals are to achieve 0.081 mg/L TP and 0.72 mg/L TN at the Roosevelt Bridge. The TMDL states that, "based on data in the period from 1996 to 2005, the cumulative load from all sources to the North Fork of the St. Lucie Estuary is 103,747 lbs/year total nitrogen and 11,672 lbs/year total phosphorus" (St. Lucie River and Estuary Basin Technical Stakeholders 2013). To reach these TMDLs, goal Load Allocations (LAs) for nonpoint sources are a 28.8% reduction of TN and a 58.1% reduction of TP.

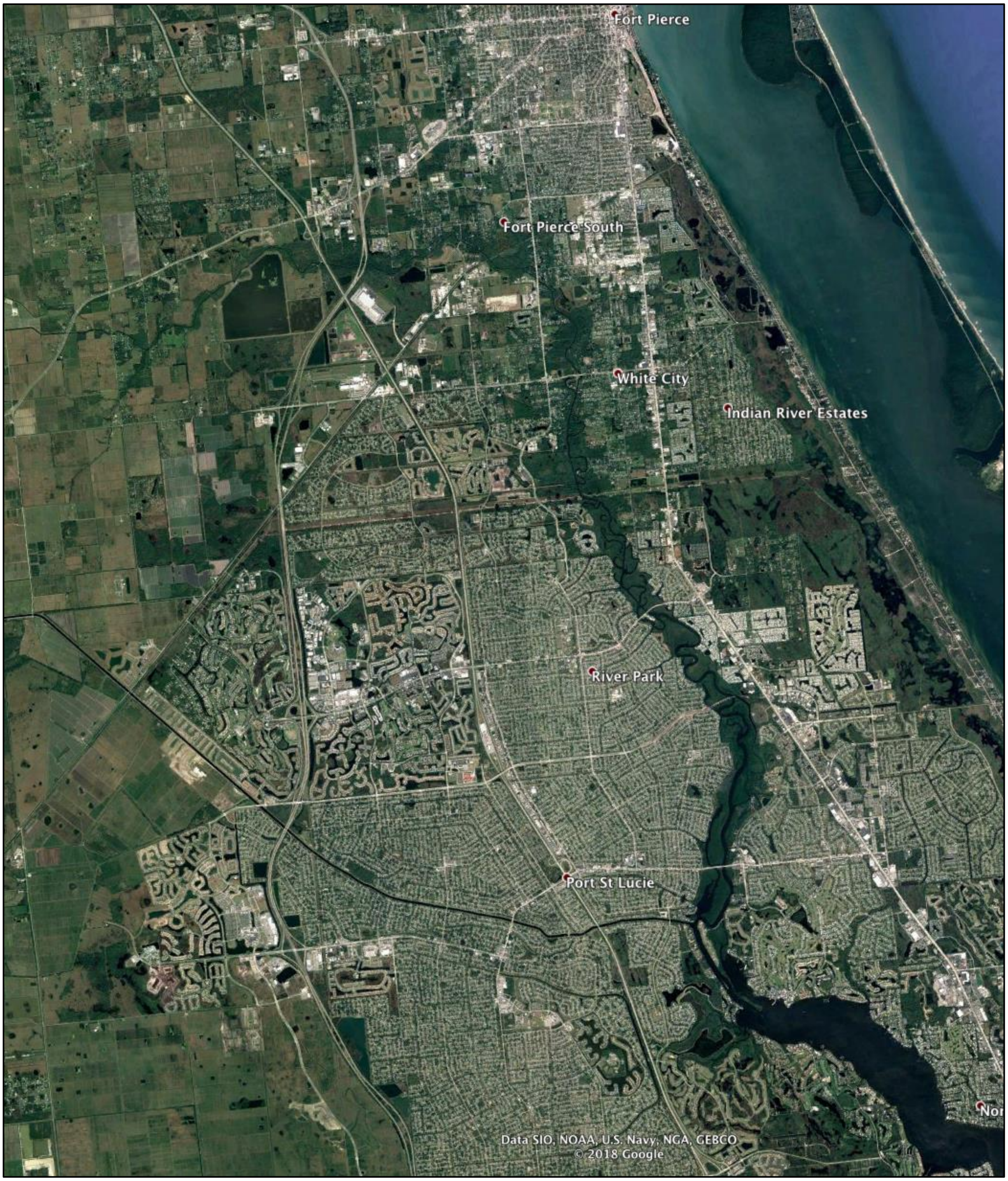

Figure 5. Satellite image of the North Fork of the St. Lucie River showing heavy residential land-use; for a detailed breakdown of land-use, see **Table 2**.

1.3 Microbial Source Tracking

Bacterial impairments and concentrations are important to consider because the presence and magnitude of certain indicator bacteria can be used as a measure of the safety and suitability of the water for various recreational uses. Sources of bacteria to surface waters can be point source (wastewater treatment plant discharge) and non-point source (septic tanks, leaking sewer pipes, sewage overflows, urban runoff, pet waste, livestock, agriculture, and wildlife; **Fig. 6**, Byappanahalli et al. 2012). Given the large number of factors to consider it is important to understand that urban water quality is highly variable and dependent on weather conditions, rainfall catchment, and drainage infrastructure (Tran et al. 2015).

Fecal coliforms are bacteria found in the lower intestines of warm-blooded organisms and generally enter the environment through fecal matter. Some strains are not harmful, however some can cause illness. The most common fecal coliform is *E. coli*, which is thought to be a better indicator of human health risk than fecal coliforms (USEPA 2012). Enterococci are a subgroup within the fecal streptococcus group that occur within human digestive systems and are able to survive in salt water. It is interesting to note that decaying macrophytes can be a source of enterococci to a system (**Fig. 6,** Byappanahalli et al. 2012), which may also be an important point to consider in the North Fork. The use of fecal indicator bacteria (FIB) to assess contamination in water bodies is challenging for a number of reasons including difficulty in discriminating between sources (e.g. enteric or environmental) and short survival times (Scott et al. 2002; Tran et al. 2015). This uncertainty creates the need for multiple lines of evidence and a suite of source tracking tools.

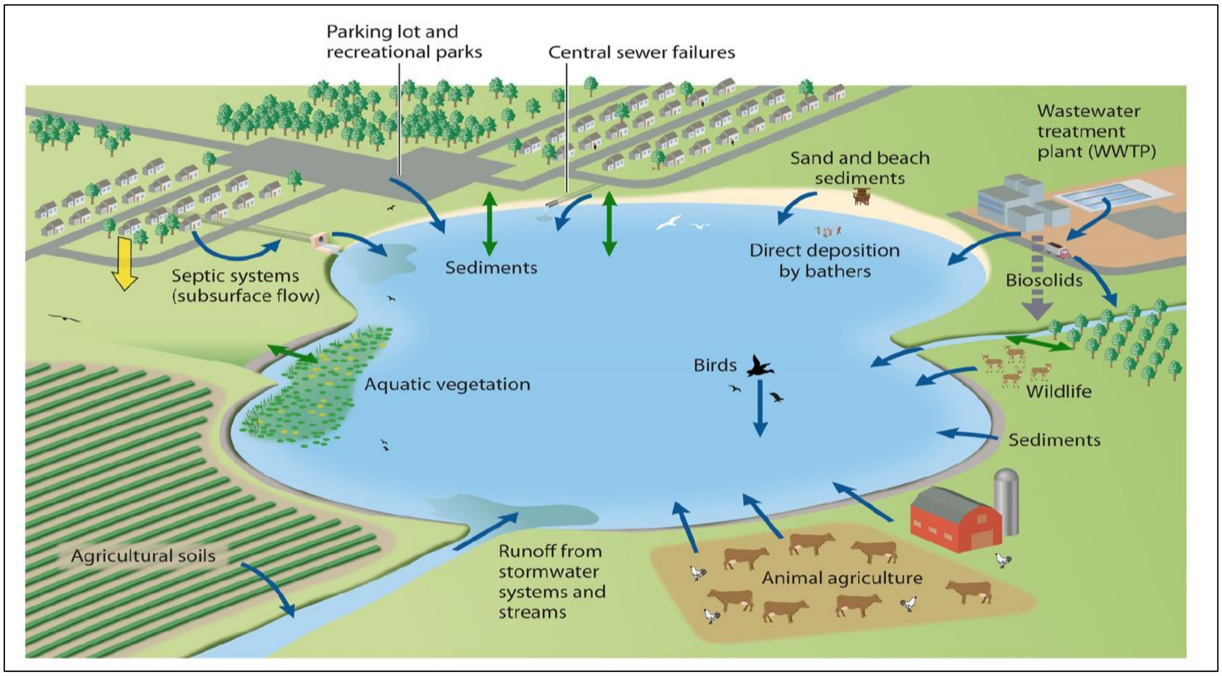

Figure 6. Sources of enteric bacteria to water bodies (blue arrows), as well as sinks where enterococci are immobilized (yellow arrows), and areas of flux, in which enterococci can transition from a reservoir to the water column and vice versa (green arrows; from Byappanahalli et al. 2012).

Molecular markers can be useful in determining the source of the bacterial impairment (Scott et al. 2002). For example, certain types of genetic material (DNA) can be amplified using quantitative polymerase chain reaction (qPCR), a laboratory technique that allows for both the identification and quantification of DNA. Specific qPCR targets can be chosen to help determine if the source of bacteria is most likely human (HF183) or birds (Gull2, GFD). The Bacteroidales HF183 DNA marker is a human associated bacteria that does not survive well in oxygenated environments, however the DNA still remains present and detectable. In raw sewage HF183 can be detected at very high levels, in the tens of millions in Genomic Equivalent Units per 100 mL of sample (GEU/100 mL), while septic tank effluent ranges in the hundreds of thousands GEU/100mL, and treated wastewater is highly variable and ranges from non-detectable quantities to tens of thousands GEU/100mL (Matthews 2016). Established guidelines for interpreting HF183 results do not exist. However relatively speaking a GEU/100 mL detection in the millions can be considered a "high" signal, above ten thousand GEU/100 mL as a "moderate" signal, and less than ten thousand GEU/100 mL as a "low" signal (Matthews 2016). The Gull2 DNA marker, measured in Target Sequence Copies per 100 mL (TSC/100mL), is based on a seagull associated bacteria species, *Catellicoccus marimammalium*, that is also found in other coastal bird species. The GFD marker is an unclassified *Helicobacter sp*. and can also be useful in detecting gulls, as well as many other bird species including goose, chicken, pigeon, egret, crow, and others. If detectable, these various molecular markers allow for discrimination between human and avian bacteria sources within a water body. Unfortunately, there are limitations to these molecular analyses. For example, suspended organic compounds, sediments, or complex biomolecules can inhibit qPCR (Sidstedt et al. 2015). HF183 is particularly sensitive to humic acid (Green and Field 2012), which is common in many rivers and streams. It is also of note that due to holding times septic tank effluent and sewage may have fewer MST molecular markers than fresh waste, and thus reduced amplification efficiency (Boehm et al. 2013).

Chemical markers are also useful in MST studies to help determine the source of bacterial contamination. The use of chemical markers has some advantages over molecular markers including increased reliability of detection, source specificity, and stability (Lim et al. 2017). Chemical markers useful for source tracking studies include indicators of human waste like the artificial sweetener, sucralose, and pharmaceuticals including pain relievers, such as acetaminophen, and anticonvulsants with broad applications, such as carbamazepine and primidone. Sucralose has been documented in treated wastewater and reclaimed water in concentrations between 10 to 40 µg/L (FDEP 2014) and in untreated wastewater, such as septic tank effluent, in concentrations ranging from 2 to 67 µg/L (Buerge et al. 2009; Lapointe et al. 2016; Yang et al. 2016; Snider et al. 2017). The overlap between the observed concentrations in the different wastewater types indicates difficulty in discriminating sources purely by concentration. This is in part because chemical concentrations vary due to relative inputs and degradation.

However, the relationships and presence of these human source indicators can be very useful in determining sources of pollution to a system. During wastewater treatment, sucralose does not break down, however acetaminophen is almost completely removed. Similar to sucralose, carbamazepine and primidone are also regarded as "conservative" tracers that are not removed during wastewater treatment (Dong et al. 2015). The detection of conservative tracers, like sucralose and carbamazepine, reveals that there is wastewater (treated or untreated) in the waterbody, while the presence of acetaminophen more strongly suggests a raw or untreated wastewater source, such as septic system effluent (Lv et al. 2014; Matthews 2016). As such, the relative concentrations of sucralose and acetaminophen can be helpful in determining if the pollution source is treated or untreated wastewater (Matthews 2016). However, in septic systems with advanced treatment technologies, wastewater may be partially treated and some pharmaceuticals removed (Wilcox et al. 2009). Furthermore, many pharmaceuticals and chemical compounds, except for sucralose, degrade during transport out of the holding tank through the drainfield soil (**Fig 7**, Yang et al. 2016).

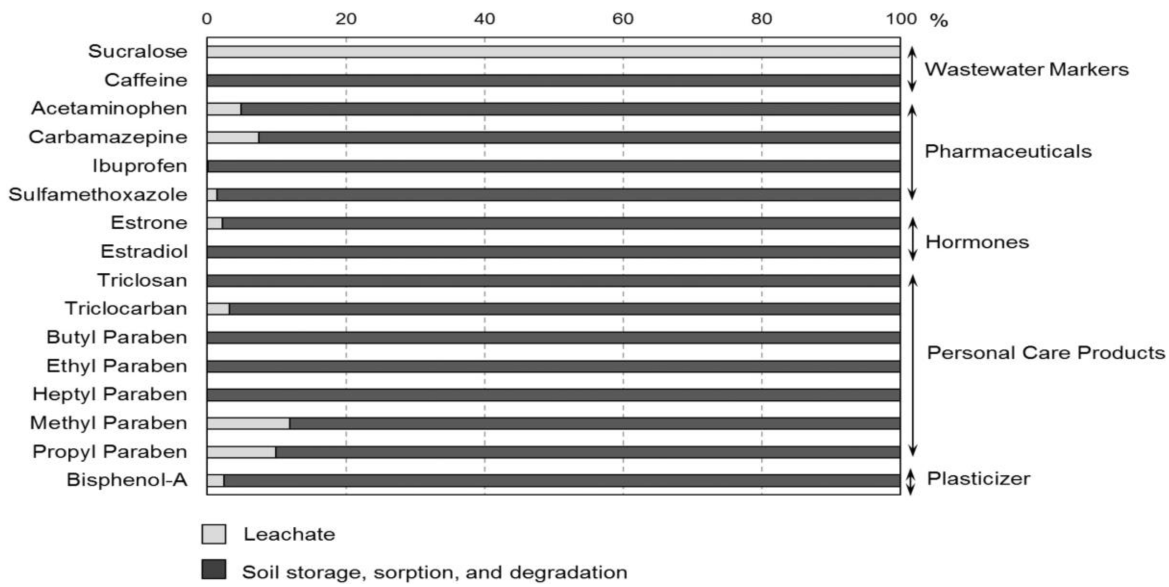

Figure 7. Average percent reduction of micropollutant loads from septic tank effluent to leachate, showing how sucralose remains present in septic tank leachate whereas more labile tracers, such as acetaminophen, and even the more chemically stabile carbamazepine, break down in septic drainfields; suggesting that sucralose is a suitable tracer of septic system impacts to groundwaters (from Yang et al. 2016).

Other chemicals can be useful tracers to assess sources of contamination to a waterbody. As such, MST testing can also be done for herbicides or insecticides of varied uses. The differential uses of these chemicals may help reveal sources of run-off to the system. For example, fluridone is used to control aquatic weeds, such as water lettuce. Therefore, the presence of fluridone suggests that chemical macrophyte control has occurred in a water body. This may be relevant to water quality as decaying macrophytes can be another source of bacteria in aquatic systems (Byappanahalli et al. 2012). Diuron, another herbicide, is used for roadside vegetation control and agricultural applications. The presence of diuron would suggest terrestrial runoff is affecting a system. Fenuron is an herbicide used for weed control on non-crop land, so it suggests more of a residential influence, while linuron is an herbicide used primarily in agricultural areas. Imidacloprid is a pesticide used to treat crops for insects, as well as homes for termites and fleas. The multiple uses of imidacloprid make it a good general tracer for terrestrial runoff. The presence or absence of these various chemicals can allow for inferences regarding where aquatic bacteria and other pollutants may be sourced from.

2. Methods

2.1 Site Descriptions

Surface water sampling at 21 sites was conducted by CPSL in conjunction with St. Lucie County, SLC DOH, and FDEP, following established FDEP standard operating procedures (SOPs) for the collection of surface water (Rule 62-160, FAC; **Fig 8**). Sites sampled included: tributary sites in Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites (R1 upstream – R7 downstream); and canal sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22; **Fig. 8**). See **Appendix 1** for detailed site information including GPS coordinates and **Supplemental Information** (**SI 1-21**) for satellite imagery of all sites. The estimated flow rates for each canal were obtained from CPSL and considered to estimate the relative potential of the canals to impact water quality in the North Fork.

Tributary Sites

Tenmile Creek

TM2 is the most upstream site within Tenmile Creek. The site is primarily characterized by agricultural land-use, with some low-density residential usage as well. TM1 is farther downstream and the land-use is predominantly residential, with a lesser amount of agricultural influence than TM2. Progressing from TM2 downstream to TM1 there are several houses directly on the water.

Fivemile Creek

FM2 is the most upstream site within Fivemile Creek. There is mixed land-use influencing FM2 of primarily agriculture and commercial properties, with a smaller proportion of residential properties. FM1 is farther downstream and is characterized by mostly residential land-use, with some commercial properties including a Walmart shopping plaza. Several houses are located directly on the water in close proximity to FM1.

River Sites

The R1 site is the most upstream river site and is located north of Midway Road. This area is primarily residential, with a heavy reliance on septic systems, and several houses located directly on the water. Historic SLC DOH sampling events confirmed consistently high enterococcus concentrations at this site.

R2 is located in a predominantly residential area. An elementary and middle school are also located upstream of R2. There is a large area reliant of septic systems for wastewater just northeast of R2.

The R3 site is located in an area of dense residential land-use with some commercial properties interspersed. Several houses are located directly on the water, including the Hidden River Development, which uses septic systems for wastewater.

R4 is located at the convergence of D11, D12, and D13 canal drainages. This site is in a dense residential area with several houses directly on the water, some using septic systems. The area consists of several small basins with mixed septic system and sewered components.

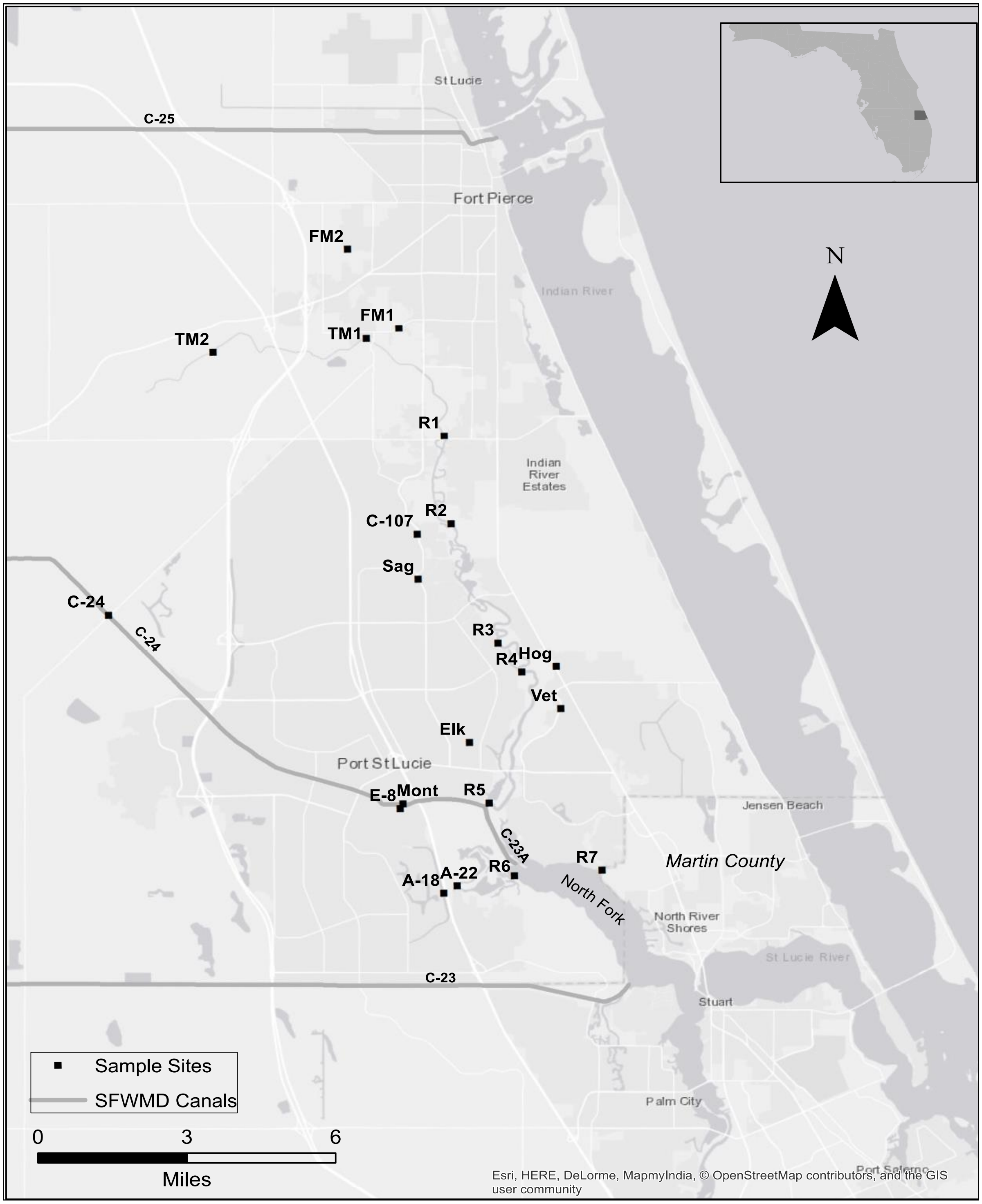

Figure 8. Sampling sites for Port St. Lucie Microbial Source Tracking (MST) study in the North Fork of the St. Lucie Estuary, including tributary sites in Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites (R1 upstream – R7 downstream); and canal sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22).

R5 is located at the convergence of the Elkcam Waterway and Kingsway Waterway, which are two large basins that represent mixed septic system and sewer components. The area supports dense residential land-use with several houses in close proximity to the water, but not directly on the water. However, canals that drain just upstream of the R5 site do have waterfront houses on septic systems.

R6 is located in the Five Fingers Area with drainage from Horseshoe Canal, Southbend Area, and Tesoro. Horseshoe Canal and Southbend are large residential basins with mixed septic and sewer components, while Tesoro is entirely sewer and has two golf courses. This site is also located directly across from Club Med. Several houses utilizing septic systems near R6 are directly on the water.

The R7 site is located in the Howard Creek Basin, which represents a mixed septic system and sewer basin. There are two golf courses upstream of this site and the Club Med resort, which had several advisories during the wet season. Within the area of influence at R7, there are several houses with septic systems directly on the water.

Canal Sites

C-107 is a drainage canal that carries some agricultural runoff from the west, as well as municipal drainage. This area has mixed wastewater infrastructure, with parcels using both septic systems and centralized sewer in the basin. The area has dense residential land-use with several houses directly on the water, some using septic systems. At this site, samples were collected upstream of a concrete outfall structure.

Sag is located in a dense residential land-use area with a combination of septic systems and sewered properties within the basin, with many houses directly on the water using septic systems. Samples at Sag were collected upstream of a concrete outfall structure. Previous sampling by CPSL found high fecal coliform and nutrient concentrations at this site.

Hog is located at the H-60 structure. The Hog site is primarily serviced by CPSL centralized sewer, with only 7 septic systems permitted in this basin. In this basin, there are several industrial lift stations that are not the responsibility of CPSL. Hog receives water flow from the Savannahs preserve area, where there are houses with septic systems. Samples were collected upstream of a concrete outfall structure. Previously, high fecal counts were observed by CPSL.

Vet is located at the U16-D016 drainage structure. The area is mostly residential, with some businesses, and many houses located directly on the water. This site receives drainage from a sewered residential area east of US-1, and water is pumped and drains through ~50 acres of vegetated area before discharging to the river. Samples were collected upstream of culvert pipes that go under Veterans Memorial Drive and directly into the river. High fecal coliform and TN were observed during the CPSL sampling program. After this study was concluded, a control structure and storm water pond were installed at this location.

Elk is a large basin, receiving drainage from St. Lucie West, which is entirely sewered, and the central part of the city that is a mixture of septic systems and sewered parcels. The area is predominantly residential, with many houses directly on the water. Samples at Elk were collected upstream of a concrete outfall structure.

Mont is a large drainage basin, which is comprised of dense residential area land-use with mixed septic system and sewered components. Many houses in Mont are located directly on the water, some using septic systems. Samples were collected upstream of a concrete outfall structure.

The C-24 canal is entirely influenced by agricultural land-use. The site is located outside the city limits. C-24 samples were collected directly from the canal, ~7 miles upstream of the outfall structure to the North Fork.

E-8 canal is a large drainage basin. This site represents dense residential land-use with mixed septic system and sewered components. At E-8 there are many houses on the water using septic systems. Samples at E-8 were collected upstream of a concrete outfall structure.

A-18 is located at a structure in Horseshoe Canal, which drains a large portion of western CPSL. The area has mixed wastewater infrastructure with both septic systems and sewered parcels. There are also many houses on the water using septic systems. Samples at A-18 were collected upstream of a concrete outfall structure.

A-22 receives drainage from a large part of the Southbend area and a portion of Horseshoe Canal. The area is mostly residential land-use, with a combination of both septic systems and sewered components. At A-22, there are many houses located directly on the water. Previous sampling by CPSL found high fecal coliform counts at this site. A-22 drains into the North Fork across from Club Med, which is a SLC DOH sampling point and had bacterial advisories during the wet season.

2.2 Data Collection

Rainfall data over the study period (May 2016 – October 2017) was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (https://www.ncdc.noaa.gov/data-access). The station selected, Port St. Lucie 2.4N, FL US (GHCND:US1FLSL0022) was centrally located in the study area (27.3138°, -80.3563°) in the River Park neighborhood of CPSL and had fairly complete coverage over the study period (96%). Daily total precipitation was plotted to indicate seasonal rainfall inputs relative to sampling events.

For MST purposes, all sites were sampled three times during the 2016 wet season (June 23, July 25, August 22), twice during the 2017 dry season (February 16, March 16), and once during a rain event in the 2017 wet season (July 19). For a table of all sample dates with the parameters collected, see **Appendix 2**. After sample collection, calibrated YSI sondes were used to measure pH, salinity, temperature, conductivity, and DO at each site. All seasonal samples were collected on the last of the outgoing tide because tidal pumping can increase contaminant loading to surface waters from groundwater (Lapointe et al. 1990) and to ensure that downstream contamination sources would not affect samples (FDEP 2014). The rain event, however, was sampled at the beginning of the outgoing tide due to logistical constraints.

Microbial sampling during this project included fecal coliforms at all sites, *E. coli* at tributaries and canals, and enterococci for river samples. Fecal coliform samples were analyzed by CPSL Utility Systems Department Laboratory, following EPA standard method (SM) 9222D. *E. coli* and enterococcus samples were analyzed by Flowers Chemical Laboratories, Inc., Port St. Lucie, FL. *E. coli* was analyzed following SM 9223 B. Enterococci was analyzed following EPA SM1600 during the wet season 2016 (reported in cfu/100mL) and then by American Society for Testing and Materials standard (ASTM) D6503 – 99 (reported in MPN/100mL) for all subsequent samples. Bacteria data were compared to the TPTV for either *E. coli* (410) or enterococci (130), as well as the previous FDEP single sample standard for fecal coliform bacteria (800).

MST samples were shipped to the FDEP Central Laboratory for determination of molecular marker and chemical concentrations, following standard methods available on the FDEP website (https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops). All MST samples were analyzed for presence and concentration of the molecular markers, HF183 and Gull2; additionally dry season and rain event samples were analyzed for the GFD marker. All MST samples were also analyzed for concentrations of sucralose, acetaminophen, carbamazepine, primidone, fluridone, diuron, fenuron, linuron, and imidacloprid. Additional markers were tested for on 03/16/2017 and 07/19/2017 including three herbicides, 2,4-Dichlorophenoxyacetic acid (2,4-D), bentazon, and triclopyr, and one pharmaceutical, the psychoactive stimulant meta-Chlorophenylpiperazine (mCPP). For descriptive purposes, sucralose detections were classified as "moderate" from 0.1-1.0 µg/L and "significant" at greater than 1.0 µg/L (FDEP 2014). See **Appendix 3** for the minimum detection limit (MDL) and practical quantitation limit (PQL) for MST analyses from each sample date. Any samples flagged by the FDEP Central Laboratory as below detection limits (U) or less than the criterion of detection (T) were removed from the dataset prior to analyses. However, samples flagged as between the laboratory MDL and the laboratory PQL (I) included in analyses.

Supplemental dissolved nutrient sampling was conducted during one of the 2017 dry season sampling events (March 16), during the 2017 rain event sampling (July 19), and an independent wet season sampling event conducted on October 9, 2017 (see **Appendix 2**). These samples were shipped to the University of Maryland Chesapeake Biological Laboratory for analysis of reactive nutrients, including ammonium (NH_4), nitrate + nitrite (NO_3), and soluble reactive phosphorus (SRP), following standard methods (online here: http://nasl.cbl.umces.edu/methods/WCC.html). Detection limits for dissolved nutrients were 0.003 mg/L for ammonium, 0.0001 mg/L for nitrate + nitrite, and 0.0003 mg/L for SRP. 2017 wet season nutrients were compared against 2016 MST data to approximate how these tracers relate to nutrient loading under wet season conditions. During the 2017 wet season dissolved nutrient sampling event, TM2 samples were collected slightly upstream (~1.5 mi) from previous sampling events due to site access.

Between June and September 2017, targeted, fine-scale bacterial sampling of surface water was conducted by CPSL following FDEP SOPs at multiple locations within the larger area surrounding three of the canal sites (Sag, Hog, and Vet) to better decipher localized sources of fecal pollution. For these targeted sampling events surface water samples were collected at the following areas: nine sites within the Sag system, (Sag 1-9), ten sites within the Hog system (Hog 1-10), and six sites within the Vet system (Vet 1-6). These samples were analyzed for *E.*

coli at Flowers Chemical Laboratories, Inc. as above. The results of these sampling events were considered in regards to the MST and nutrient data to further assess potential sources of fine-scale pollution within these canal sites.

2.3 Data Analyses

Microbial source tracking data and nutrient concentrations were initially compared by considering overall site averages, following which seasonal averages were compared by site. Relationships between the relative concentrations of various parameters were then investigated to look for relationships between variables. Site visits and local knowledge from CPSL were used to groundtruth data interpretation. The combined microbial source tracking, water quality, and land-use data were considered together to make inferences regarding potential sources of bacterial contamination in the North Fork, to propose possible solutions to this chronic public health issue, and to make recommendations for the direction of future investigations.

3. Results

3.1 Flow Rates

Based on 10 year – 1 day flow estimates, Mont has the highest flow rate, followed by A-22, C-107, Sag, E-8, Elk, A-18, Hog, and Vet (**Table 3**). When the 25 Year – 3 Day flow rate is considered, A-22 has the greatest flow rate, followed by C-107, Mont, Elk, Sag, Vet, E-8, A-18, and Hog (**Table 3**). The 10 Year – 1 day flow rate will be considered with the purpose of evaluating the relative influence of canal discharges on adjacent river water quality. The flow of the C-24 canal site is variable and controlled by South Florida Water Management District (SFWMD), so was not considered. The relative magnitude of the effect the canal sites might exert upon the North Fork can be examined by considering their relative flow rates. C-107, Mont, and A-22 have the highest flow rates and therefore are expected to have the greatest impact on the North Fork, while Sag, Elk, and E-8 have median flow rates, and Hog, Vet, and A-18 have the lowest flow rates.

Table 3. Estimated flow rates for canals draining into the North Fork of the St. Lucie Estuary.

Canal	**10 Year - 1 Day Flow (ft^3/sec)**	**25 Year - 3 Day Flow (ft^3/sec)**	**100 Year - 3 Day Flow (ft^3/sec)**
C-107	447	719	804
Sagamore	306	335	370
Hogpen Slough	111	171	217
Veterans Memorial	93	335	382
Elkcam	204	428	519
Monterrey	595	709	734
C-24	N/A	N/A	N/A
E-8	214	298	345
Horseshoe A-18	194	222	261
Southbend/Horseshoe A-22	490	855	1089

3.2 Rainfall

All sampling events were conducted on days with little to no precipitation (0-0.5 inch; **Fig. 9**). During the wet season approximately 15 inches of rain fell in the two months prior to the first sampling event, with about 2 inches of rainfall in the week before samples were collected. Rainfall was lower during the dry season, with approximately 2.5 inches of rain in the two months prior to the first dry season sampling event. There was 0-0.16 inches of rain recorded in the week leading up to each dry season sampling event. The rain event sampling was preceded by about 14 inches of rain in the two months prior to the sampling event, with ~2.5 inches of rain in the week before samples were collected. The supplemental wet season nutrient sampling was preceded by approximately 21 inches of recorded rainfall in the two months prior to this sampling event, however, there appears to be a data gap due to Hurricane Irma in September 2017. It is estimated that over 20 inches of rain fell on Port St. Lucie during Hurricane Irma on September 10-11, 2017, but this station only recorded 6 inches of rain during this timeframe. Approximately 6 inches of rainfall were recorded in the week before the supplemental wet season nutrient sampling event.

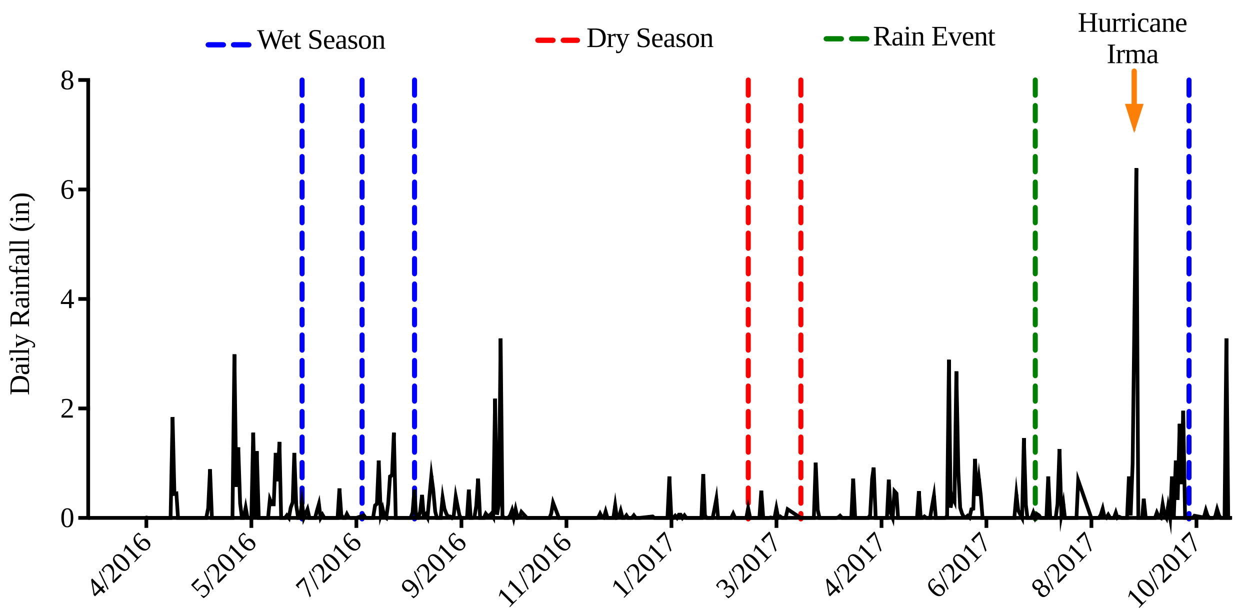

Figure 9. Daily precipitation (inches) in central Port St. Lucie over duration of the study period; dashed bars represent sampling events (colored by season as indicated); September 10-11, 2017, Hurricane Irma (indicated by the orange arrow) passed through the study areas and over 20 inches of rainfall is estimated to have fallen in the study area.

3.3 Environmental Parameters

Slight variability was observed in measured environmental parameters between study sites (**Table 4**) and seasons (**Appendix 4**). When the overall site averages from the project are considered, some trends remain consistent, though variability was observed for some parameters over the course of the study. At tributary sites, DO was generally higher downstream (TM1 and FM1) than upstream (TM2 and FM2). Overall, Tenmile Creek had higher conductivity (2987 ± 3516) and salinity (1.06 ± 0.79 ppt) than Fivemile Creek (811 ± 642 conductivity and 0.61 ± 0.28 ppt salinity; **Table 4**). River sites were fairly consistent for pH, temperature, and DO, however, salinity and conductivity increased from the upstream sites at the head of the North Fork to downstream in the Middle Estuary (**Table 4**). Salinity varied from mostly fresh at R1 (0.77 ±0.31 ppt) to more saline at R7 (7.82 ± 10.36 ppt; **Appendix 4**). Generally speaking, canal sites had the lowest conductivities and salinities, as well as the greatest variability in DO (**Table 4**).

Table 4. Environmental parameters observed in surface water during sampling events in the North Fork of the St. Lucie Estuary collected from tributaries of the North Fork (Tenmile Creek and Fivemile Creek), the main stem of the North Fork of the St. Lucie Estuary (river sites, R1 upstream – R7 downstream), and canals draining into the North Fork, showing the average ± standard error (SE) of all field measurements made during the wet and dry season sampling events, as well as the rain sampling event; SE presented to show the variability in physical conditions at each site over the study period.

Type	Site	Count	pH	Conductivity	Dissolved Oxygen (mg/L)	Temperature (°C)	Salinity
Tributary	Tenmile 2 - upsteam	7	7.03±0.38	2120±1022	4.67±0.86	26.12±5.66	1.10±0.74
	Tenmile 1 - downstream	7	7.10±0.37	3286±3396	5.52±1.67	25.55±5.68	0.94±0.68
	Fivemile 2 - upstream	7	7.15±0.56	1175±349	4.36±1.42	26.07±4.53	0.59±0.22
	Fivemile 1 - downstream	7	7.24±0.30	1378±849	5.27±1.65	25.50±5.39	0.64±0.35
River	R1 - upsteam	7	7.53±0.41	1513±509	4.70±1.51	26.13±4.26	0.77±0.31
	R2	7	7.60±0.43	1627±705	4.94±1.68	26.67±4.09	0.82±0.45
	R3	7	7.57±0.39	3586±4214	4.99±1.77	27.39±3.98	2.05±2.63
	R4	7	7.61±0.36	4588±5959	4.80±1.69	27.53±4.07	2.70±3.78
	R5	7	7.53±0.41	8566±12635	4.53±1.48	27.54±4.20	5.47±8.48
	R6	7	7.53±0.38	10487±13871	4.71±1.56	27.33±4.58	6.77±9.69
	R7 - downstream	7	7.37±0.49	12184±14551	5.10±1.87	27.13±4.50	7.82±10.36
Canal	C-107	7	7.44±0.23	444±81	4.49±2.10	27.44±4.35	0.20±0.05
	Sagamore	5	7.38±0.18	636±72	4.66±0.90	26.18±0.77	0.30±0.03
	Hogpen Slough	7	7.39±0.46	268±52	3.71±1.29	25.44±5.31	0.13±0.03
	Veterans Memorial	7	7.39±0.59	375±170	5.10±1.49	25.96±5.78	0.18±0.12
	Elkcam	7	7.56±0.26	415±166	4.87±2.74	27.19±3.97	0.22±0.03
	Monterrey	7	7.43±0.22	619±85	5.62±2.05	26.44±4.17	0.30±0.06
	C-24	7	7.59±0.38	976±391	7.07±2.97	27.53±4.64	0.46±0.24
	E-8	7	7.61±0.27	709±60	7.66±2.84	27.60±4.47	0.33±0.06
	Horseshoe A-18	7	7.68±0.33	480±80	7.49±2.36	27.96±4.87	0.23±0.05
	Southbend/Horseshoe A-22	7	7.48±0.31	456±68	3.57±1.37	26.53±5.47	0.22±0.05

3.3 Bacterial Prevalence

Enteric bacteria were present at all sites sampled during the study period, with the highest concentrations found in canals (**Table 5**). Fecal coliform bacteria concentrations for tributaries (Tenmile and Fivemile Creek) draining into the North Fork before the R1 site were generally lower than canals, but still in exceedance of FDEP standards in many instances (**Fig. 10**). During the wet season, *E. coli* was elevated at TM1 (301±269 MPN/100 mL), FM1 (263±147 MPN/100 mL), and exceeded FDEP standards at FM2 (430±481 MPN/100 mL; **Fig. 11a**). The same trend held for fecal coliforms with no tributary sites exceeding FDEP standards (**Fig. 10a**). Both Fivemile sites (344±380 MPN/100 mL at FM1 and 552±704 MPN/100 mL at FM2) and TM1 (222±98 MPN/100 mL) had elevated *E. coli* concentrations during the dry season (**Fig. 11b**), while fecal coliforms were low (below 200 cfu/100mL) for those sampling events (**Fig. 10b**). *E. coli* concentrations exceeding FDEP standards were observed at TM1 (821 MPN/100 mL) and FM1 (545 MPN/100 mL) during the rain event (**Fig. 11c**). No clear upstream vs. downstream seasonal trends emerged in the tributary sites for fecal coliforms (**Fig. 10**). However, *E. coli* concentrations in Tenmile Creek increased downstream (higher at TM1 than TM2), while

Fivemile Creek showed the opposite pattern of decreasing downstream (lower at FM1 than FM2).

Within the main stem of the North Fork the fecal coliform concentrations generally decreased from upstream to downstream (**Fig. 10**). These concentrations were higher in the wet season, ranging from 124±128 to 842±1350 cfu/100 mL (**Fig. 10a**), than the dry season which ranged from 10±0 to 477±301 cfu/100 mL (**Fig. 10b**) and the rain event which ranged from 20 to 2250 cfu/100 mL (**Fig. 10c**). However the most upstream site (R1) was consistently high, exceeding FDEP standards in all seasons (**Fig. 10a,b,c**). Similar trends were evident in both enterococci (**Fig. 11a,b,c**) and fecal coliforms (**Fig. 10a,b,c)**. During the wet season, enterococci were almost always found in higher concentrations, ranging from 151±90 to 1039±763 cfu/100 mL (**Fig. 11a**), than fecal coliforms at the river sites, which ranged from 124±128 to 842±1350 cfu/100 mL (**Fig. 11a**).

Table 5. Bacterial concentrations observed in surface water during sampling events in the North Fork of the St. Lucie Estuary collected from tributaries of the North Fork (Tenmile Creek and Fivemile Creek), the main stem of the North Fork of the St. Lucie Estuary (river sites, R1 upstream – R7 downstream), and canals draining into the North Fork, showing the average ± standard error of all data collected in the wet and dry season sampling events, as well as the rain

Type	Site	Count	Fecal Coliform (cfu)	*Escherichia coli* (MPN)	Count	Enterococci (cfu)	Count	Enterococci (MPN)
Tributary	Tenmile 2 - upsteam	6	102±69	75±39		NA		NA
	Tenmile 1 - downstream	6	169±110	361±288		NA		NA
	Fivemile 2 - upstream	6	250±234	413±470		NA		NA
	Fivemile 1 - downstream	6	145±57	337±223		NA		NA
River	R1 - upsteam	6	779±739	NA	3	734±307	3	469±652
	R2	6	286±254	NA	3	546±102	3	68±33
	R3	6	121±66	NA	3	790±524	3	41±37
	R4	6	123±59	NA	3	1039±763	3	50±35
	R5	6	99±88	NA	3	500±121	3	42±39
	R6	6	144±218	NA	3	151±90	3	51±16
	R7 - downstream	6	428±967	NA	3	288±262	3	33±21
Canal	C-107	6	70±123	24±22		NA		NA
	Sagamore	4	8660±7239	4635±3885		NA		NA
	Hogpen Slough	6	3246±2887	1605±1165		NA		NA
	Veterans Memorial	6	2897±4028	164±129		NA		NA
	Elkcam	6	1529±3417	109±224		NA		NA
	Monterrey	6	124±75	44±21		NA		NA
	C-24	6	56±52	17±20		NA		NA
	E-8	6	68±106	24±29		NA		NA
	Horseshoe A-18	6	41±45	11±6		NA		NA
	Southbend/Horseshoe A-22	6	556±1073	63±104		NA		NA

For canals draining into the North Fork, fecal coliforms were present at generally higher concentrations than in the main stem (**Fig. 10**). In the wet season, *E. coli* was highest (above 1,000 MPN/100 mL) at Sag and Hog, but also elevated at Vet and Elk (**Fig. 11a**). Sites that exceeded the previous FDEP fecal coliform standard of 800 cfu/100mL during the wet season included Sag, Hog, Vet, Elk, and A-22 (**Fig. 10a**). During the dry season, *E. coli* was very high, approaching 6,000 MPN/100mL at Hog (**Fig. 11b).** In the dry season fecal coliform concentrations, Hog exceeded the standard again at 5650±1626 cfu/100 mL, while Vet (90 cfu/100 mL) and A-22 (290±382 cfu/100 mL) concentrations were lower than the wet season and Sag was unable to be sampled due to no flow (**Fig. 10b**). The highest *E. coli* concentrations during the rain event were observed at Sag (2600 MPN/100 mL) and Hog (275 MPN/100 mL; **Fig. 11c**). Sag exceeded the fecal coliform standard at 1840 cfu/100 mL during the rain event and elevated levels of fecal coliforms were observed at C-107 (320 cfu/10 mL), Hog (160 cfu/100 mL), Vet (280 cfu/100 mL), Mont (214 cfu/100 mL), E-8 (280 cfu/100 mL), and A-18 (90 cfu/100 mL; **Fig. 10**). See **Appendix 5** for seasonal bacteria averages.

3.4 Microbial Source Tracking

The human marker, HF183, was detected above the MDL at TM2, R1, and Sag, while the avian tracer, Gull2, was detected above the MDL at R1. Sucralose was found at every site, carbamazepine was also observed at almost all sites, except TM2, C-107, Vet, and C-24, and acetaminophen was found at many sites (**Table 6**). Chemicals relating to terrestrial weed control (diuron and fenuron) were also present globally (**Table 6**). GFD, linuron, and primidone were not detected during this study and are not displayed in **Table 6**. It is noteworthy that primidone has not been widely prescribed since the 1980s and therefore is unlikely to be present. For seasonal MST averages, see **Appendix 6**. At the downstream tributary sites (TM1 and FM1) and sites within the main stem of the river (R1-R7), dilution and tidal movement of chemicals may have affected the results, making the sources of these tracers more difficult to decipher.

Table 6. Microbial source tracking molecular markers and chemical concentrations (overall averages ± standard error, except where only there was only one detection) from surface water samples collected from tributaries of the North Fork (Tenmile Creek and Fivemile Creek), the main stem of the North Fork of the St. Lucie Estuary (river sites, R1 upstream – R7 downstream), and canals draining into the North Fork.

Type	Site	Count	Sucralose (µg/L)	Acetaminophen (µg/L)	Carbamazepine (µg/L)	Diuron (µg/L)	Fenuron (µg/L)	Flouridone (µg/L)	Imidacloprid (µg/L)
Tributary	Tenmile 2 - upsteam	6	0.019±0.002	0.006	BDL	0.020±0.020	0.009	0.004±0.007	0.123±0.112
	Tenmile 1 - downstream	6	0.031±0.015	0.017±0.017	0.0006	0.030±0.029	BDL	0.005±0.006	0.100±0.101
	Fivemile 2 - upstream	6	0.064±0.048	0.011±0.006	0.0014±0.0006	0.011±0.004	BDL	0.001±0.000	0.095±0.153
	Fivemile 1 - downstream	6	0.058±0.014	0.031±0.018	0.0008±0.0006	0.021±0.021	BDL	0.006±0.007	0.048±0.051
River	R1 - upsteam	6	0.047±0.008	0.011	0.0008±0.0004	0.036±0.034	BDL	0.004±0.005	0.078±0.078
	R2	6	0.048±0.013	0.026	0.0004±0.0000	0.061±0.069	BDL	0.003±0.003	0.083±0.099
	R3	6	0.107±0.032	BDL	0.0010±0.0004	0.032±0.035	BDL	0.005±0.004	0.125±0.113
	R4	6	0.127±0.033	0.010	0.0008±0.0003	0.039±0.037	BDL	0.005±0.004	0.094±0.096
	R5	6	0.114±0.038	0.020	0.0009±0.0003	0.069±0.051	BDL	0.013±0.012	0.120±0.061
	R6	6	0.132±0.059	BDL	0.0012±0.0003	0.116±0.067	0.012	0.013±0.011	0.119±0.059
	R7 - downstream	6	0.326±0.480	0.020	0.0010±0.0001	0.079±0.055	0.010	0.012±0.010	0.111±0.049
Canal	C-107	6	0.070±0.050	0.011±0.009	BDL	0.023±0.017	0.017±0.012	0.008±0.004	0.045±0.043
	Sagamore	4	0.928±0.595	0.038±0.033	0.0032±0.0019	0.068±0.028	0.020±0.008	0.001±0.000	0.030±0.024
	Hogpen Slough	6	0.380±0.414	0.025±0.008	0.0029±0.0019	0.047±0.075	BDL	0.012±0.004	0.010±0.005
	Veterans Memorial	6	0.080±0.086	0.018±0.003	BDL	0.588±0.813	0.036±0.020	0.016±0.009	0.020±0.012
	Elkcam	6	0.499±0.284	0.030±0.031	0.0038±0.0021	5.222±8.921	0.049±0.020	0.693±1.620	0.040±0.024
	Monterrey	6	0.848±0.367	BDL	0.0098±0.0012	0.764±0.910	0.029±0.009	0.067±0.124	0.034±0.021
	C-24	6	0.041±0.023	BDL	BDL	0.007±0.003	BDL	0.008±0.006	0.114±0.109
	E-8	6	0.462±0.209	BDL	0.0030±0.0005	3.005±6.404	0.022±0.013	0.008±0.005	0.065±0.071
	Horseshoe A-18	6	0.455±0.212	BDL	0.0045±0.0018	1.376±1.186	0.036±0.021	0.017±0.016	0.041±0.039
	Southbend/Horseshoe A-22	6	0.428±0.298	0.025	0.0043±0.0016	1.520±2.253	0.033±0.009	0.064±0.070	0.046±0.033

Type	Site	Count	HF183 (GEU/100mL)	Gull2 (TSC/100mL)	Count	2,4-D (µg/L)	Bentazon (µg/L)	MCPP (µg/L)	Triclopyr (µg/L)
Tributary	Tenmile 2 - upsteam	6	110	BDL	2	0.855±1.054	0.009±0.012	0.003±0.001	0.007±0.004
	Tenmile 1 - downstream	6	BDL	BDL	2	0.385±0.290	0.009±0.011	0.003±0.001	3.402±4.805
	Fivemile 2 - upstream	6	BDL	BDL	2	0.067±0.018	0.017±0.023	0.003±0.001	0.019±0.021
	Fivemile 1 - downstream	6	BDL	BDL	2	0.079±0.030	0.019±0.025	0.003±0.001	0.027±0.033
River	R1 - upsteam	6	400	52000	2	0.285±0.276	0.012±0.015	0.003±0.001	0.052±0.068
	R2	6	BDL	BDL	2	0.160±0.085	0.015±0.017	0.003±0.001	0.332±0.464
	R3	6	BDL	BDL	2	0.120±0.014	0.024±0.029	0.003±0.001	2.752±3.886
	R4	6	BDL	BDL	2	0.098±0.031	0.021±0.025	0.003±0.001	2.052±2.896
	R5	6	BDL	BDL	2	0.094±0.079	0.022±0.026	0.003±0.001	0.392±0.549
	R6	6	BDL	BDL	2	0.101±0.112	0.023±0.026	0.004±0.000	0.152±0.209
	R7 - downstream	6	BDL	BDL	2	0.095±0.120	0.025±0.027	0.004±0.001	0.062±0.082
Canal	C-107	6	BDL	BDL	2	0.116±0.091	0.031±0.037	0.009±0.006	0.007±0.004
	Sagamore	4	1600	BDL	1	0.095	0.028	0.002	0.004
	Hogpen Slough	6	BDL	BDL	2	0.152±0.209	0.048±0.049	0.003±0.001	0.007±0.004
	Veterans Memorial	6	BDL	BDL	2	12.519±17.652	0.021±0.021	0.004±0.001	0.024±0.019
	Elkcam	6	BDL	BDL	2	0.297±0.359	0.070±0.057	0.005±0.001	0.007±0.004
	Monterrey	6	BDL	BDL	2	0.048±0.008	0.051±0.052	0.008±0.001	0.007±0.004
	C-24	6	BDL	BDL	2	0.061±0.069	0.012±0.016	0.003±0.001	0.007±0.004
	E-8	6	BDL	BDL	2	0.200±0.028	0.034±0.035	0.021±0.024	0.007±0.004
	Horseshoe A-18	6	BDL	BDL	2	0.148±0.116	0.054±0.065	0.017±0.012	0.007±0.004
	Southbend/Horseshoe A-22	6	BDL	BDL	2	0.163±0.095	0.050±0.060	0.014±0.010	0.007±0.004

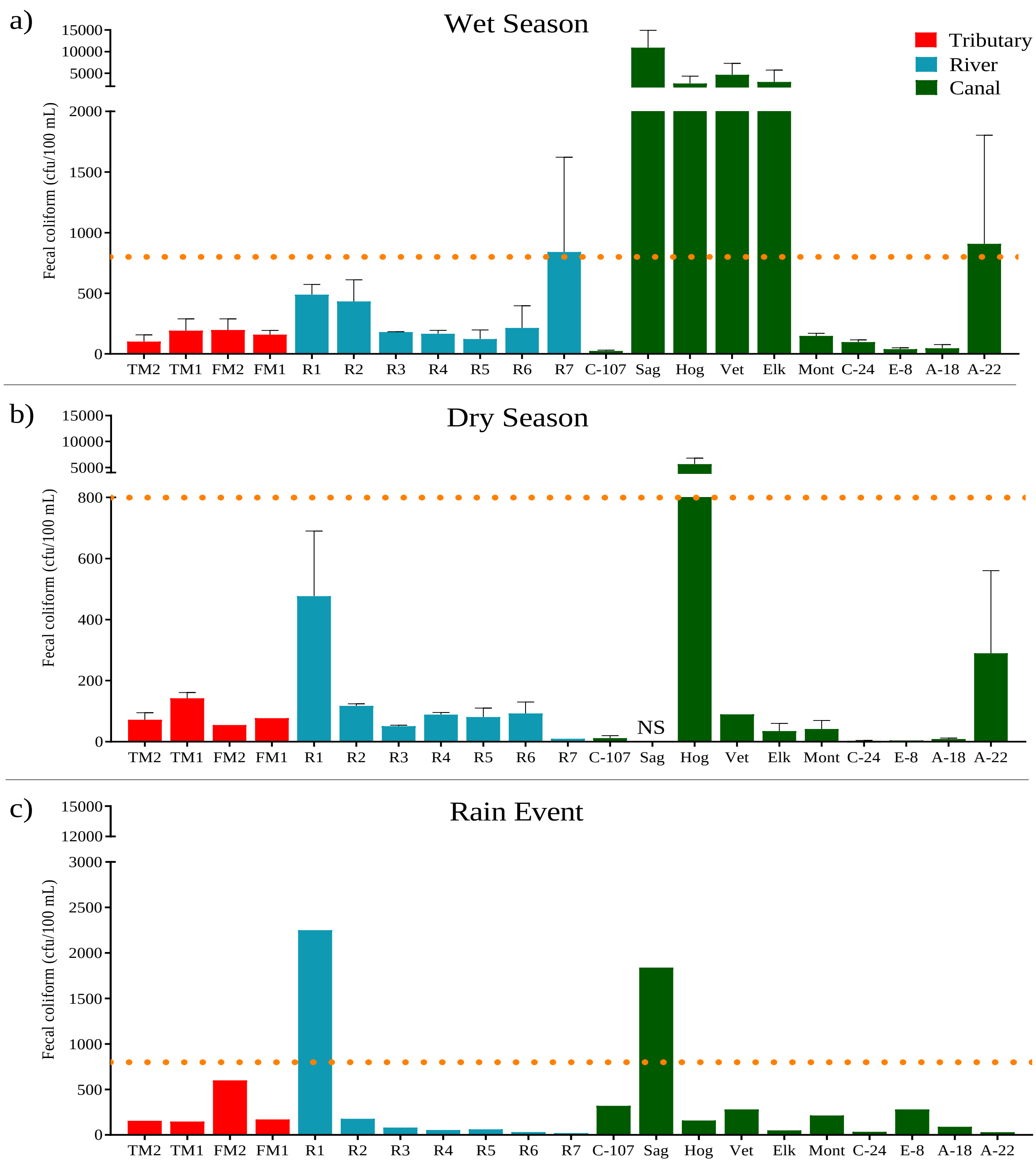

Figure 10. Fecal coliform concentrations in surface water samples (average ± standard error, except where only one sample was collected) collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22); former FDEP standards (800 cfu/100mL) are shown (updated in 2017) as a dotted orange line, NS = not sampled due to no flow.

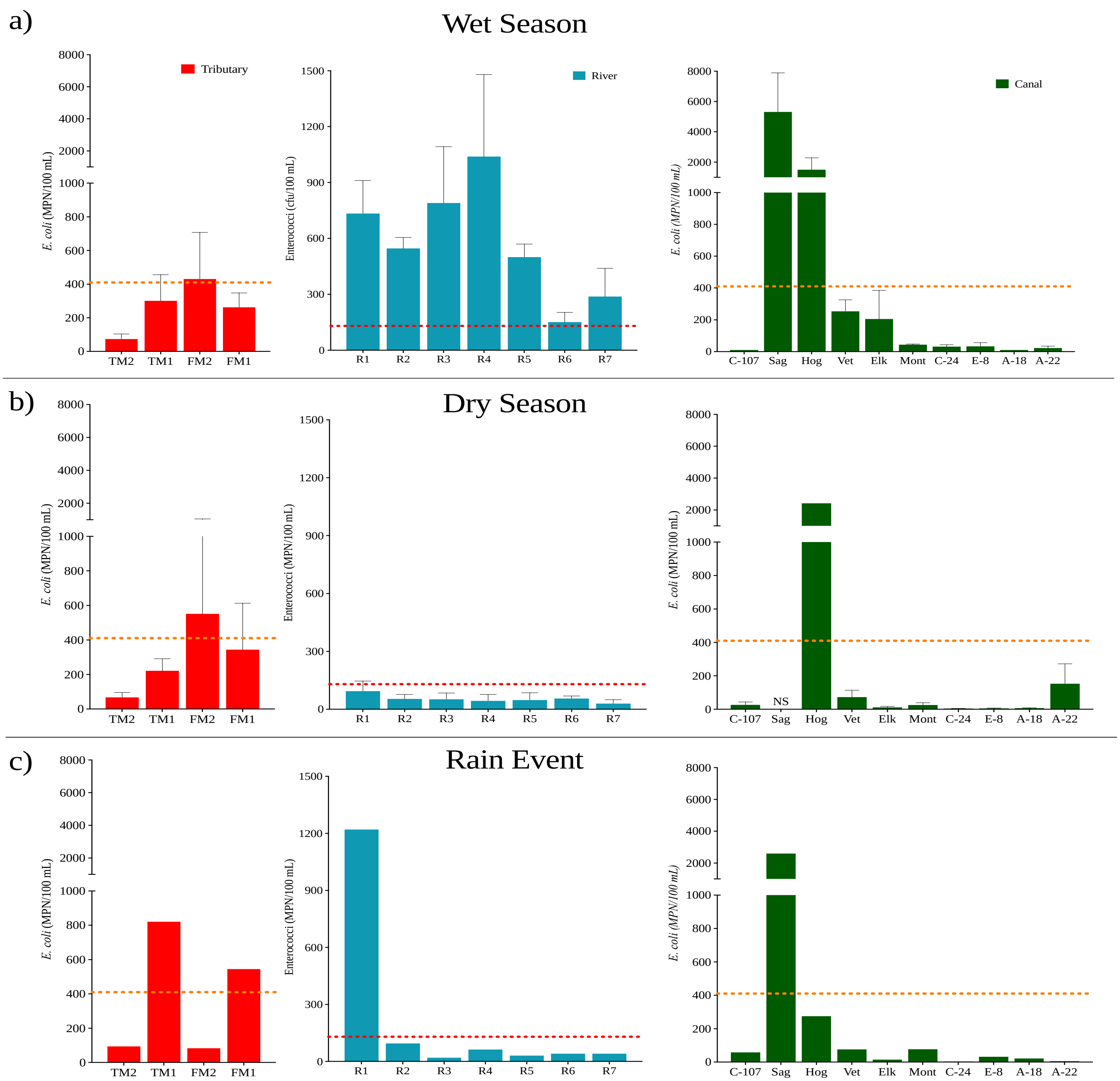

Figure 11. Concentrations of bacteria (average ± standard error, except where only one sample was collected) observed in surface water samples, including *Escherichia coli (E. coli*) from "Tributary" sites (Tenmile Creek [TM2- upstream and TM1- downstream] and Fivemile Creek [FM2- upstream and FM1- downstream]) and "Canal" sites (C-107, Sagamore (Sag), Hogpen Slough [Hog], Veterans Memorial [Vet], Elkcam [Elk], Monterrey [Mont], C-24, E-8, Horseshoe A-18 [A-18], and Southbend/Horseshoe A-22 [A-22]) draining into the North Fork of the St. Lucie Estuary and enterococci samples collected from "River" sites within the North Fork (R1 upstream – R7 downstream) during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017; FDEP surface water quality standards are shown for enterococci (130 TPTV) and *E. coli* (410 TPTV) as a dotted orange line, NS = not sampled due to no flow.

Tributary samples exhibited low concentrations of sucralose relative to river and canal samples, consistently maintaining levels below "moderate" (0.10 µg/L) across all seasons (**Fig. 12**). For tributary sites, the highest sucralose concentrations were observed in Fivemile Creek at FM1 and FM2 during each season with a marked increase from upstream to downstream in the wet season (**Fig. 12**). Acetaminophen concentrations were generally low in the tributaries during the wet season (< 0.01 µg/L) with the exception of the downstream site, FM1 (**Fig. 13a**). During the dry season only the upstream Tenmile Creek site, TM2, had a detectable acetaminophen concentration (0.029 µg/L), while only the Fivemile Creek sites (0.014 µg/L at FM1 and 0.018 µg/L at FM2) had detectable concentrations during the rain event (**Fig. 13b, c**). In the wet season, carbamazepine was detected at TM1 (0.0006 µg/L) and both Fivemile sites (0.0009 µg/L at FM1 and 0.0011±0.0004 µg/L at FM2; **Appendix 8a**), but only found at FM2 during the dry season (0.0021 µg/L) and at both Fivemile sites (0.0011 µg/L at FM1 and 0.0001 µg/L at FM2) during the rain event (**Appendix 8b,c**). Fenuron was present during the rain event at TM2 (0.009 µg/L; **Appendix 10c**). Fluridone was present during the wet and dry seasons at all tributary sites, but only at FM2 and both Tenmile sites during the rain event (**Appendix 11a**). Imidacloprid was observed at all tributary sites in all seasons with the greatest concentrations found during the rain event (0.4 µg/L at FM1), followed by the wet season (0.132 µg/L at TM1), and the lowest concentrations in the dry season (0.014±0.012 µg/L at FM1); the highest concentration of the study was found at FM1 during the rain event (**Appendix 12**). Diuron was found at all tributary sites during the wet season and rain event (**Appendix 9a,c**).

For river samples, there was seasonal variation observed in the human source tracers, sucralose and acetaminophen (**Fig. 12, 13**). Sucralose was present at all sites and during all seasons, maintaining the consistent trend that the lowest concentrations were detected at upstream sites, R1 and R2, with the higher concentrations (at or approaching the FDEP "moderate" classification of 0.1 to 1.0 µg/L) found downstream at R3 through R7 (**Fig. 12**). During the wet season, sucralose levels at R3 through R7 were approximately "moderate," ranging from 0.086±0.009 µg/L at R3 to 0.113±0.042 µg/L at R4 (**Fig. 12a**). However, during the dry season sucralose was slightly higher for R3 through R6, with concentrations from 0.112±0.026 µg/L at R5 to 0.145±0.021 µg/L at R3, and variation into the "significant" classification (above 1.0 µg/L) at R7 (**Fig. 12b**). Rain event sucralose concentrations were similar to the wet season (**Fig. 12a,c**). Acetaminophen concentrations were highest during the dry season (0.026 µg/L at R2), but were undetectable at all sites during the wet season and rain event, with the exception of the most upstream site, R1 (0.011 µg/L), during the rain event (**Fig. 13**). Carbamazepine was observed at all river sites during the wet season at similar levels to the tributaries (<0.002 µg/L), with fewer detections in the dry season, and relatively low detections at R3-R7 (<0.002 µg/L) during the rain event (**Appendix 8c**). During all seasons, diuron was present in low concentrations in the river and increased slightly downstream (**Appendix 9**). Fenuron was present at the most downstream sites, R6 (0.012 µg/L) and R7 (0.010 µg/L) during the rain event (**Appendix 10c**). Fluridone concentrations in river samples ranged from BDL at R1 to 0.031 µg/L at R5 during the rain event, with concentrations at all river sites during the wet and dry seasons maintaining levels below 0.02 µg/L (**Appendix 11a,b,c**). In the river, imidacloprid was found at all sites during the wet season and rain event, with low amounts (<0.03 µg/L) detected at R1-R4 in the dry season (**Appendix 12**). Imidacloprid generally decreased downstream during the rain event (**Appendix 12**).

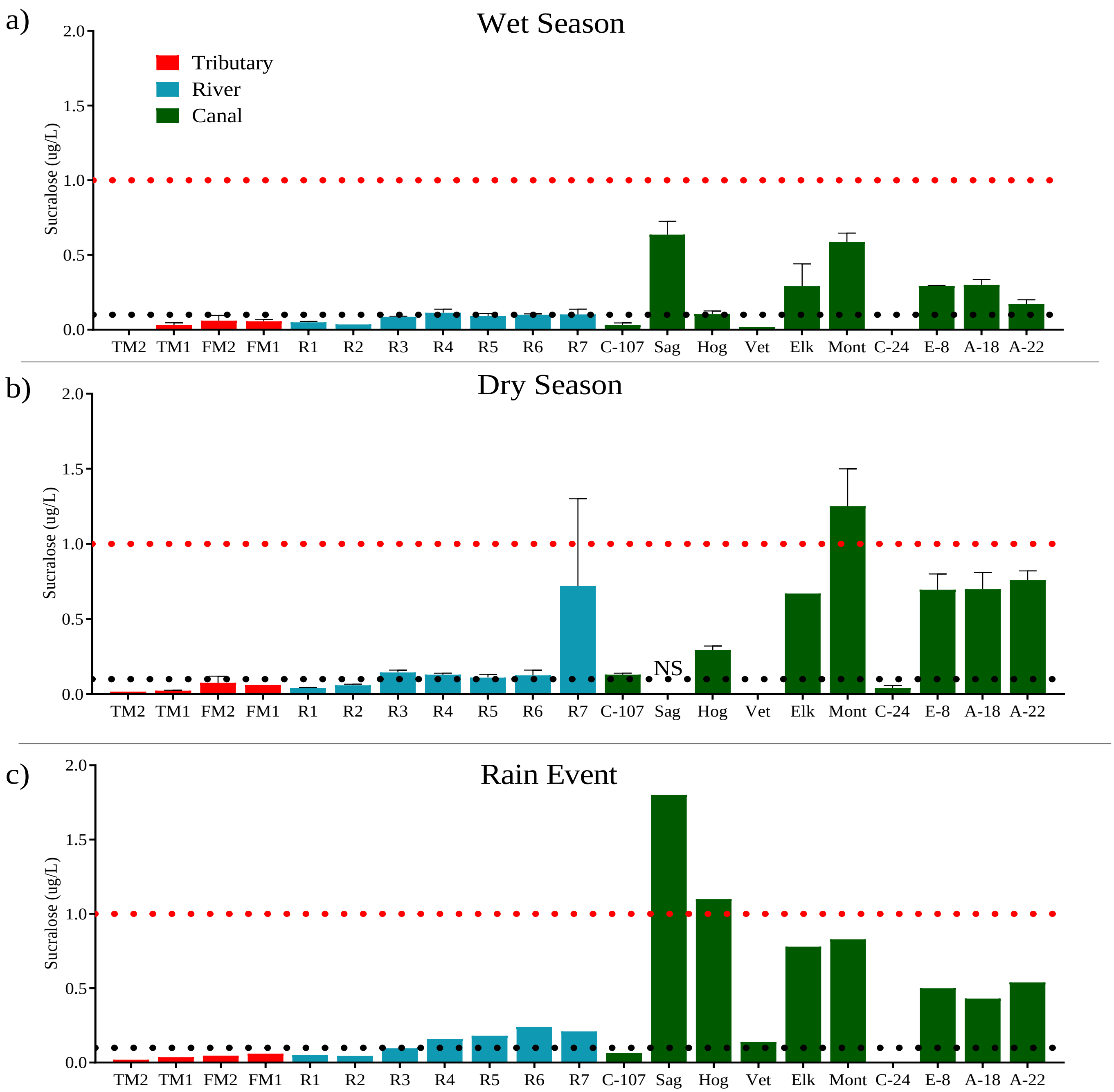

Figure 12. Sucralose concentrations of surface water samples (average ± standard error, except where only one sample was collected or a single detection occurred) collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22); showing the FDEP classification for "moderate" sucralose (0.1 µg/L) presence as a dotted black line and "significant" sucralose presence (1.0 µg/L) as a dotted red line, NS = not sampled due to no flow.

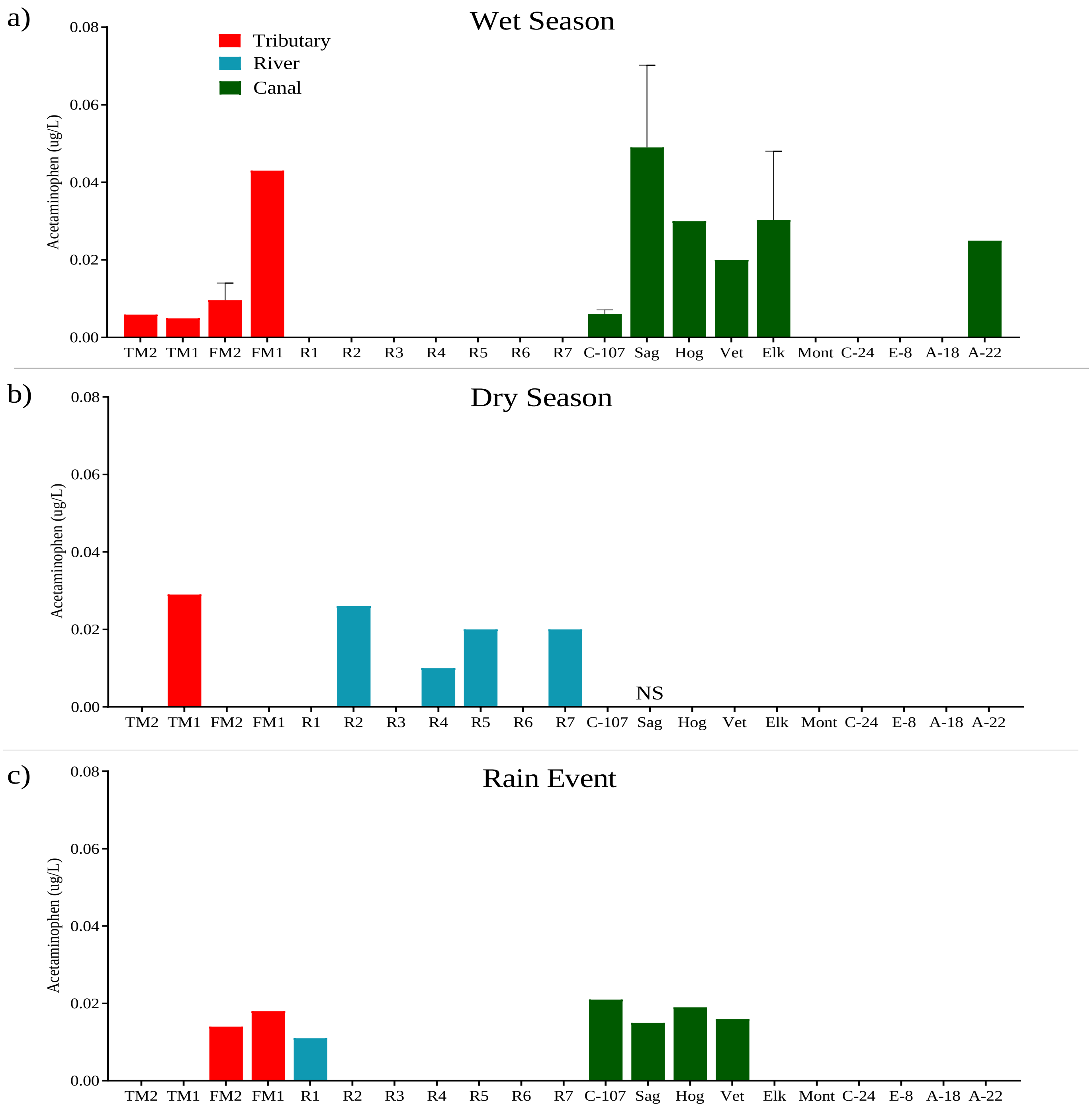

Figure 13. Acetaminophen concentrations of surface water samples (average ± standard error, except where only one sample was collected or a single detection occurred) collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

Sucralose concentrations in the canal samples were usually higher than the river samples (**Fig. 12**). The wet season generally had the lowest observed sucralose concentrations, ranging from BDL at C-24 to 0.637±0.156 μg/L at Sag, with most canal sites at or approaching "moderate" levels (**Fig. 12a**). "Significant" sucralose concentrations were found at Mont (1.250±0.354 μg/L) during the dry season and at Sag (1.800 μg/L) and Hog (1.100 μg/L) during the rain event (**Fig. 12b,c**). Acetaminophen was not detected at any canal sites during the dry season and the greatest values were observed at Sag (0.049±0.037 μg/L) during the wet season (**Fig. 13a,b**). Carbamazepine concentrations were highest in the canal sites during all seasons, particularly Mont, A-18, and A-22, however no carbamazepine was observed in any season at C-107, Vet, or C-24 (**Appendix 8**). Diuron was elevated in canal sites with lower concentrations observed during the wet season at C-107 (0.007±0.002 μg/L), Sag (0.063±0.033 μg/L), and Hog (0.007±0.002 μg/L), and no detections at C-24 (**Appendix 9**). Fenuron was present during all seasons in canal sites with the highest concentrations during the rain event at Vet (0.069 μg/L) and Elk (0.065 μg/L) and no detections at Hog or C-24 (**Appendix 10**). The greatest concentrations of fluridone were present at Mont (0.113±0.180 μg/L) during the wet season and at Elk (2.002±2.825 μg/L) during the dry season (**Appendix 11a,c**). Imidacloprid concentrations were highest during the rain event (0.170 μg/L at E-8), followed by the wet season (0.135±0.128 μg/L at C-24), then the dry season (0.005 μg/L at Mont; **Appendix 12**). C-24 (0.114±0.109 μg/L), E-8 (0.065±0.071 μg/L), and A-22 (0.046±0.033 μg/L) had the highest overall imidacloprid concentrations of the canal sites (**Table 6**).

3.5 Dissolved nutrients

All four tributary sites (TM1, TM2, FM1, FM2) had high average concentrations (>0.1 mg/L) of ammonium, SRP, and DIN, while Tenmile Creek (both TM1 and TM2) had elevated average concentrations of nitrate (0.16±0.06 mg/L at TM1 and 0.13±0.06 mg/L at TM2) and higher average DIN:SRP ratios (4.83±1.00 at TM1 and 5.30±2.12 at TM2) relative to Fivemile Creek (0.08±0.06 mg/L and 0.06±0.02 mg/L nitrate at FM1 and FM2, respectively, and 3.94±2.48 and 2.73±1.02 DIN:SRP at FM1 and FM2, respectively; **Table 7**). The overall trend for average ammonium, SRP, and DIN concentrations at the river sites was a general decrease in concentration from upstream (0.12±0.15 mg/L at R1) to downstream (0.04±0.04 mg/L at R7), while average nitrate concentrations were relatively similar at each river site, ranging from 0.09±0.07 mg/L at R5 to 0.12±0.10 mg/L at R6 (**Table 7**). The greatest average DIN:SRP ratios at the river sites were observed at the downstream sites, R6 (5.24±4.40) and R7 (6.14±8.02; **Table 7**). Among the canal sites, E-8 (0.11±0.13 mg/L) had the highest average ammonium concentrations, while Sag exhibited high average concentrations of nitrate (0.12±0.17 mg/L), SRP (0.13±0.11 mg/L), and DIN (0.20±0.24 mg/L), and C-24 had the highest average SRP concentrations (0.16±0.14 mg/L; **Table 7**). The average DIN:SRP ratios for canal sites were highly variable, ranging from 2.46 ± 1.53 at C-24 to 102.51 ± 143.83 at Vet (**Table 7**).

Seasonal variation was observed in ammonium concentrations at tributary, river, and canal sites, with the highest concentrations observed during the wet season and the lowest concentrations typically observed during the dry season (**Fig. 14**). See **Appendix 7** for seasonal dissolved nutrient data by site. The tributary sites had high ammonium concentrations during the wet season, ranging from 0.23±0.01 mg/L to 0.28±0.01 mg/L, while the upstream site in Tenmile Creek, TM2, displayed the highest concentrations during the dry season (0.09±<0.01 mg/L) and the rain event (0.16±0.01 mg/L; **Fig. 14a,b,c**). The upstream river sites, R1 and R2, consistently had the greatest ammonium concentrations relative to the farther downstream river sites regardless of season and there was a general decrease in concentration from north to south among the river sites (**Fig. 14a,b,c**). The canal sites Mont (0.23±0.01 mg/L) and E-8 (0.25±0.01 mg/L) had the highest ammonium concentrations during the wet season relative to the other canal sites, while A-22 exhibited the highest concentrations during the dry season (0.08±0.01 mg/L) and the rain event (0.09±0.01 mg/L; **Fig. 14a,b,c**).

Table 7. Nutrient concentrations for surface water samples (overall average ± standard error) collected from tributaries of the North Fork (Tenmile Creek and Fivemile Creek), the main stem of the North Fork of the St. Lucie Estuary (river sites, R1 upstream – R7 downstream), and canals draining into the North Fork.

Type	Site	Count	Ammonium (mg/L)	Nitrate +Nitrite (mg/L)	Soluble Reactive Phosphorus (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	DIN:SRP
Tributary	Tenmile 2 - upsteam	9	0.16±0.07	0.13±0.06	0.15±0.11	0.29±0.10	5.30±2.12
	Tenmile 1 - downstream	9	0.14±0.12	0.16±0.06	0.14±0.07	0.30±0.15	4.83±1.00
	Fivemile 2 - upstream	9	0.12±0.13	0.06±0.02	0.17±0.18	0.17±0.15	2.73±1.02
	Fivemile 1 - downstream	9	0.10±0.10	0.08±0.06	0.14±0.15	0.18±0.16	3.94±2.48
River	R1 - upsteam	9	0.12±0.15	0.10±0.05	0.16±0.10	0.23±0.20	2.98±0.79
	R2	9	0.10±0.10	0.10±0.08	0.18±0.13	0.19±0.16	2.04±1.56
	R3	9	0.08±0.09	0.10±0.08	0.16±0.13	0.17±0.15	2.01±1.60
	R4	9	0.05±0.06	0.10±0.07	0.17±0.13	0.15±0.12	1.89±1.19
	R5	9	0.05±0.04	0.09±0.07	0.16±0.14	0.13±0.11	2.84±3.54
	R6	9	0.03±0.02	0.12±0.10	0.08±0.02	0.15±0.12	5.24±4.40
	R7 - downstream	9	0.04±0.04	0.10±0.09	0.09±0.05	0.14±0.12	6.14±8.02
Canal	C-107	9	0.07±0.06	0.04±0.06	0.01±0.01	0.11±0.12	53.64±65.28
	Sagamore	6	0.07±0.08	0.12±0.17	0.13±0.11	0.20±0.24	7.88±10.75
	Hogpen Slough	9	0.03±0.02	0.06±0.06	0.04±0.03	0.09±0.06	11.63±11.07
	Veterans Memorial	9	0.03±0.02	0.04±0.04	0.01±0.02	0.07±0.04	102.51±143.83
	Elkcam	9	0.06±0.08	0.05±0.08	0.01±0.01	0.11±0.16	33.28±28.06
	Monterrey	9	0.09±0.12	0.08±0.09	0.04±0.04	0.18±0.21	24.94±20.51
	C-24	9	0.06±0.06	0.05±0.05	0.16±0.14	0.11±0.08	2.46±1.53
	E-8	9	0.11±0.13	0.06±0.10	0.04±0.06	0.17±0.23	36.20±37.39
	Horseshoe A-18	9	0.04±0.06	0.06±0.09	0.01±0.01	0.10±0.15	18.33±7.70
	Southbend/Horseshoe A-22	9	0.10±0.03	0.09±0.08	0.03±0.02	0.19±0.11	26.90±22.82

Overall, Tenmile Creek had higher nitrate concentrations relative to Fivemile Creek, with the highest average concentrations observed upstream at TM2 (0.13±0.06 mg/L), decreasing downstream at TM1 (0.16±0.06 mg/L; **Table 7**). The wet season nitrate concentrations at the tributary sites were all higher than the concentrations observed during the dry season, with TM1 exhibiting the highest concentration during both the wet (0.17±<0.01 mg/L) and dry (0.10±0.01 mg/L) seasons, however the downstream sites in both tributaries (0.17±<0.01 mg/L at TM1 and 0.14±<0.01 mg/L at FM1) were similar in the wet season (**Fig. 15a,b**). Nitrate concentrations at the river sites were elevated (>0.1 mg/L) and fairly consistent during the wet season and rain event, but were relatively low (<0.1 mg/L) during the dry season with the highest concentration at the upstream site, R1 (0.05±<0.01 mg/L; **Fig. 15a,b,c**). The highest nitrate concentrations at the canal sites were observed during the wet season at Sag (0.24±0.01 mg/L), Elk (0.15±<0.01 mg/L), Mont (0.18±<0.01 mg/L), E-8 (0.17±<0.01 mg/L), A-18 (0.17±0.04 mg/L), and A-22 (0.18±<0.01 mg/L), while Hog (0.12±<0.01 mg/L) and Vet (0.08±0.05 mg/L) had the highest concentrations during the dry season, and C-24 (0.11±<0.01 mg/L) was highest during the rain event (**Fig. 15a,b,c**).

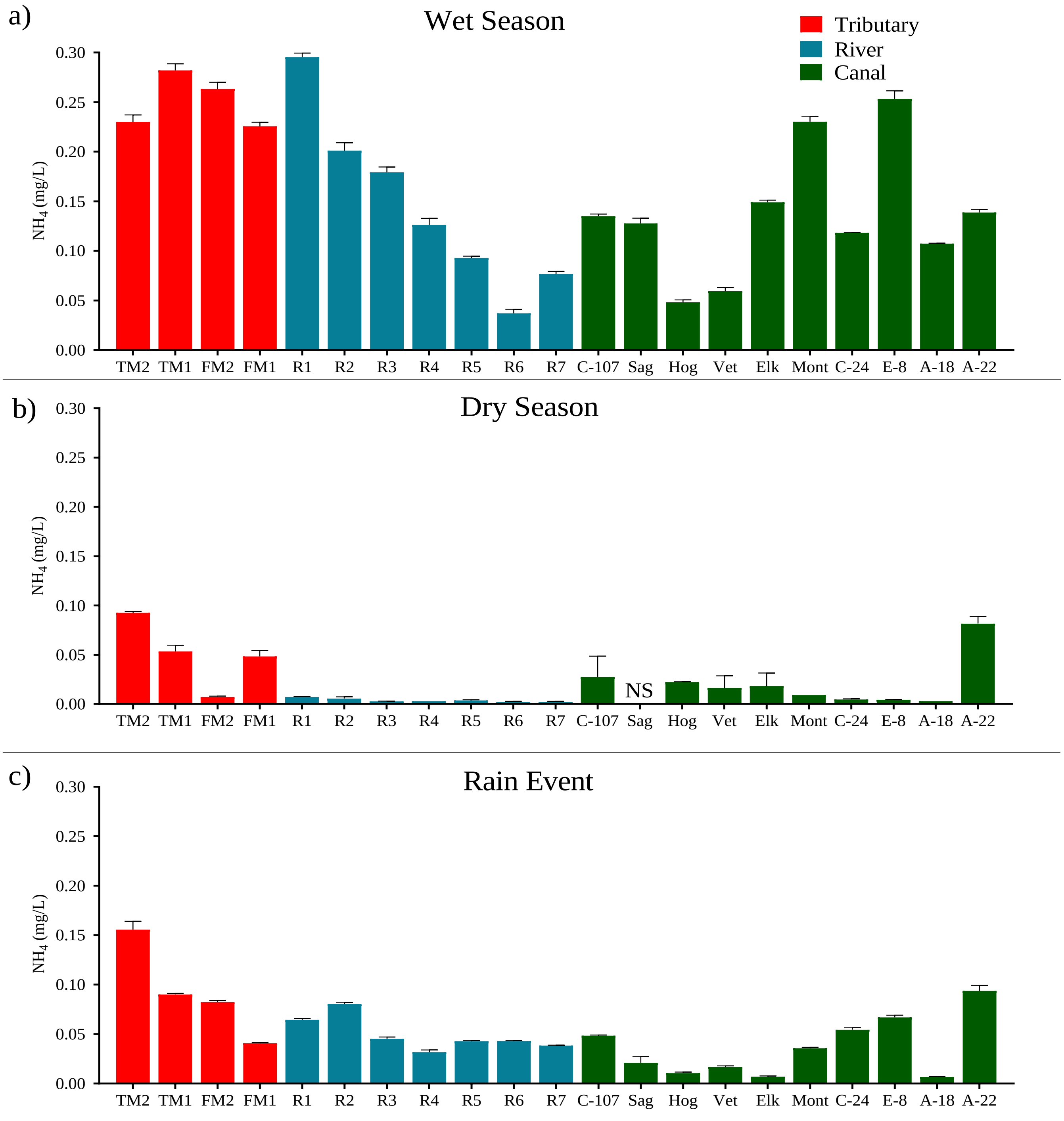

Figure 14. Ammonium (NH_4) concentrations (average mg/L ± standard error) observed in surface water collected during the a) 2017 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

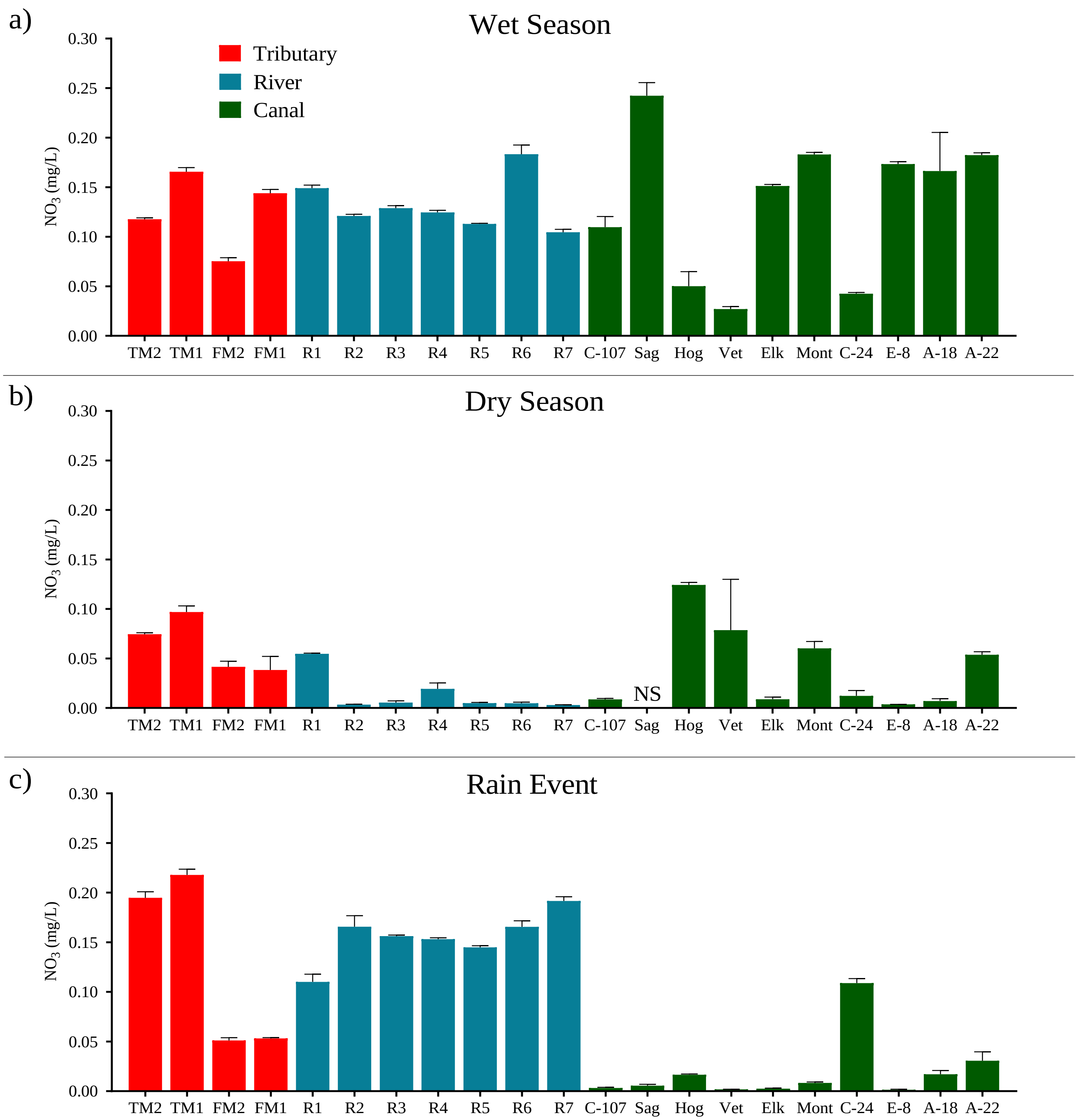

Figure 15. Nitrate + nitrite (NO_3) concentrations (average mg/L ± standard error) observed in surface water collected during the a) 2017 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

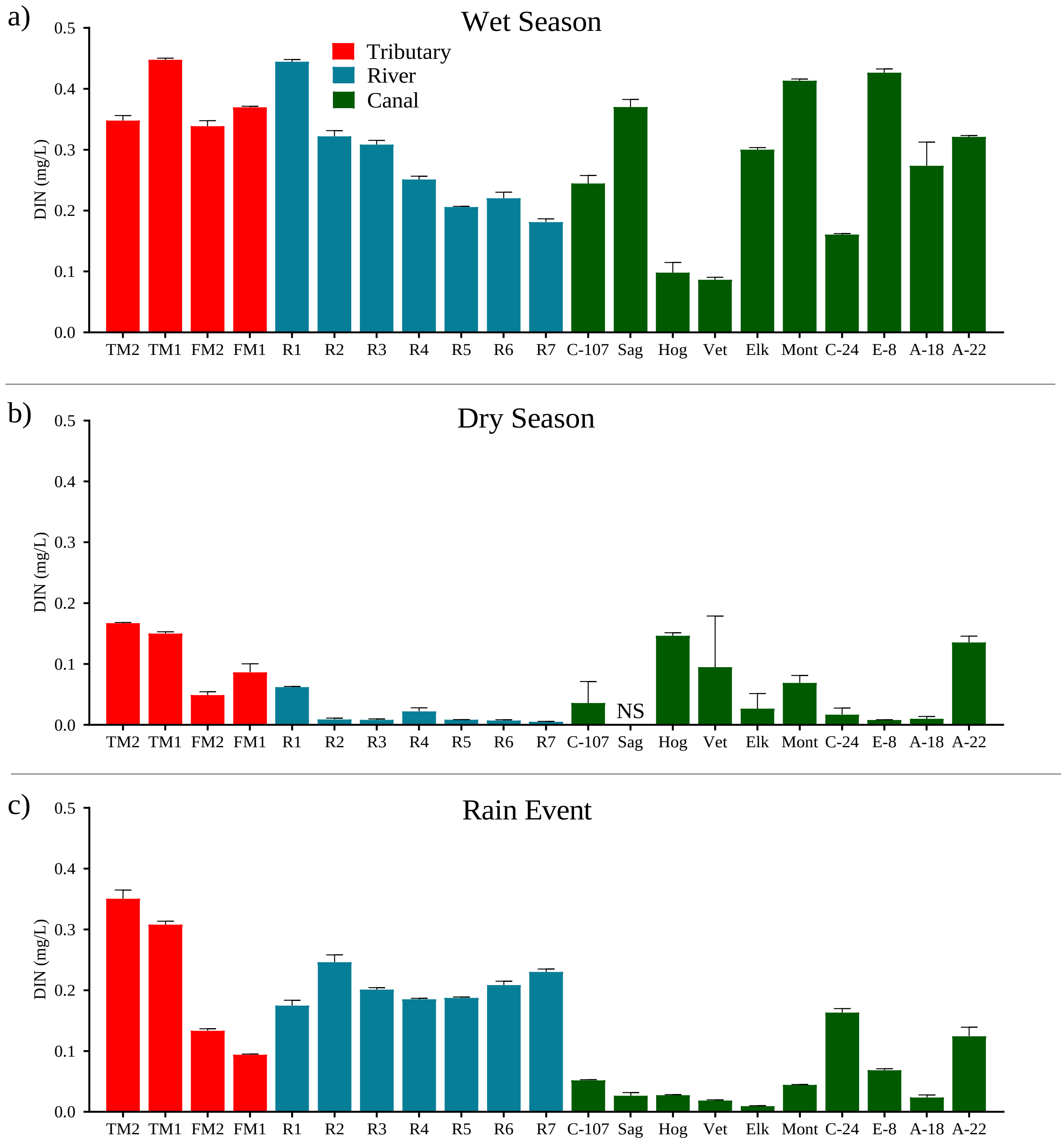

Figure 16. Dissolved inorganic nitrogen (DIN) concentrations (average mg/L ± standard error) observed in surface water collected during the a) 2017 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

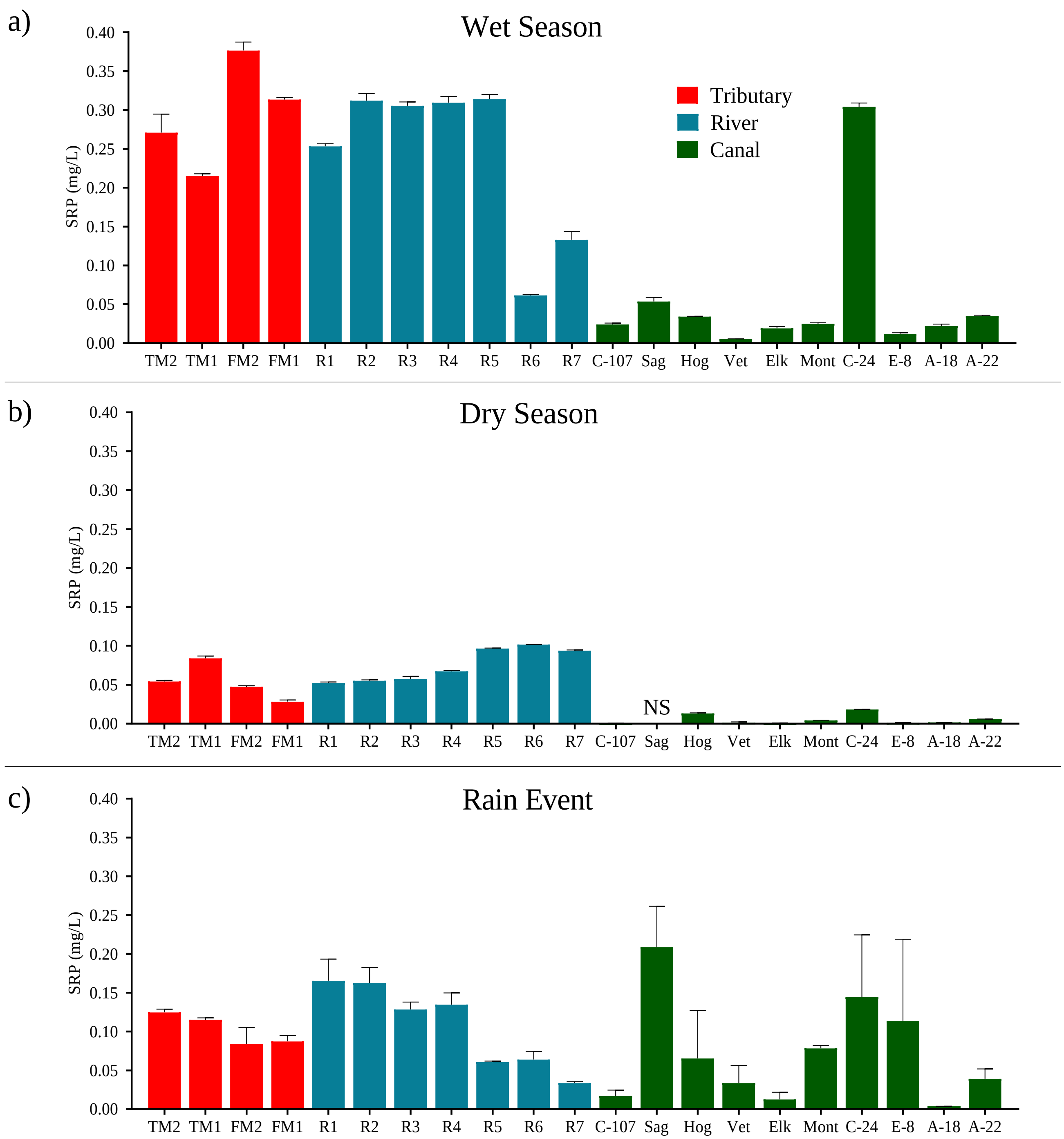

Figure 17. Soluble reactive phosphorus (SRP) concentrations (average mg/L ± standard error) observed in surface water collected during the a) 2017 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

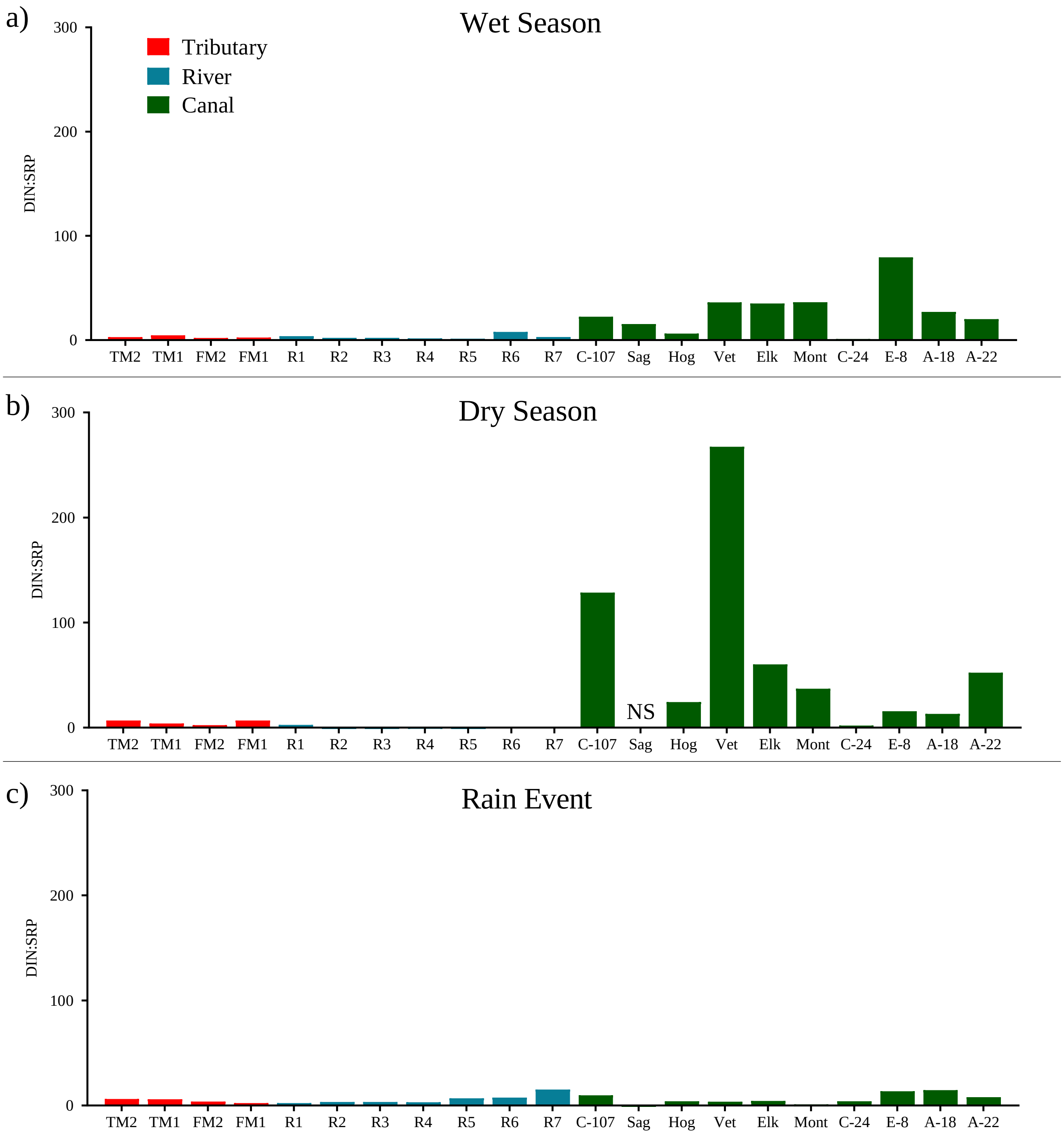

Figure 18. Ratio of dissolved inorganic nitrogen (DIN) to soluble reactive phosphorus (SRP) observed in surface water collected during the a) 2017 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore (Sag), Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

DIN concentrations were elevated (>0.3 mg/L) in both tributaries during the wet season and the lowest concentrations were observed at FM2 (0.05±0.01 mg/L) during the dry season (**Fig. 16a,b**). Tenmile Creek exhibited the highest DIN concentrations relative to Fivemile Creek during the dry season (0.15±<0.01 mg/L at TM1 and 0.17±<0.01 mg/L at TM2) and the rain event (0.31±0.01 mg/L at TM1 and 0.35±0.01 mg/L at TM2; **Fig. 16b,c**). During the wet season, DIN concentrations at the river sites generally decreased from north to south, with R1 (0.44±<0.01 mg/L) having the highest concentrations and R7 (0.18±0.01 mg/L) having the lowest concentrations (**Fig. 16a**). In the dry season, the river sites had the lowest DIN concentrations, with the highest concentration of 0.06±<0.01 mg/L observed upstream at R1 (**Fig. 16b**). DIN concentrations were relatively similar during the rain event at all river sites, ranging from 0.17±0.01 mg/L at R1 to 0.25±0.01 mg/L at R2 (**Fig. 16c)**. The highest DIN concentrations at the canal sites were observed during the wet season, specifically at Sag (0.37±0.01 mg/L), Elk (0.30±<0.01 mg/L), Mont (0.41±<0.01 mg/L), E-8 (0.43±0.01 mg/L), A-18 (0.27±0.04 mg/L), and A-22 (0.32±<0.01 mg/L; **Fig. 16a**), with relatively lower (<0.2 mg/L) DIN values at the canal sites during the dry season and the rain event; Hog (0.15±<0.01 mg/L) and A-22 (0.14±0.01 mg/L) had the greatest concentrations during the dry season, while C-24 (0.16±0.01 mg/L) and A-22 (0.12±0.01 mg/L) had the highest concentrations during the rain event (**Fig. 16b,c**).

Seasonal differences were observed in SRP concentrations at tributary, river, and canal sites (**Fig. 17**). Tributary sites had the greatest SRP concentrations during the wet season and the lowest in the dry season (**Fig 17a,b**). In the wet season, higher SRP concentrations were observed in Fivemile Creek (0.31±<0.01 mg/L at FM1 and 0.38±0.01 mg/L at FM2) than Tenmile Creek (0.21±<0.01 mg/L at TM1 and 0.27±0.02 mg/L at TM2), with SRP decreasing from the upstream site (FM2) to the downstream site (FM1; **Fig. 17a**). During both the dry season and the rain event SRP concentrations were higher at Tenmile Creek than Fivemile Creek (**Fig. 17b,c**). SRP concentrations were generally highest at the river sites during the wet season, with the upstream sites R1 (0.25±<0.01 mg/L) through R5 (0.31±0.01 mg/L) having relatively high concentrations and the most downstream sites, R6 (0.06±<0.01 mg/L) and R7 (0.13±0.01 mg/L), having much lower concentrations (**Fig. 17a**). Dry season SRP concentrations were usually lower at the river sites and showed a slight increase in concentration from upstream (0.05±<0.01 mg/L at R1) to downstream (0.09±<0.01 mg/L at R7; **Fig. 17b**). Conversely, during the rain event SRP concentrations at the river sites mostly decreased from upstream (0.17±0.03 mg/L at R1) to downstream (0.03±<0.01 mg/L; **Fig. 17c**). At canal sites, the lowest SRP concentrations (<0.05 mg/L) were observed during the dry season and, with the exception of C-24, the canal sites also had relatively low concentrations (<0.1 mg/L) during the wet season (**Fig. 17a,b**). The highest SRP concentrations for canal sites during the rain event were observed at Sag (0.10±0.05 mg/L), with C-24 (0.14±0.08 mg/L) and E-8 (0.11±0.11 mg/L) also having elevated values (**Fig. 17c**).

The DIN:SRP ratios observed were lowest in the tributary and river sites, while the canal sites usually had the highest ratios (**Fig. 18**). DIN:SRP ratios at the tributary sites were all relatively low among sites and between seasons, ranging from 1.99±0.07 at FM2 during the wet season to 6.82±0.12 at TM2 during the dry season (**Fig. 18**). At the river sites, the rain event exhibited the highest DIN:SRP ratios, with a trend of increasing from upstream (2.43±0.31 at R1) to downstream (15.24±0.76 at R7; **Fig. 18c**). During the wet season, R6 (7.93±0.44) had the highest

DIN:SRP ratios among the river sites and R1 (2.63±0.04) had the highest ratio during the dry season (**Fig. 18a,b**). The canal sites had the lowest DIN:SRP ratios during the rain event and relatively high (>10) DIN:SRP ratios for most sites during the wet season and the dry season (**Fig. 18a,b,c**). E-8 (79.36±6.55) exhibited the highest DIN:SRP ratios among the canal sites during the wet season and C-107 (128.64±77.36) and Vet (267.52±205.44) had the highest ratios during the dry season (**Fig. 18a,b**).

Overall, dissolved nutrient concentrations were higher during the wet season and the rain event than during the dry season, especially for tributary and river sites. At the river sites, ammonium showed trends of decreasing concentrations from upstream to downstream during the wet season, and the concentrations observed at R1, the most upstream site, were similar to the values observed at the tributaries during the wet season. More variability was observed in the canal sites, but generally the dissolved nutrient concentrations were higher in the wet season and rain event than during the dry season.

3.6 Targeted Bacterial Sampling

The Sagamore area has a mix of parcels that are sewered and parcels reliant on septic systems and there are multiple waterfront parcels with septic systems in this basin (**Appendix 13a)**. Every site sampled within the Sagamore basin exceeded the FDEP *E. coli* standard during at least one sampling event with exceedances ranging from 399 to 9,210 MPN/100mL (**Appendix 13b**). Within Sagamore, rainfall appears to influence *E. coli* counts. For some sites, in particular, Sag-3, the lowest counts were determined on sample dates preceded by the least amount of rainfall (June 19, 2017 and September 19, 2017), however on these same dates high counts (greater than 100 MPN/100mL) were observed at other sites (Sag-4 through 6). On the sample dates preceded by heavier rainfall (June 7, 2017 and July 13, 2017), all sites sampled exceeded standards (**Appendix 13b**). Sag-4 through Sag-6 exceeded standards during every sampling event (**Appendix 13b**).

The Hogpen area consists of mostly sewered parcels with only a few utilizing septic systems (**Appendix 14a**). Within the Hogpen area, the sites Hog-5, Hog-8, Hog-9, and Hog-10 had bacteria counts that exceeded the FDEP *E. coli* standard with counts ranging from 548 to 2,420 MPN/100mL (**Appendix 14b**). The highest bacteria counts were observed on the sampling date preceded by the greatest rainfall (July 13, 2017) at two sewered sites Hog-5 and Hog-10 that also receive water flow from the Savannahs preserve area, which supports several residential neighborhoods and businesses on septic systems. Hog-5 exceeded standards for 3 of 4 sampling events (**Appendix 14b**). Hog-8 and Hog-9 are downstream of waterfront septic systems, which may influence water quality at these sites (**Appendix 14a,b**).

The Veterans Memorial area is largely sewered having only a few parcels using septic systems, which would not be expected to widely influence the local water quality (**Appendix 15a**). In this area, the sites Vet-1, Vet-2, Vet-3, Vet-5, and Vet-6 all exceeded the FDEP *E. coli* standard for bacteria with counts ranging from 461 to 2,420 MPN/100mL (**Appendix 15b**). The most widespread bacteria were observed following the heaviest rainfall event (**Appendix 15b**).

4. Discussion

4.1 Flow Rates

C-107 and Sag drain into the North Fork upstream of R3, so nitrogen enrichment from the high NH_4 concentrations observed at these canal sites are expected to have the greatest effect at R3, especially the contributions from C-107 due to its high flow. Sag also exhibited high bacteria, sucralose, acetaminophen, and SRP concentrations, suggesting a moderate impact downstream at R3 for these factors. Although they are low flow sites, Hog, which drains in close proximity to R4, and Vet, which drains upstream of R5, had high levels of bacteria and acetaminophen and may have contributed to concentrations observed downstream at these river sites. Mont and E-8 both had high concentrations of NH_4 and sucralose. As these canal sites drain in close proximity to R5 and upstream of R6 the influence is expected to be greatest at these river sites. Mont also experienced high NO_3 concentrations and is expected to have a higher impact on water quality than E-8 on R5 and R6 due to its high flow rate. A-22 and A-18 drain just upstream of R6 and R7, so the poor water quality, demonstrated by high NH_4 and DIN observed at A-22 and the high NO_3 and sucralose observed at both A-22 and A-18, is expected to have an impact on these river sites. In particular A-22 has a high flow rate and is expected to have the greatest effect at these river sites.

4.2 Site Summaries

4.2.1 Tenmile Creek

Generally bacteria concentrations were lower than standards in Tenmile Creek, with fecal coliforms and *E. coli* increasing from the upstream site to the downstream site for both seasons, however during the rain event there was a slight decrease from downstream to upstream. Interestingly, the human molecular marker (HF183) amplified at the upstream site, TM1, which helps link these bacteria to human wastewater. Sucralose and acetaminophen were present at both sites, while carbamazepine was only detected at the downstream site during the wet season. Sucralose concentrations were below “moderate” in Tenmile Creek for all seasons, representing some of the lowest values found in the study (similar to R1, R2, C-24, and Vet), especially compared to canal sites. However, the effects of dilution in this system should be considered relative to canals given that this is a larger, flowing, tidally influenced stream. Acetaminophen was present at TM2 (upstream) during the wet season and at TM1 (downstream) during the wet and dry seasons. These human source tracers were detected in relatively low quantities compared to other sites. However, the amplification of HF183 at the upstream site suggests that there is a human wastewater source that could be contributing to the FIB load of this system, even at the most upstream site with more dominant agricultural land-use. Diuron and imidacloprid were all found at both sites, however fenuron was only present at the upstream site. These chemical tracers reflect the potential for surface runoff to affect the water quality in Tenmile Creek, and thus downstream in the North Fork. Surficial runoff can increase nutrient loading from pet waste and fertilizers, which have been suggested to be important to water quality in this area (White and Turner 2012). Fluridone was present in the wet season in low concentrations, which suggests that decaying macrophytes could also contribute to bacteria in Tenmile Creek during this season.

Tenmile Creek had generally high dissolved nutrient concentrations compared to other study sites. DIN concentrations were among the highest in the study. In the wet season concentrations increased downstream, suggesting a downstream source of nutrients. During the dry season and the rain event there wasn’t as much of a difference between the sites for DIN, with a slight decrease headed downstream, which could be driven by a precipitation of suspended solids enhanced by the mixing of freshwater and saltwater as suggested by Yang et al. (2008) for this system. Ammonium increased downstream and concentrations were some of the highest observed in the study, with concentrations similar to past research in this watershed and nearby urban areas (Yang et al. 2008; Lapointe et al. 2012, 2017). Higher ammonium in conjunction with heavier rainfall may be a result of increased nitrogen-loading from septic systems due to an elevated water table that doesn't allow for proper nitrification (conversion to nitrate in aerobic conditions) in the drainfield before elution. Similarly, nitrate concentrations were elevated downstream regardless of season, which may reflect increased nitrogen-loading from septic tank effluent, where nitrification has occurred (or partially occurred). Tenmile Creek also had some of the highest phosphorus concentrations in the study and SRP was higher upstream in the wet season and the rain event, which may be reflective of the influence of agricultural land-use upstream. The DIN:SRP ratios were relatively similar between upstream and downstream sites, with slightly higher ratios upstream during the dry season and rain event and slightly higher ratios downstream during the wet season. DIN:SRP was similar to values for Fivemile Creek and the river sites, showing slight nitrogen-limitation. This is similar to ratios observed by Yang et al. (2008), who attributed the low ratios to high background phosphorus. The high nutrients observed in Tenmile Creek indicate this tributary is a major source of nutrient loading to the North Fork.

4.2.2 Fivemile Creek

In Fivemile Creek, fecal coliforms and *E. coli* usually decreased from upstream to downstream, except for *E. coli* during the rain event and fecal coliforms during the dry season which showed the opposite pattern. No molecular markers (human or avian) amplified in Fivemile Creek, which does not allow for any discrimination or elimination of a probable bacteria source. However, sucralose, acetaminophen, and carbamazepine were present at both sites. Sucralose concentrations were approaching "moderate" levels for the wet and dry season, but were lower during the rain event. Acetaminophen was detected at both Fivemile sites during the wet season and at FM2 (upstream) during the rain event. Carbamazepine levels were similar to those found in Tenmile Creek and the river sites. The presence of these three compounds suggests strongly that there is a wastewater influence, such as septic tank effluent, within Fivemile Creek. Diuron and imidacloprid were also found at both sites in Fivemile Creek demonstrating that surficial runoff is another issue impacting water quality in this creek. Fluridone was detected at FM1 only during the wet season, therefore seasonally decaying macrophytes may also contribute to bacterial loading in Fivemile Creek.

Similar to Tenmile Creek, dissolved nutrient concentrations were relatively high in Fivemile Creek. DIN concentrations were usually higher downstream. Ammonium concentrations in Fivemile Creek were some of the highest observed in the study and were greater upstream during the wet season and the rain events, illustrating the influence of increased rainfall and the rising high water table. These values were also similar to past studies in this stream and urbanized areas showing influence of wastewater (Yang et al. 2008; Lapointe et al. 2012, 2017). Nitrate concentrations were highest downstream during the wet season, and were relatively similar between sites during the dry season and rain event, with slightly higher concentrations upstream during the dry season and slightly lower concentrations upstream during the rain event. The higher nitrate concentrations observed during the rain event may be due to increased nitrogen-loading from high water tables flushing out septic drainfields, similar to the pattern that was observed in Tenmile Creek. Another similarity to Tenmile Creek was that SRP concentrations were generally highest upstream, reflective of the agricultural influence, with the exception of the rain event. DIN:SRP ratios were often higher downstream, except during the rain event where higher DIN:SRP ratios were observed upstream. Compared to canals the DIN:SRP was low and reflected slight nitrogen-limitation. The high nutrient concentrations show that Fivemile Creek is another major source of nutrients to the North Fork.

4.2.3 North Fork of St. Lucie River

Within the North Fork, bacteria were highly variable. Overall highest average concentrations of fecal coliforms were observed at the most upstream site, R1, followed by the most downstream site, R7, and then R2. Enterococci were highest at R4, followed by R3 and R1. Higher enterococci counts were determined by cfu rather than MPN, suggesting that the methodology used in sample enumeration can potentially have an effect on the study results. Wet season FIB counts were generally higher than the dry season or the rain event, with all river samples exceeding the FDEP standard in the wet season. R1 was elevated for all sampling events for either fecal coliforms or enterococci. R7 exceeded standards for both FIB in the wet season, but FIB was low during the other sampling events. Sucralose and carbamazepine were present at all river sites, and acetaminophen was found at all but R3 and R6. Sucralose concentrations increased downstream in all seasons, with R1 and R2 below “moderate” levels and R3-R5 at “moderate”; the variance at R7 extended into “significant” during the dry season, showing an increase in wastewater influence downstream. Acetaminophen was detected at R2, R4, R5, and R6 during the dry season and at R1 during the rain event. Diuron and imidacloprid were present at all river sites and fenuron was found at R6 and R7 demonstrating that runoff affects water quality and that these chemicals accumulate downstream in the river. Fluridone was found at low levels in the river, with the greatest concentrations observed during the wet season. There was a slight increase in fluridone headed downstream from R1-R7, suggesting that it may accumulate downstream from multiple upstream sources. Therefore decaying macrophytes either directly, or indirectly as a result of downstream transport, may contribute to bacterial loading in the North Fork.

Dissolved nutrient concentrations were greatest upstream and decreased going downstream, likely as a result of dilution within the main stem of the river. Overall, DIN concentrations were usually elevated upstream and decreased downstream, with the exception of the rain event which had relatively similar concentrations among all sites. Ammonium and SRP concentrations were also elevated at upstream sites and declined at the downstream sites, while nitrate concentrations were relatively similar at all river sites. The greatest overall DIN:SRP ratios were observed at downstream sites reflecting increased N-loading via downstream residential canals. Seasonally, ammonium concentrations were generally greatest at upstream sites, with the highest concentrations observed during the wet season, further supporting increased leaching of septic tank effluent with heavy precipitation. Nitrate concentrations were relatively similar among river sites, with peak concentrations observed during the wet season and rain event. The highest SRP concentrations were generally observed upstream reflecting the more agricultural land-use, except during the dry season where concentrations gradually increased from upstream to downstream. The greatest DIN:SRP ratios were observed during the rain event, with the highest ratios observed downstream, while the lowest ratios were observed during the dry season. This upstream nutrient loading can create downstream conditions favorable for algal blooms

(Lapointe et al. 2015, 2017.) Overall, nutrient concentrations increased during the wet season and rain event illustrating the influence of upstream sources.

4.2.4 C-107

Bacteria concentrations were relatively low at C-107 compared to other study sites, though they were slightly elevated during the rain event. No molecular markers amplified at C-107, however sucralose and acetaminophen were present. Sucralose was elevated in the dry season at the "moderate" level, reflecting increased concentration without the dilution effect of rainfall. Despite sucralose concentrations being comparatively low, acetaminophen was detected during the wet season and during the rain event at some of the highest levels found in the study. These chemical tracers demonstrate the influence of untreated wastewater, such as septic tank effluent, at C-107. Chemical tracers, including diuron, fenuron, and imidacloprid were found at C-107 demonstrating that surface runoff is influential at this site. Fluridone was detected in trace amounts which suggests decaying macrophytes may be another source of bacteria in this system.

Dissolved nutrient concentrations at C-107 were variable. Overall DIN was higher in the wet season and lower during the dry season and rain event. Ammonium was elevated in the wet season and lower during the dry season and rain event, but was one of the highest canal sites for these samplings. Nitrate was elevated during the wet season, suggesting an influence of denitrified wastewater from septic systems, but was much lower during the dry season and the rain event. SRP was low at C-107 in the wet season and the rain event and barely detectable during the dry season, suggesting rainfall influences the SRP source at this site. Greater DIN:SRP ratios were observed during the dry season, with lower, but still relatively high DIN:SRP in the wet season and the rain event. These ratios reflect phosphorus-limitation, likely driven by nitrogen inputs and selective phosphorus removal from septic systems at this site.

4.2.5 Sagamore

During the events when Sag could be sampled, it had the highest average bacteria concentrations of all sites for both types of FIB and exceeded fecal coliform standards. The human molecular marker amplified at Sag, furthermore sucralose, acetaminophen, and carbamazepine were all present. The highest sucralose concentrations observed during the study were at Sag during the rain event, exceeding "significant" criteria, and concentrations were "moderate" in the wet season. Acetaminophen was detected during the wet season and during the rain event. These chemical tracers strongly support a wastewater influence from septic systems at Sag. Diuron, fenuron, and imidacloprid were found at Sag, suggesting inputs of surficial runoff.

Dissolved nutrient concentrations support the same conclusions as the chemical tracers. DIN (both ammonium and nitrate) concentrations were both elevated at Sag during the wet season, indicative of increased flow of septic tank effluent. SRP concentrations were higher during the rain event. DIN:SRP ratios were highest during the wet season. Sag had the greatest average nitrate, which is a reliable septic tank effluent tracer. The second highest SRP concentrations relative to the other canal sites were also found at Sag, with elevated nitrate concentrations during the wet season and the greatest SRP concentrations during the rain event of all sites.

Targeted, fine-scale sampling for *E. coli* within the Sag drainage basin helped to reveal trends in surface water quality. Bacteria levels were consistently high and varied with rainfall within the

Sag basin, suggesting that high water tables and surface runoff affect water quality at these sites. The lower FIB counts at Sag post Irma may reflect flushing due to previous heavy rainfall. The ubiquitous presence of enteric bacteria combined with molecular markers and chemical tracers, and dissolved nutrient concentrations in the Sagamore area suggests the influence of local septic systems in this neighborhood, many of which are located directly on the waterfront. The persistently degraded water quality at Sag indicates that it should be prioritized for future monitoring and restoration.

4.2.6 Hogpen Slough

Hog had the second highest average bacteria concentration for both FIB. This site exceeded former DEP fecal coliform standards during the wet and dry seasons, and had elevated levels of *E. coli*. No molecular markers amplified at Hog, however sucralose, acetaminophen, and carbamazepine were all detected. Sucralose was "moderate" in the wet and dry season and "significant" during the rain event. Acetaminophen was present during the wet season and during the rain event. The bacteria exceedances and presence of human source tracers reflect a wastewater influence. There are only seven permitted septic systems in this area, suggesting an outside source is introducing wastewater. At this site, surface runoff is also an issue as evidenced by the detection of diuron and imidacloprid at Hog. There were also low detections of fluridone suggesting decaying macrophytes may also influence water quality at this site.

Dissolved nutrients at Hog showed seasonal trends. Ammonium concentrations were highest during the wet season at Hog, while nitrate concentrations were greatest during the dry season. Ammonium was low compared to other canal sites in the wet season, but average during the dry season and rain event. Nitrate was low during the wet season and rain event, but was the highest of all sites during the dry season. SRP concentrations were greatest during the rain event. DIN:SRP ratios were elevated during the dry season showing greater phosphorus-limitation.

In the Hogpen Slough drainage basin Hog-5, the site with the most bacterial exceedances during the fine-scale sampling, does not have septic systems. In fact, this neighborhood is largely sewered, so the high prevalence of *E. coli* is surprising. However, this area receives water input from the Savannahs preserve, which is thought to transport effluent from other neighborhoods that are on septic systems (CPSL 2018, personal communication). In Hogpen Slough, during the heaviest rain event, the sites with septic systems had increased bacteria. The consistently high FIB counts at Hog-5, but not downstream at Hog-6 seems to suggest a localized source of bacteria at this site. It appears there may be a relationship between bacterial counts at the upstream site, Hog-10, and the downstream site, Hog-5, but more sampling would be required to determine this.

4.2.7 Veterans Memorial

Vet was another site with persistent bacterial problems. Average fecal coliforms were high (above 800 cfu/100mL) at Vet. Fecal coliforms and *E.coli* were highest during the wet season, exceeding former FDEP standards, but lower during the dry season and the rain event. No molecular markers amplified at Vet, but sucralose and acetaminophen were present. The highest sucralose concentration was "moderate" and was observed during the rain event. Acetaminophen was found during the wet season and during the rain event. The presence of these chemical tracers, coupled with the high bacteria levels, indicates untreated wastewater influenced by rainfall as the source of contamination at Vet. Diuron, fenuron, and imidacloprid were detected at Vet revealing the influence of surficial runoff on water quality at this site. Fluridone was detected in moderate concentrations, suggesting that decaying macrophytes may contribute slightly to bacteria in this basin.

Dissolved nutrient concentrations at Vet helped to interpret the MST data. Ammonium concentrations were highest during the wet season, while nitrate concentrations were elevated during the dry season. SRP concentrations were greatest during the rain event, further supporting the influence of rainfall on water quality in this basin. The greatest DIN:SRP ratios for all sites were observed at Vet during the dry season. As seen with the microbial source tracers, dissolved nutrient concentrations at Vet appear to be influenced by precipitation.

In the Veterans Memorial area fine-scale sampling, bacteria concentrations were highly variable and not as tightly coupled with rainfall as the other areas. This area also includes the Tiffany Stormwater Pump Station, built by GDC in 1977, which is responsible for maintaining stormwater drainage in this area. The area received 3” of rain in the seven days prior to sampling on July 13, 2017, the date with the most exceedances, indicating that rainfall affects water quality in this area. As such, on-going improvements to the downstream conveyance system of the Tiffany Stormwater Pump Station and the planned stormwater treatment area in Veterans Memorial will likely be beneficial to local water quality. Furthermore, when water is pumped through this area it flows through approximately 50 acres of vegetation (CPSL, personal communication). Contact with vegetation may affect bacterial concentrations at this site if decaying macrophytes are present (Byappanahalli et al. 2012), which is suggested by the presence of fluridone.

4.2.8 Elkcam

Average fecal coliforms were high (above 800 cfu/100mL) at Elk. Fecal coliforms exceeded former FDEP standards in the wet season, but were much lower during the dry season and the rain event. *E. coli* was highest during the wet season and minimal during the dry season and rain event. No molecular markers amplified at Elk. Sucralose, acetaminophen, and carbamazepine were found at Elk. Sucralose was “moderate” at all seasons, with the highest concentration during the rain event, and acetaminophen was detected during the wet season. Elk is influenced by septic and sewer systems, so the presence of acetaminophen during the wet season suggest that untreated septic effluent is introduced via runoff. Diuron, fenuron, and imidacloprid were present at Elk illustrating the influence of surficial runoff at this site. Decaying macrophytes may represent another important influence on water quality at Elk, based on the high concentrations of fluridone detected during the wet and dry seasons.

At Elk, dissolved nutrient concentrations varied seasonally. DIN and SRP concentrations were highest during the wet season at this site. DIN (both ammonium and nitrate) averages were roughly average for canal sites, while SRP averages were relatively low compared to other canal sites. DIN:SRP ratios were highest during the dry season. The high nutrient concentrations during the wet season further support the influence of increased loading from septic tank effluent and surficial runoff at this site.

4.2.9 Monterrey

Overall FIB averages at Mont were not high. The highest observed fecal coliform concentrations were in the wet season and the rain event, and *E. coli* was minimal for all events. No molecular markers amplified at Mont. Sucralose was "moderate" during the wet season and the rain event, and "significant" during the dry season, reflecting a wastewater influence. Carbamazepine concentrations were consistently highest at Mont in every season compared to all other sites, indicating a high influx of anticonvulsants into the watershed. There is not a large medical facility in this watershed to explain the higher levels of carbamazepine, however there is a pediatric office (466 SW Port St. Lucie Blvd.), podiatrist (691 SW Port St. Lucie Blvd), and a spinal center (683 SW Port St. Lucie Blvd.) in the area. Diuron, fenuron, and imidacloprid were detected at Mont, demonstrating surficial runoff influences local water quality. The high levels of fluridone during the wet season and moderate levels during the dry season and rain event reflect the potential for decaying macrophytes to contribute to bacterial abundance at this site.

Mont showed seasonal variability in the concentrations of dissolved nutrients. DIN concentrations peaked during the wet season, while SRP concentrations were elevated during the rain event. The lowest DIN:SRP ratios were observed during the rain event, and ratios were similar during the wet and dry seasons, with slightly higher ratios during the dry season. Overall average concentrations for ammonium and nitrate were relatively high compared to other canal sites, suggesting Mont, which has the highest flow rate of all the canals, may be an important source of nutrient loading to the North Fork. The higher nutrient concentrations during the wet season and rain event are suggestive of increased loading from septic systems and surficial runoff at this site.

4.2.10 C-24

The bacteria concentrations at C-24 were minimal. The highest FIB concentrations were observed in the wet season, but were still below standards. No molecular markers amplified at C-24. Sucralose was present at C-24 and concentrations were below "moderate" criteria for all seasons. Diuron, fluridone, and imidacloprid were detected at C-24, while acetaminophen and carbamazepine were not found at C-24 during this study. The low levels of sucralose and lack of pharmaceuticals indicate a minimal influence of wastewater at this site. However, the presence of herbicides and the pesticide, imidacloprid, suggest that runoff (likely from agricultural land-use) is influencing water quality at this site.

At C-24, there was seasonal variation in dissolved nutrient concentrations. Overall, DIN concentrations were elevated during the wet season and rain event. Ammonium concentrations were greatest during the wet season at C-24, while nitrate concentrations were elevated during the rain event. The highest average SRP concentrations were observed at C-24 relative to the other canal sites, which reflects the agricultural influence at this site. DIN:SRP ratios were relatively low compared to the other canal sites, with the highest ratios observed during the rain event.

4.2.11 E-8

Low bacteria concentrations were observed at E-8. The highest FIB concentrations were observed in the wet season and rain event, but were still below standards. No molecular markers amplified at E-8. Sucralose concentrations were "moderate" for all seasons and carbamazepine was also detected, supporting the influence of wastewater at this site. Runoff is also indicated at E-8 as diuron, fenuron, and imidacloprid were all detected. Fluridone was also detected in low concentrations during all seasons.

Dissolved nutrient concentrations were influenced by rainfall at E-8. DIN concentrations (both ammonium and nitrate) were highest during the wet season at E-8, while SRP concentrations were highest during the rain event. The greatest average ammonium concentrations were observed at E-8 relative to the other canal sites, which could indicate a wastewater influence. DIN:SRP ratios were highest during the wet season, exceeding the ratios observed for all other sites during that season.

4.2.12 Horseshoe A-18

A-18 did not have high FIB concentrations. At this site, fecal coliforms peaked in the wet season and rain event, but still well below former FDEP standards. *E. coli* was minimal for all events. The overall lowest average fecal coliform and *E. coli* concentrations were observed at A-18. No molecular markers amplified at A-18. Sucralose concentrations were "moderate" for all seasons and carbamazepine was also detected, demonstrating that wastewater influences water quality at A-18. The chemicals diuron, fenuron, and imidacloprid, which are related to runoff, were also detected at A-18. Moderate levels of fluridone were observed in the wet season, with lower concentrations in the dry season and during the rain event.

Similar to other sites in the study, ammonium, nitrate, and SRP concentrations were all elevated during the wet season at A-18. The greatest DIN:SRP ratios were observed during the wet season, with the lowest ratios observed during the dry season. High wet season nutrient concentrations are suggestive of increased nutrient loading from septic systems and the influence of surficial runoff at A-18.

4.2.13 Southbend/Horseshoe A-22

Average fecal coliform concentrations were elevated, but not above 800cfu/100mL, at A-22. Fecal coliforms exceeded former FDEP standards in the wet season, were relatively high in the dry season, and were lowest during the rain event. *E. coli* was highest during the dry season, but was generally low for all events. No molecular markers amplified at A-22. Sucralose, acetaminophen, and carbamazepine were present. Sucralose concentrations were "moderate" for all seasons, while acetaminophen was only detected during the wet season. The high sucralose concentrations reflect the prevalence of wastewater, and the presence of acetaminophen during the wet season suggests that untreated wastewater presence is increased by rainfall. The area is mixed septic and sewer, so septic effluent is likely the source of untreated wastewater. Diuron, fenuron, fluridone, and imidacloprid were present at A-22 showing that surficial runoff and decaying macrophytes are other potential sources of bacteria at A-22.

DIN concentrations (both ammonium and nitrate) were elevated during the wet season at A-22. The lowest SRP concentrations were observed during the dry season, with similar SRP concentrations observed during the wet season and rain event. At A-22, the highest DIN:SRP ratios were observed during the dry season. The high nutrient concentrations during the wet season and rain event support that increased precipitation leads to more contaminant loading at A-22.

4.3 Project Summary

When the river, canal, and tributary sites are considered together it is apparent that while bacteria concentrations are highly variable, there are sites that consistently exceed standards and/or regularly have high FIB concentrations. Sites of concern for bacterial contamination include: tributary sites in Tenmile Creek (downstream site, TM1) and Fivemile Creek (both FM1 and FM2); the most upstream river site (R1); and the canal sites, Sag, Hog, Elk, and Vet. The presence of human tracers at every site suggests a widespread influence of wastewater in the North Fork. This is supported by a statewide study in Florida that found flowing waters have detectable levels of sucralose in 35% of canals, 52% of streams, and 72% of rivers, and detectable levels of either acetaminophen, carbamazepine, or primidone in 8% of canals, 26% of streams, and 27% of rivers (Silvanima et al. 2018, in review). The influence of wastewater is further reinforced by the nutrient data at many sites. Septic tank effluent is a likely source of this contamination, however sewer overflows, reuse water, and illegal dumping are other possibilities at some sites. Septic tank effluent has been considered a contributing source to microbial pollution in other parts of Florida (Paul et al. 1995; Arnade 1999; Lipp et al. 2001). Higher SRP in Tenmile Creek, Fivemile Creek, and the C-24 canal relate to agricultural land-use. All sites reflect the probable influence of surface runoff on water quality due to the ubiquitous presence of herbicides and imidacloprid (pesticide). The report by Silvanima et al. (2018, in review) estimates flow waters have detectable amounts of imidacloprid in 50% of canals, 37% of streams, and 65% of rivers, yet in this study all sites had detectable amounts. Additionally, the average concentrations of these pesticides are higher than those estimated from statewide probabilistic surveys (James Silvanima, FDEP, personal communication). The data collected in this study will allow for more focused future investigations into what is driving the persistent bacterial contamination and eutrophication of the North Fork.

Molecular markers did not amplify at many sites for either human or avian markers. It is possible that there are other sources of bacteria, such as dog, cattle, or plant, that were not tested in this study. The human DNA tracer, HF183, amplified at TM2, R1, and Sag, demonstrating that human waste is likely present at these sites. The seabird tracer, Gull2, amplified at R1, suggesting that birds may be influencing water quality at this site as well. Samples for which the FDEP Laboratory suspected possible matrix interference with qPCR amplification were diluted or purified and re-analyzed, which often raised the MDL for the analysis, thereby potentially affecting the number of measurable detections. The lack of amplification of the molecular markers (both human and avian) suggests that these sources may not be major contributors to the fecal pollution in the North Fork. However, non-detections could also represent a false negative result and do not necessarily allow for elimination of these sources as factors contributing to the impairment of this system. Perricone (2017) also encountered a lack of amplification for HF183 in the St. Lucie Estuary at sites with a history of bacterial impairment and attributed this to either a non-human source, low levels of the marker, or sample matrix interference. Therefore, it may be useful to assess the viability of molecular markers in this system by also testing future water samples for a general bacteroides signal to ensure amplification, before analyzing the species or genera specific markers (Troy Scott, personal communication). This would enable non-detections to more conclusively eliminate bacteria sources.

Previously, HF183 has been amplified, along with significant sucralose and fecal coliform concentrations, at multiple sites in the South Fork of the St. Lucie Estuary in Martin County (FDEP 2014). Seasonal sucralose concentrations found in the North Fork were comparable to those found in the South Fork and other septic tank effluent contaminated surface waters of South Florida. For example, in Martin County sucralose was detected in surface water in concentrations ranging from 0.1 to 5.5 μg/L during the dry season and 0.1 to 4.9 μg/L during the wet season (Lapointe et al. 2017). Additionally, during the wet season at surface water sites in Port Charlotte, sucralose concentrations ranged from 0.1 to 0.2 μg/L (Lapointe et al. 2016). In this study sucralose ranged from below detection limits (BDL) at TM2 and C-24 to 0.637 ± 0.156 μg/L at Sag in the wet season. In the dry season, Vet was BDL while Mont was elevated at 1.250 ± 0.354 μg/L. During the rain event, C-24 was BDL and the highest concentration was 1.8 μg/L at Sag. This range of concentrations is similar to what has been found in other urban areas of Florida.

Sucralose was found at every site, however, the additional presence of acetaminophen at some sites strongly indicates untreated wastewater, such as septic tank effluent, is affecting the local water quality. Interestingly, the anticonvulsant carbamazepine, which is used to treat a variety of neurological disorders, was also observed at almost all sites, except TM2, C-107, Vet, and C-24, though it should be noted that carbamazepine has a much lower detection limit than sucralose and acetaminophen. The widespread presence of carbamazepine further emphasizes the global influence of human wastewater throughout this system. As carbamazepine is not always broken down during wastewater treatment (Dong et al. 2015) it has become ubiquitous in surface water for many locales affected by wastewater (Andreozzi et al. 2002; Glassmeyer et al. 2005). As such, its presence in the North Fork is not surprising. Seasonal variation was also observed with wet season and rain sampling events generally yielding more detections and higher concentrations of carbamazepine. The highest carbamazepine levels were found in canals, particularly Mont. Five canal sites (Sag, Hog, Vet, Elk, and Mont) exhibited high levels of FIB, sucralose, and/or acetaminophen during this study. Sag, Elk, and Mont are all located in residential areas with high densities of septic systems, so the high sucralose concentrations observed at these sites, as well as the high FIB concentrations observed at Sag, illustrate the potential influence of residential wastewater. Interestingly, Hog and Vet are located in areas that are 90-95% sewered, but had high levels of FIB and acetaminophen during the wet season and the rain event, indicating the presence of untreated wastewater with increased precipitation. It is noteworthy that Hog receives runoff from the Savannahs residential area of Fort Pierce, FL, which includes several large businesses using septic systems. The combined use of these human tracers was effective and allowed for detection of wastewater in areas where it would not have been suspected to be a problem, such as Hog and Vet.

Overall, the human source tracers results reveal seasonal variation for sucralose, carbamazepine, and acetaminophen at the tributary, river, and canal sites. These seasonal variations reflect the influence that seasonal rainfall and high water table differences can have on surface water quality. The results suggest that untreated wastewater is more prevalent during the dry season in the river sites, specifically R2, R4, R5, and R7, than the wet season or rain event, which may be an effect of dilution. In the tributaries and canals, higher acetaminophen concentrations were observed during the wet season, indicating untreated wastewater is more prevalent during that season due to increased loading from septic systems inundated by high water tables. These higher concentrations during the wet season also reflect potentially shorter residence times in septic systems, where acetaminophen is not broken down. The tributary sites, FM1 and FM2, and the canal sites, Sag, Hog, and Vet, were consistently elevated both during the rain event and the wet season, suggesting these sites may be prone to untreated wastewater exposure.

The majority of the sites in this study are within dense residential areas. CPSL Utilities Department estimates that the study area within city service areas contains 18,243 parcels with septic systems, 5,312 of which are within 50 feet of a waterway (2017). There are also an estimated 492 septic systems within the Tenmile Creek watershed (White and Turner 2012) and approximately 1,021 "known," 726 "likely," and 27 "somewhat likely" septic systems in the Fivemile Creek watershed (WBID 3194D) based on estimates from the FL DOH Florida Water Management Inventory database, though these are likely underestimations as records for septic systems installed prior to the 1970s in Florida are not readily available (USEPA 1996). Regardless, the high densities of septic systems within these watersheds indicate that septic tank effluent has the potential to cause detriment to water quality in the North Fork. Furthermore, the chemical tracers and dissolved nutrient data lend support to septic systems as a contributing factor to pollution in the North Fork.

High nitrogen found in surface waters near high density septic systems has been reported in a number of locations (Lapointe and Clark 1992; Hatt et al. 2004; Lapointe et al. 2015, 2016, 2017). Simple calculations can be done to estimate the contribution of septic systems to eutrophication in the North Fork. Bicki et al. (1984) estimated that the average person eliminates 18 lbs of nitrogen per year. To account for attenuation and make a conservative estimate, this estimated loading can be reduced by 50% to 9 lbs of nitrogen per person per year (as done in Lapointe and Herren 2016). This value is similar to the 9.7 lbs/person/year used by FDEP (Polley 2014). Multiplying 9 lbs N per person, per year by an average of 2.5 people per the 18,243 parcels on septic systems in CPSL yields an estimated 410,468 lbs nitrogen per year originating from septic systems along the North Fork. Bicki et al. (1984) also estimated that the average person eliminates 1.66 lbs of phosphorus per year. Again, to account for attenuation and be conservative this value can be reduced by 50% to 0.83 lbs of phosphorus per year (Lapointe and Herren 2016). Multiplying 0.83 lbs phosphorus per person, per year by an average of 2.5 people per 18,243 parcels on yields an estimated 37,854 lbs phosphorus per year from septic systems. If the Tenmile Creek (492) and Fivemile Creek (1,021) estimates are also considered it brings the total to 444,510 lbs nitrogen per year and 40,994 lbs phosphorus a year. Therefore septic systems may be a significant source of nutrient pollution to the North Fork.

There are other potential sources of the persistent human chemical tracers, sucralose and carbamazepine, besides wastewater that should be investigated in the North Fork. First of all, reclaimed water is another potential source of human source tracers. However, reclaimed water probably has minimal effects on bacterial concentration and nutrient loading because it is thoroughly treated and disinfected before application. Furthermore, within the study area, reuse water is minimally applied (CPSL Utilities Department 2016). There is a reuse water application site downstream of R6 at the Tesoro Club golf course, however, this is across the river from R7 and would not be expected to contribute to water quality at this site. There is another water reuse site at Santa Lucia River Club golf course, which is upstream of R7 and could potentially have downstream effects at that site. Future studies could be conducted to determine if this application has any downstream effects. Groundwater is another potential source of these chemical tracers. A statewide study in Florida by Seal et al. (2016) found sucralose was detected in 25% of unconfined groundwater aquifers, and 12% had detections of carbamazepine, acetaminophen, or primidone. The application of Class AA biosolids within the study area could also be contributing to the presence of these chemical tracers. Class AA biosolids are processed human waste that is made available to the general public for purchase as fertilizer. During the production of Class AA biosolids, pathogens are supposed to be eliminated. However, the potential exists for these compounds to contribute human chemical tracers and/or nutrients to adjacent surface waters, and impact critical ecosystems (King et al. 2011). This has not been well studied, but studies regarding these relationships combined with data on local sale of Class AA biosolids would allow for better interpretation of this MST data.

Chemical tracers relating to land-use were informative as to what factors may be influencing water quality. For example, Tenmile Creek progresses from agricultural land-use upstream to residential/urban downstream and *E. coli* concentrations increase from upstream to downstream, suggesting that the source of this contamination is more likely attributable to residential/urban sources, such as surficial runoff and wastewater, than agriculture. The predominance of residential or urban influence on water quality is further supported by the lack of detection for the agricultural tracer, linuron, at any sites in this study. Furthermore, the effects of residential land-use are also supported by the detection of acetaminophen at the downstream site (TM1) during the dry season. The chemical tracers diuron and imidacloprid, which have agricultural applications, were both consistently detected during this study, indicating that there may be agricultural influence on the North Fork. It is important to note, however, that diuron and imidacloprid are also used for non-crop purposes, so these results require more information about the use of these chemicals in the North Fork region in order to determine the source of the chemicals. Relatively high SRP concentrations were observed at C-24 during the wet season. C-24 has a greater agricultural influence than other sites within the study area, which is supported by land-use data.

The presence of the terrestrial herbicides, diuron and fenuron, at canal sites reveals that stormwater runoff may also be affecting water quality at these sites. The aquatic herbicide, fluridone, was found in the highest concentrations in canal sites, which is not surprising given that it is used to control aquatic weeds. Decaying macrophytes can contribute to bacterial concentrations in surface waters (Byappanahalli et al. 2012), so the use of this herbicide to kill nuisance aquatic plants, such as water lettuce, could potentially be compounding local water quality issues. The ubiquitous presence of the residential and agricultural pesticide, imidacloprid, during the wet season and rain event further suggest that surficial runoff is also impacting water quality in the North Fork.

Seasonal differences demonstrated the influence of stormwater runoff and increased wastewater loading from septic tank effluent and other sources on the dissolved nutrient content in the North Fork. Throughout the Indian River Lagoon, increased rainfall has been found to increase nitrogen-loading from septic systems (Lapointe et al. 2015, Barile 2018). Similarly, during the wet season and the rain event, generally higher DIN (both ammonium and nitrate) suggests there is more movement of nutrients from septic systems into adjacent surface waters of the North Fork. Furthermore, the high ammonium observed in the North Fork is similar to values observed in canals of the North River Shores area that are lined with septic systems (Lapointe et al. 2012). When septic systems do not function properly because of high water tables, an increase in ammonium is often observed because rather than undergoing nitrification the ammonium moves directly into the groundwater (Lapointe et al. 1990, 2015; Lapointe and Krupa 1995). In the Florida Keys, wastewater has been shown to move quickly (~3 hrs) from septic systems to adjacent canal waters using bacteriophages (Paul et al. 2000). Similar tracing studies could be conducted in CPSL to determine residence of wastewater in septic systems and connectivity between septic systems and adjacent surface waters.

It is important to note that since nutrient sampling was not initially included in the study design, trends between wet season nutrient data and microbial source tracers cannot be directly compared since the data were collected during different sampling events. Future studies should seek to utilize dissolved nutrient data combined with simultaneously collected bacterial abundance and microbial source tracers (both molecular and chemical). Furthermore, additional data regarding the nutrient budget in the North Fork would allow for greater understanding of the factors leading to poor water quality. For example, phosphorus has been identified as the primary limiting nutrient for bacterial growth in blackwater streams in the southeastern United States (Mallin et al. 2004). This suggests that an in-depth understanding of phosphorus loading in the North Fork is essential to mitigating the chronic bacterial issues. Groundwater data was not collected in this study, but would allow for greater understanding of how septic systems are affecting the North Fork by illustrating if there is movement of nutrients, chemicals, and fecal bacteria into adjacent surface waters. In Martin County, distinctive aqueous isotope signals were used to determine the source of dissolved nutrients, which linked the ammonium in groundwater downstream of drainfields to the signal in surface water and algae (Lapointe et al. 2017). Similar isotope studies would provide critically needed data for sustainable management and continued development of the North Fork. These data would also allow for discrimination between nutrient sources fueling eutrophication and contributing bacterial pollution.

5. Conclusions

Using multiple lines of evidence, the results of this MST study allow for the following conclusions to be made regarding pollution sources of the North Fork.

- High levels of fecal bacteria are present at many sites within the North Fork, its tributaries, and canals draining into the river.
- The widespread detection of sucralose and carbamazepine demonstrates that wastewater is a factor contributing to the impairment of the North Fork.
    - The presence of acetaminophen at several sites with high densities of septic systems, such as TM1, FM1, C-107, and Sag, indicates septic systems are a source of the wastewater contamination.
    - The presence of sucralose and carbamazepine in areas that are mostly sewered support the possibility of downstream contamination from other areas (eg. the Savannahs) or other sources, such as groundwater, sewer overflows, and leaking sewer pipes.
    - These data reveal that wastewater is negatively influencing water quality throughout the North Fork.
- Seasonal variation in nutrient and bacterial concentrations reflect the impact of increased rainfall on surface runoff and subsurface flow of septic tank effluent to surface waters.

    - Higher nutrient concentrations were observed during the wet season due to increased loading from septic systems and surface runoff.
    - The high levels of fecal bacteria and nutrient pollution are relatable to seasonal high water tables and poor soil conditions.
    - The highest ammonium and nitrate was found during the wet season, specifically at tributary sites in Tenmile and Fivemile Creeks and urbanized canals influenced by septic systems.
- The influence of surficial urban/residential runoff was evidenced by the presence of terrestrial herbicides and pesticides.
    - Runoff can contribute bacteria and nutrients to the system by the addition of pet waste, lawn clippings, fertilizers, and other substances.
    - The influx of seasonal residents may also account for some of the variation between the wet and dry seasons, due to the population surge.
- The ubiquitous presence of the aquatic herbicide, fluridone, suggests that the methods used to clear macrophytes from this system may also affect the water quality via increased nutrient loading and bacterial concentrations from decaying plants.
- Sites with high flow and greater fecal bacteria abundance, the presence of chemical or molecular source tracer, and/or elevated nutrient concentrations should be closely monitored as their impact on the North Fork may be significant.

6. Recommendations

Based on the conclusions above, we provide the following recommendations for consideration for future research, monitoring, and management.

6.1 Recommendations for Further Research and Monitoring

- Continued investigation is recommended at sewered and non-sewered sites using coupled groundwater and surface water monitoring of aqueous nitrogen isotopes and other tracers, such as sucralose, to further investigate the couplings between septic systems and surface water, as done recently in Martin County (Lapointe et al. 2017).
    - These studies would be enhanced greatly by the addition of viral tracing studies using bacteriophages, in which the rate of septic effluent transport into adjacent waters and ultimate fate could be determined.
- Detailed studies using stable carbon, nitrogen, and sulfur isotopes of macrophytes, particulate organic matter, and sediment are needed to assess the relative contribution of fertilizers, agriculture, wastewater, and other sources to the North Fork.
    - This could also help determine the source of the high dissolved nutrients in Tenmile Creek and Fivemile Creek.
- Reclaimed water and Class AA biosolid use within this system should be investigated as a potential contributing source of dissolved nutrients and chemical wastewater tracers (e.g. sucralose and carbamazepine).
    - Studies could be performed near residential or public land application sites to determine if there is an effect of biosolids leaching into adjacent surface waters.
- A study of the potential effects of aquatic plant control methods is needed to assess relative contributions to local herbicide, bacterial, and nutrient pollution.
    - Laboratory and/or field studies are needed to quantify the impact decaying water lettuce exerts on water quality of the North Fork.

- A long-term water quality monitoring program using dissolved nutrients, stable isotopes, and human source tracers is highly recommended.
    - Long-term monitoring data would provide a baseline to gauge water quality status and trends as a result of septic-to-sewer programs, stormwater treatment, or other infrastructure improvements made within the system.

6.2 Recommendations for Water Quality Improvement

- Although CPSL has been very proactive in implementing an ambitious septic-to-sewer program, increasing the focus on connecting septic systems to centralized wastewater treatment at hotspots identified in this study (Sag, Hog, Elk, and Mont in particular), as well as other septic systems in close proximity to waterways, is recommended to mitigate the water quality issues.
- Stormwater improvements should be continued and expanded in residential and urban areas to alleviate runoff issues.
- Ongoing water quality research and monitoring to continuously evaluate the effectiveness of infrastructure improvements is highly recommended. Long-term monitoring would allow for continued re-assessment to ensure that management actions achieve environmental goals.

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9. Appendix

Appendix 1. Detailed site information by site type for the microbial source tracking study in the North Fork of the St. Lucie River, including the original name used for the site in FDEP lab reports, the site abbreviation used in this report, and site coordinates (decimal degrees).

Type	Site	Lab Report Site Name	Site Abbreviation	Latitude	Longitude
Tributary	Tenmile 2 - upsteam	Tenmile 2	TM2	27.401047	80.419933
	Tenmile 1 - downstream	Tenmile 1	TM1	27.403761	80.365692
	Fivemile 2 - upstream	Fivemile 2	FM2	27.447711	80.374464
	Fivemile 1 - downstream	Fivemile 1	FM1	27.408486	80.357953
River	R1 - upsteam	R1	R1	27.375294	80.343265
	R2	R2	R2	27.348224	80.341107
	R3	R3	R3	27.311232	80.325987
	R4	R4	R4	27.302195	80.318319
	R5	R5	R5	27.261744	80.329209
	R6	R6	R6	27.239145	80.321053
	R7 - downstream	R7	R7	27.240534	80.292789
Canal	C-107	C1	C-107	27.345049	80.352197
	Sagamore	C2	Sag	27.331174	80.352054
	Hogpen Slough	C3	Hog	27.303842	80.307058
	Veterans Memorial	C4	Vet	27.290844	80.305552
	Elkcam	C5	Elk	27.280641	80.335582
	Monterrey	C6	Mont	27.261731	80.357441
	C-24	C7	C-24	27.320454	80.453519
	E-8	C8	E-8	27.260247	80.358349
	Horseshoe A-18	C9	A-18	27.233944	80.344243
	Southbend/Horseshoe A-22	C10	A-22	27.236234	80.339907

Appendix 2. Sampling dates by season for microbial source tracking compounds and nutrient concentrations; NS = this parameter was not sampled for on this date.

Season	Microbial Source Tracking	Nutrient Concentrations
Wet 2016	6/23/2016	NS
	7/25/2016	NS
	8/22/2016	NS
Dry 2017	2/16/2017	NS
	3/16/2017	3/16/2017
Rain 2017	7/19/2017	7/19/2017
Wet 2017	NS	10/9/2017

Appendix 3. Minimum detection limits (MDLs) and practical quantitation limits (PQLs) of microbial source tracking molecular and chemical tracers by sampling date from the Florida Department of Environmental Protection Central Laboratory.

Season	Analyte	Units	MDL	PQL
Wet	GULL2-qPCR	TSC/100 mL	1700	5.20E+03
	HF183-qPCR	GEU/100 mL	170	510
	Acetaminophen	ug/L	0.004	0.016
	Carbamazepine	ug/L	0.0004	0.0016
	Diuron	ug/L	0.004	0.016
	Fenuron	ug/L	0.008	0.032
	Fluridone	ug/L	0.0004	0.0016
	Imidacloprid	ug/L	0.004	0.016
	Linuron	ug/L	0.004	0.016
	Primidone	ug/L	0.008	0.032
	Sucralose	ug/L	0.02	0.08
Dry	GFD-purified-qPCR	TSC/100 mL	10000	5.00E+04
	GULL2-qPCR	TSC/100 mL	21000	1.00E+05
	HF183-qPCR	GEU/100 mL	71	350
	2,4-D	ug/L	0.004	0.016
	Acetaminophen	ug/L	0.004	0.016
	Bentazon	ug/L	0.004	0.016
	Carbamazepine	ug/L	0.0004	0.0016
	Diuron	ug/L	0.04	0.16
	Fenuron	ug/L	0.008	0.032
	Fluridone	ug/L	0.0004	0.0016
	Imidacloprid	ug/L	0.004	0.016
	Linuron	ug/L	0.004	0.016
	MCPP	ug/L	0.004	0.016
	Primidone	ug/L	0.008	0.032
	Sucralose	ug/L	0.01	0.04
	Triclopyr	ug/L	0.01	0.04
Rain	GFD-purified-qPCR	TSC/100 mL	170	840
	GULL2-qPCR	TSC/100 mL	8400	42000
	HF183-qPCR	GEU/100 mL	140	710
	2,4-D	ug/L	0.002	0.008
	Acetaminophen	ug/L	0.008	0.032
	Bentazon	ug/L	0.0008	0.0032
	Carbamazepine	ug/L	0.0004	0.0016
	Diuron	ug/L	0.0008	0.0032
	Fenuron	ug/L	0.008	0.032
	Fluridone	ug/L	0.0004	0.0016
	Imidacloprid	ug/L	0.0008	0.0032
	Linuron	ug/L	0.004	0.016
	MCPP	ug/L	0.002	0.008
	Primidone	ug/L	0.004	0.016
	Sucralose	ug/L	0.01	0.04
	Triclopyr	ug/L	0.004	0.016

Appendix 4. Seasonal environmental parameters (average ± standard error, except where only one measurement was made) observed in surface water during sampling events in the North Fork of the St. Lucie Estuary, showing the wet and dry season sampling events, as well as the rain sampling event; NS = not sampled due to no flow, NA = this parameter was not analyzed.

Type	Site	*Wet Season 2016*					*Dry Season 2017*				
		pH	**Conductivity**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Salinity**	**pH**	**Conductivity**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Salinity**
Tributary	Tenmile 2 - upsteam	7.19±0.02	1610±354	4.48±0.39	29.50±1.27	0.77±0.10	6.95±0.21	3365±233	5.35±0.92	19.10±3.25	2.02±0.30
	Tenmile 1 - downstream	7.23±0.10	1230±311	6.31±0.57	28.95±0.78	0.52±0.08	7.00±0.42	2935±346	6.15±0.92	18.50±3.39	1.78±0.37
	Fivemile 2 - upstream	7.17±0.05	1026±48	5.16±1.07	28.30±0.28	0.50±0.01	7.35±0.78	1445±346	4.65±0.78	20.30±0.99	0.81±0.22
	Fivemile 1 - downstream	7.33±0.04	970±156	6.08±0.95	29.00±0.85	0.45±0.06	7.00±0.00	2285±1011	5.80±0.99	18.90±3.68	1.08±0.16
River	R1 - upsteam	7.39±0.26	1523±367	4.53±0.34	28.83±0.31	0.71±0.17	8.00±0.00	2046±132	6.55±0.07	20.05±2.05	1.17±0.13
	R2	7.50±0.26	1448±355	4.86±0.87	29.27±0.68	0.66±0.16	8.10±0.00	2474±474	6.88±0.03	20.80±1.13	1.40±0.31
	R3	7.58±0.24	1243±321	4.91±1.08	29.93±1.24	0.56±0.14	7.95±0.07	9703±1088	7.00±0.00	21.80±0.99	5.86±0.83
	R4	7.60±0.26	1213±327	4.81±1.02	30.20±1.47	0.54±0.14	7.95±0.07	13241±1648	6.65±0.35	21.85±0.92	8.19±1.27
	R5	7.51±0.36	1262±494	4.42±0.68	30.43±1.59	0.56±0.22	7.85±0.07	27024±1664	6.20±0.14	21.75±1.06	17.84±1.65
	R6	7.56±0.15	2977±2121	4.51±0.85	30.47±1.29	1.39±1.01	7.85±0.07	30649±1208	6.50±0.42	20.95±2.05	20.87±1.89
	R7 - downstream	7.64±0.25	5246±3713	4.61±2.20	30.13±1.53	2.54±1.82	7.55±0.07	32999±3429	6.70±0.28	20.85±1.48	22.72±3.39
Canal	C-107	7.47±0.06	451±85	2.75±0.83	30.13±0.51	0.20±0.04	7.45±0.21	451±66	7.30±0.42	21.20±1.84	0.24±0.02
	Sagamore	7.49±0.09	599±45	4.74±0.92	26.00±0.79	0.29±0.02	NS	NS	NS	NS	NS
	Hogpen Slough	6.96±0.32	246±78	3.81±0.32	29.07±0.50	0.11±0.04	7.80±0.14	296±4	4.80±0.99	18.05±3.46	0.17±0.01
	Veterans Memorial	6.84±0.15	303±9	4.26±1.44	29.23±1.00	0.13±0.01	8.10±0.14	508±345	6.80±0.85	17.90±3.96	0.30±0.23
	Elkcam	7.37±0.02	476±68	2.78±1.32	29.60±0.44	0.21±0.03	7.90±0.00	458±47	8.60±0.14	21.50±1.70	0.24±0.01
	Monterrey	7.33±0.09	594±36	5.78±1.89	29.23±0.15	0.26±0.02	7.70±0.00	732±16	7.20±2.40	20.50±1.84	0.38±0.05
	C-24	7.40±0.17	827±118	6.17±1.00	30.57±0.65	0.36±0.05	8.05±0.07	1358±11	8.65±0.07	20.90±1.41	0.74±0.01
	E-8	7.59±0.26	706±47	7.62±3.33	30.40±0.40	0.31±0.03	7.90±0.00	772±50	9.10±1.27	21.25±2.05	0.41±0.04
	Horseshoe A-18	7.62±0.07	501±61	6.96±1.26	31.35±1.39	0.21±0.02	8.10±0.14	547±32	8.30±0.14	21.10±1.41	0.29±0.03
	Southbend/Horseshoe A-22	7.22±0.23	440±73	2.83±1.76	29.50±0.50	0.20±0.03	7.75±0.07	523±50	4.76±0.64	18.95±4.03	0.29±0.00

Type	Site	*Rain Event 2017*					*Wet Season 2017*				
		pH	**Conductivity**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Salinity**	**pH**	**Conductivity**	**Dissolved Oxygen (mg/L)**	**Temperature (°C)**	**Salinity**
Tributary	Tenmile 2 - upsteam	7.60	2928	4.20	30.00	1.37	7.49	971	3.69	29.80	0.40
	Tenmile 1 - downstream	7.63	1638	4.01	30.30	0.74	7.53	10003	2.69	29.30	0.40
	Fivemile 2 - upstream	6.03	1150	NA	28.30	0.53	7.58	689	2.16	29.90	0.30
	Fivemile 1 - downstream	7.21	3126	5.60	30.00	1.47	7.77	677	2.60	28.90	0.30
River	R1 - upsteam	6.60	1380	NA	29.10	0.63	6.93	739	2.26	27.70	0.40
	R2	6.40	1800	NA	29.70	0.63	6.95	656	2.33	27.90	0.30
	R3	7.02	1080	NA	28.30	0.50	6.86	587	2.30	28.00	0.30
	R4	6.30	1420	NA	29.30	0.65	6.96	562	2.18	28.00	0.30
	R5	7.01	525	4.27	29.90	0.23	6.81	490	2.00	27.70	0.20
	R6	7.82	437	3.16	30.60	0.19	6.74	252	2.23	28.00	0.10
	R7 - downstream	7.10	747	3.67	25.80	0.35	6.36	428	2.85	27.80	0.20
Canal	C-107	7.46	290	3.77	27.6	0.13	7.72	326	4.33	29.4	0.1
	Sagamore	7.34	382	4.98	29.8	0.17	7.33	635	5.4	27.1	0.3
	Hogpen Slough	7.34	55	4.26	29.9	0.24	7.76	255	1.17	27.2	0.1
	Veterans Memorial	7.11	514	4.38	28.9	0.23	7.7	319	4.35	28.4	0.1
	Elkcam	7.11	1308	11.5	30.6	0.58	7.65	507	4.26	28.6	0.2
	Monterrey	7.31	659	9.18	30.7	0.29	7.52	572	3.23	27.5	0.3
	C-24	7.21	378	11.22	30.5	0.16	7.72	327	2.2	28.6	0.1
	E-8	6.42	2060	NA	28.3	0.98	7.43	642	3.4	28.8	0.3
	Horseshoe A-18	7.57	1193	3.91	28.6	0.55	7.47	384	3.74	29	0.2
	Southbend/Horseshoe A-22	7.54	1443	3.92	29.4	0.66	7.35	390	3.82	28.7	0.2

Appendix 5. Seasonal bacterial concentrations (average ± standard error, except for where only one measurement was made) observed in surface water samples collected in the North Fork of the St. Lucie Estuary, showing the wet and dry season, as well as the rain sampling events; NS = not sampled due to no flow, NA = this parameter was not analyzed.

Type	**Site**	*Wet Season*				*Dry Season*				*Rain Event*			
		Count	**Fecal Coliform (CFU)**	***Escherichia coli*** **(MPN)**	**Enterococci (CFU)**	**Count**	**Fecal Coliform (CFU)**	***Escherichia coli*** **(MPN)**	**Enterococci (MPN)**	**Count**	**Fecal Coliform (CFU)**	***Escherichia coli*** **(MPN)**	**Enterococci (MPN)**
Tributary	Tenmile 2 - upsteam	3	103±95	74±52	NA	2	73±32	68±40	NA	1	157	94	NA
	Tenmile 1 - downstream	3	193±169	301±269	NA	2	143±26	222±98	NA	1	149	821	NA
	Fivemile 2 - upstream	3	198±159	430±481	NA	2	55	552±704	NA	1	600	84	NA
	Fivemile 1 - downstream	3	159±60	263±147	NA	2	77	344±380	NA	1	172	545	NA
River	R1 - upsteam	3	489±145	NA	734±307	2	477±301	NA	94±74	1	2250	NA	1220
	R2	3	433±308	NA	546±102	2	118±8	NA	54±33	1	178	NA	95
	R3	3	181±5	NA	790±524	2	51±4	NA	52±45	1	82	NA	20
	R4	3	167±49	NA	1039±763	2	89±10	NA	44±47	1	56	NA	63
	R5	3	124±128	NA	500±121	2	81±41	NA	48±53	1	62	NA	31
	R6	3	215±317	NA	151±90	2	93±52	NA	56±19	1	32	NA	41
	R7 - downstream	3	842±1350	NA	288±262	2	10±0	NA	30±28	1	20	NA	41
Canal	C-107	3	25±11	10±0	NA	2	12±11	27±25	NA	1	320	58	NA
	Sagamore	3	10933±6900	5313±4459	NA	0	NS	NS	NA	1	1840	2600	NA
	Hogpen Slough	3	2671±2912	1505±1352	NA	2	5650±1626	2420±0	NA	1	160	275	NA
	Veterans Memorial	3	4705±4492	253±125	NA	2	90	73±58	NA	1	280	76	NA
	Elkcam	3	3018±4748	205±312	NA	2	35±35	12±8	NA	1	50	15	NA
	Monterrey	3	149±39	44±6	NA	2	42±40	26±18	NA	1	214	77	NA
	C-24	3	99±32	31±21	NA	2	3±1	4±2	NA	1	36	2	NA
	E-8	3	40±20	33±40	NA	2	4±0	5±3	NA	1	280	32	NA
	Horseshoe A-18	3	47±53	10±0	NA	2	9±4	7±3	NA	1	90	22	NA
	Southbend/Horseshoe A-22	3	908±1552	22±21	NA	2	290±382	154±168	NA	1	30	4	NA

Appendix 6. Seasonal microbial source tracking molecular and chemical tracer concentrations (average ± standard error, except where only 1 detection occurred) observed in surface water samples collected in the North Fork of the St. Lucie Estuary, canals draining into the North Fork, and tributaries (Tenmile Creek and Fivemile Creek), showing the wet season (2016), dry season (2017), and the rain sampling events; NS = not sampled due to no flow, NA = not analyzed, an asterisk by the tracer for the dry season indicates only samples collected on 03/16/2017 were analyzed for this compound (n=1).

Wet Season 2016		Human Tracer	Bird Tracer	Artificial Sweetener	Phamacueticals			Herbicides						Pesticide
Site	**Count**	**HF183 (GEU/100mL)**	**Gull2 (TSC/100mL)**	**Sucralose (µg/L)**	**Acetaminophen (µg/L)**	**Carbamazepine (µg/L)**	**MCPP (µg/L)**	**2,4-D (µg/L)**	**Bentazon (µg/L)**	**Diuron (µg/L)**	**Fenuron (µg/L)**	**Flouridone (µg/L)**	**Triclopyr (µg/L)**	**Imidacloprid (µg/L)**
Tenmile 2 - upsteam	3	BDL	BDL	0.034±0.022	0.005	0.0006	NA	NA	NA	0.010	BDL	0.008±0.006	NA	0.091±0.104
Tenmile 1 - dowstream	3	BDL	BDL	BDL	0.006	BDL	NA	NA	NA	0.009±0.006	BDL	0.007±0.008	NA	0.132±0.129
Fivemile 2 - upstream	3	BDL	BDL	0.056±0.020	0.043	0.0011±0.0004	NA	NA	NA	0.007	BDL	0.010±0.008	NA	0.036±0.035
Fivemile 1 - downstream	3	BDL	BDL	0.061±0.060	0.010±0.008	0.0009	NA	NA	NA	0.008	BDL	0.001±0.000	NA	0.047±0.039
R1 - upsteam	3	BDL	BDL	0.049±0.011	BDL	0.0008±0.0004	NA	NA	NA	0.012	BDL	0.005±0.005	NA	0.079±0.075
R2	3	BDL	BDL	0.036±0.000	BDL	0.0004±0.0000	NA	NA	NA	0.012	BDL	0.005±0.004	NA	0.074±0.057
R3	3	BDL	BDL	0.086±0.009	BDL	0.0011±0.0004	NA	NA	NA	0.010±0.006	BDL	0.009±0.002	NA	0.132±0.119
R4	3	BDL	BDL	0.113±0.042	BDL	0.0010±0.0003	NA	NA	NA	0.009±0.002	BDL	0.008±0.002	NA	0.122±0.098
R5	3	BDL	BDL	0.094±0.024	BDL	0.0010±0.0004	NA	NA	NA	0.039±0.026	BDL	0.013±0.009	NA	0.129±0.071
R6	3	BDL	BDL	0.100±0.010	BDL	0.0011±0.0004	NA	NA	NA	0.124±0.075	BDL	0.018±0.012	NA	0.122±0.072
R7 - downstream	3	BDL	BDL	0.103±0.061	BDL	0.0010±0.0002	NA	NA	NA	0.073±0.024	BDL	0.016±0.006	NA	0.116±0.058
C-107	3	BDL	BDL	0.033±0.022	0.006±0.002	BDL	NA	NA	NA	0.007±0.004	0.010±0.001	0.008±0.001	NA	0.028±0.015
Sagamore	3	1600	BDL	0.637±0.156	0.049±0.037	0.0035±0.0022	NA	NA	NA	0.063±0.033	0.017±0.006	0.001±0.000	NA	0.030±0.029
Hogpen Slough	3	BDL	BDL	0.105±0.035	0.030	0.0034±0.0013	NA	NA	NA	0.007±0.002	BDL	0.015±0.002	NA	0.008±0.005
VeteraNA Memorial	3	BDL	BDL	0.019	0.020	BDL	NA	NA	NA	0.457±0.413	0.031±0.015	0.021±0.009	NA	0.015±0.015
Elkcam	3	BDL	BDL	0.291±0.260	0.030±0.031	0.0051±0.0020	NA	NA	NA	9.190±12.163	0.054±0.023	0.051±0.013	NA	0.044±0.029
Monterrey	3	BDL	BDL	0.587±0.104	BDL	0.0103±0.0012	NA	NA	NA	0.113±0.152	BDL	0.113±0.180	NA	0.037±0.013
C-24	3	BDL	BDL	BDL	BDL	BDL	NA	NA	NA	0.004	BDL	0.009±0.008	NA	0.135±0.128
E-8	3	BDL	BDL	0.293±0.006	BDL	0.0030±0.0006	NA	NA	NA	5.371±9.205	0.015±0.005	0.012±0.004	NA	0.038±0.016
Horseshoe A-18	3	BDL	BDL	0.300±0.062	BDL	0.0059±0.0008	NA	NA	NA	0.874±0.744	BDL	0.027±0.018	NA	0.024±0.009
Southbend/Horseshoe A-22	3	BDL	BDL	0.170±0.053	0.025	0.0054±0.0013	NA	NA	NA	0.940±1.178	0.038±0.008	0.120±0.056	NA	0.030±0.008
Dry Season 2017														
Site	**Count**	**HF183 (GEU/100mL)**	**Gull2 (TSC/100mL)**	**Sucralose (µg/L)**	**Acetaminophen (µg/L)**	**Carbamazepine (µg/L)**	**MCPP* (µg/L)**	**2,4-D (µg/L)**	**Bentazon* (µg/L)**	**Diuron (µg/L)**	**Fenuron (µg/L)**	**Flouridone (µg/L)**	**Triclopyr* (µg/L)**	**Imidacloprid (µg/L)**
Tenmile 2 - upsteam	2	110	BDL	0.024±0.003	0.029	BDL	0.004	1.600	0.018	BDL	BDL	0.001	0.010	0.016
Tenmile 1 - dowstream	2	BDL	BDL	0.017	BDL	BDL	0.004	0.590	0.017	BDL	BDL	0.001	6.800	0.021
Fivemile 2 - upstream	2	BDL	BDL	0.061	BDL	BDL	0.004	0.054	0.033	BDL	BDL	0.001±0.001	0.033	0.020±0.002
Fivemile 1 - downstream	2	BDL	BDL	0.077±0.062	BDL	0.0021	0.004	0.058	0.037	BDL	BDL	0.00066	0.050	0.014±0.012
R1 - upsteam	2	BDL	52000	0.043±0.002	BDL	BDL	0.004	0.480	0.023	BDL	BDL	0.001	0.100	0.020±0.014
R2	2	BDL	BDL	0.060±0.008	0.026	BDL	0.004	0.220	0.027	BDL	BDL	0.001	0.660	0.007±0.004
R3	2	BDL	BDL	0.145±0.021	BDL	BDL	0.004	0.110	0.044	0.032±0.005	BDL	0.001±0.001	5.500	0.008
R4	2	BDL	BDL	0.130±0.014	0.010	0.0005	0.004	0.076	0.039	0.039±0.000	BDL	0.002±0.001	4.100	0.006±0.000
R5	2	BDL	BDL	0.112±0.026	0.020	0.0005	0.004	0.038	0.041	0.068±0.024	BDL	0.003±0.000	0.780	BDL
R6	2	BDL	BDL	0.126±0.049	BDL	BDL	0.004	0.021	0.041	0.067±0.028	BDL	0.003±0.000	0.300	BDL
R7 - downstream	2	BDL	BDL	0.720±0.820	0.020	BDL	0.004	0.010	0.044	0.038±0.004	BDL	0.001±0.001	0.120	BDL
C-107	2	BDL	BDL	0.130±0.014	BDL	BDL	0.004	0.052	0.057	0.034±0.015	0.018	0.010±0.006	0.010	0.023
Sagamore	0	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Hogpen Slough	2	BDL	BDL	0.295±0.035	BDL	0.0013±0.0001	0.004	0.004	0.083	0.022±0.008	BDL	0.012±0.000	0.010	0.017
VeteraNA Memorial	2	BDL	BDL	BDL	BDL	BDL	0.004	0.037	0.036	0.029±0.017	0.028±0.013	0.014±0.011	0.010	0.025±0.011
Elkcam	2	BDL	BDL	0.670±0.000	BDL	0.0018±0.0006	0.006	0.550	0.110	1.845±2.341	0.033±0.009	2.002±2.825	0.010	0.031±0.032
Monterrey	2	BDL	BDL	1.250±0.354	BDL	0.0088±0.0013	0.007	0.053	0.088	1.750±0.212	0.022	0.021±0.003	0.010	0.005
C-24	2	BDL	BDL	0.041±0.023	BDL	BDL	0.004	0.012	0.023	BDL	BDL	0.011±0.003	0.010	0.006
E-8	2	BDL	BDL	0.695±0.148	BDL	0.0027±0.0006	0.004	0.180	0.058	0.059±0.035	0.022±0.001	0.003±0.000	0.010	0.014
Horseshoe A-18	2	BDL	BDL	0.700±0.156	BDL	0.0024±0.0001	0.009	0.066	0.100	2.600±1.131	0.021	0.007±0.003	0.010	0.023
Southbend/Horseshoe A-22	2	BDL	BDL	0.760±0.085	BDL	0.0026±0.0004	0.007	0.096	0.092	2.940±4.045	0.025	0.010±0.006	0.010	BDL
Rain Event 2017														
Site	**Count**	**HF183 (GEU/100mL)**	**Gull2 (TSC/100mL)**	**Sucralose (µg/L)**	**Acetaminophen (µg/L)**	**Carbamazepine (µg/L)**	**MCPP (µg/L)**	**2,4-D (µg/L)**	**Bentazon (µg/L)**	**Diuron (µg/L)**	**Fenuron (µg/L)**	**Flouridone (µg/L)**	**Triclopyr (µg/L)**	**Imidacloprid (µg/L)**
Tenmile 2 - upsteam	1	BDL	BDL	0.036	BDL	BDL	0.002	0.110	0.001	0.051	BDL	0.000	0.004	0.210
Tenmile 1 - dowstream	1	BDL	BDL	0.020	BDL	BD:	0.002	0.180	0.001	0.042	0.009	0.001	0.004	0.200
Fivemile 2 - upstream	1	BDL	BDL	0.060	0.018	0.0001	0.002	0.079	0.001	0.036	BDL	0.001	0.004	0.140
Fivemile 1 - downstream	1	BDL	BDL	0.047	0.014	0.0011	0.002	0.100	0.001	0.013	BDL	BDL	0.004	0.4
R1 - upsteam	1	400	BDL	0.050	0.011	BDL	0.002	0.090	0.002	0.060	BLD	BDL	0.004	0.190
R2	1	BDL	BDL	0.046	BDL	BDL	0.002	0.100	0.003	0.110	BDL	0.001	0.004	0.260
R3	1	BDL	BDL	0.096	BDL	0.0007	0.002	0.130	0.004	0.100	BDL	0.001	0.004	0.220
R4	1	BDL	BDL	0.160	BDL	0.0007	0.002	0.120	0.004	0.100	BDL	0.002	0.004	0.190
R5	1	BDL	BDL	0.180	BDL	0.0009	0.002	0.150	0.004	0.160	BDL	0.031	0.004	0.093
R6	1	BDL	BDL	0.240	BDL	0.0012	0.004	0.180	0.005	0.190	0.012	0.021	0.004	0.110
R7 - downstream	1	BDL	BDL	0.210	BDL	0.0010	0.003	0.180	0.005	0.180	0.010	0.025	0.004	0.094
C-107	1	BDL	BDL	0.064	0.021	BDL	0.013	0.180	0.004	0.036	0.037	0.002	0.004	0.120
Sagamore	1	BDL	BDL	1.800	0.015	0.0022	0.002	0.095	0.028	0.082	0.030	BDL	0.004	0.027
Hogpen Slough	1	BDL	BDL	1.100	0.019	0.0054	0.002	0.300	0.013	0.180	BDL	0.005	0.004	0.008
VeteraNA Memorial	1	BDL	BDL	0.140	0.016	BDL	0.003	25.000	0.007	2.100	0.069	0.006	0.037	0.026
Elkcam	1	BDL	BDL	0.780	BDL	0.0038	0.004	0.043	0.030	0.071	0.065	0.004	0.004	0.047
Monterrey	1	BDL	BDL	0.830	BDL	0.01	0.009	0.042	0.014	0.093	0.035	0.023	0.004	0.055
C-24	1	BDL	BDL	BDL	BDL	BDL	0.002	0.110	0.001	0.009	BDL	0.001	0.004	0.160
E-8	1	BDL	BDL	0.500	BDL	0.0036	0.038	0.220	0.009	1.800	0.047	0.006	0.004	0.170
Horseshoe A-18	1	BDL	BDL	0.430	BDL	0.0044	0.026	0.230	0.009	0.430	0.050	0.007	0.004	0.110
Southbend/Horseshoe A-22	1	BDL	BDL	0.540	BDL	0.0043	0.021	0.230	0.008	0.420	0.024	0.006	0.004	0.095

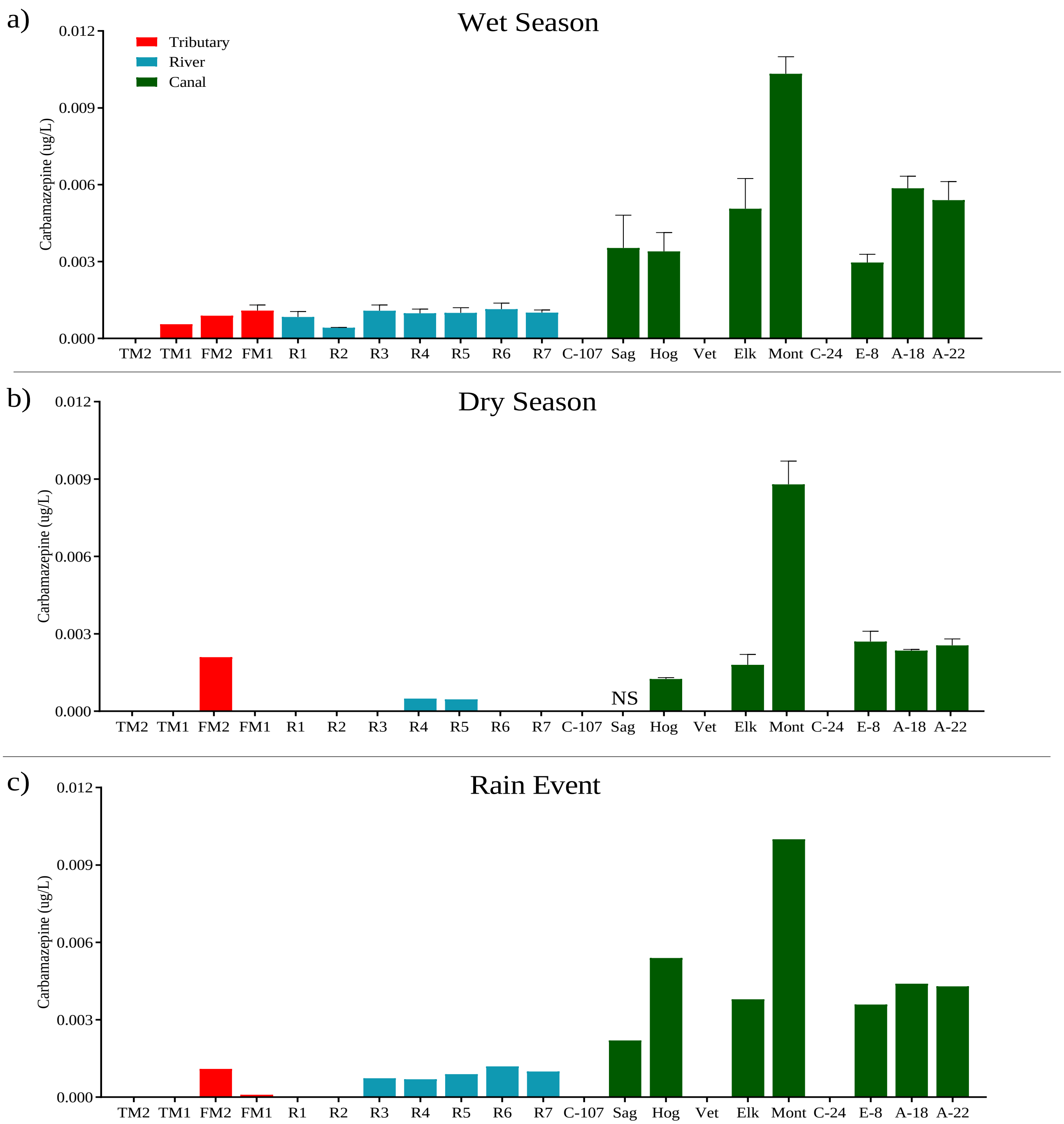
Appendix 7. Seasonal dissolved nutrient concentrations (average ± standard error) observed in surface water samples collected in North Fork of the St. Lucie Estuary, canals draining into the North Fork, and tributaries (Tenmile Creek and Fivemile Creek), showing the dry season (2017), the rain sampling (2017), and the wet season (2017) sampling events; NS = not sampled due to no flow.

Type	Site	Dry Season 2017					
		Count	Ammonium (mg/L)	Nitrate +Nitrite (mg/L)	Soluble Reactive Phosphorus (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	DIN:SRP
Tributary	Tenmile 2 - upsteam	3	0.09±<0.01	0.07±<0.01	0.05±<0.01	0.17±<0.01	6.82±0.12
	Tenmile 1 - downstream	3	0.05±0.01	0.10±0.01	0.08±<0.01	0.15±<0.01	3.97±0.09
	Fivemile 2 - upstream	3	0.01±<0.01	0.04±0.01	0.05±<0.01	0.05±0.01	2.29±0.28
	Fivemile 1 - downstream	3	0.05±0.01	0.04±0.01	0.03±<0.01	0.09±0.01	6.80±1.03
River	R1 - upsteam	3	0.01±<0.01	0.05±<0.01	0.05±<0.01	0.06±<0.01	2.63±0.04
	R2	3	0.01±<0.01	<0.01±<0.01	0.06±<0.01	0.01±<0.01	0.37±0.08
	R3	3	<0.01±<0.01	0.01±<0.01	0.06±<0.01	0.01±<0.01	0.31±0.06
	R4	3	<0.01±<0.01	0.02±0.01	0.07±<0.01	0.02±0.01	0.74±0.20
	R5	1	<0.01±<0.01	<0.01±<0.01	0.10±<0.01	0.01±<0.01	0.20±0.01
	R6	3	<0.01±<0.01	<0.01±<0.01	0.10±<0.01	0.01±<0.01	0.16±0.03
	R7 - downstream	3	<0.01±<0.01	<0.01±<0.01	0.09±<0.01	0.01±<0.01	0.13±0.01
Canal	C-107	3	0.03±0.02	0.01±<0.01	<0.01±<0.01	0.04±0.02	128.64±77.36
	Sagamore	0	<0.01±<0.01	<0.01±<0.01	<0.01±<0.01	<0.01±<0.01	<0.01±<0.01
	Hogpen Slough	3	0.02±<0.01	0.12±<0.01	0.01±<0.01	0.15±<0.01	24.35±0.36
	Veterans Memorial	3	0.02±0.01	0.08±0.05	<0.01±<0.01	0.10±0.05	267.52±205.44
	Elkcam	3	0.02±0.01	0.01±<0.01	<0.01±<0.01	0.03±0.01	60.34±20.74
	Monterrey	3	0.01±<0.01	0.06±0.01	<0.01±<0.01	0.07±0.01	37.05±1.68
	C-24	3	<0.01±<0.01	0.01±0.01	0.02±<0.01	0.02±0.01	2.07±0.72
	E-8	3	<0.01±<0.01	<0.01±<0.01	<0.01±<0.01	0.01±<0.01	15.68±2.07
	Horseshoe A-18	3	<0.01±<0.01	0.01±<0.01	<0.01±<0.01	0.01±<0.01	13.04±2.48
	Southbend/Horseshoe A-22	3	0.08±0.01	0.05±<0.01	0.01±<0.01	0.14±0.01	52.31±4.62

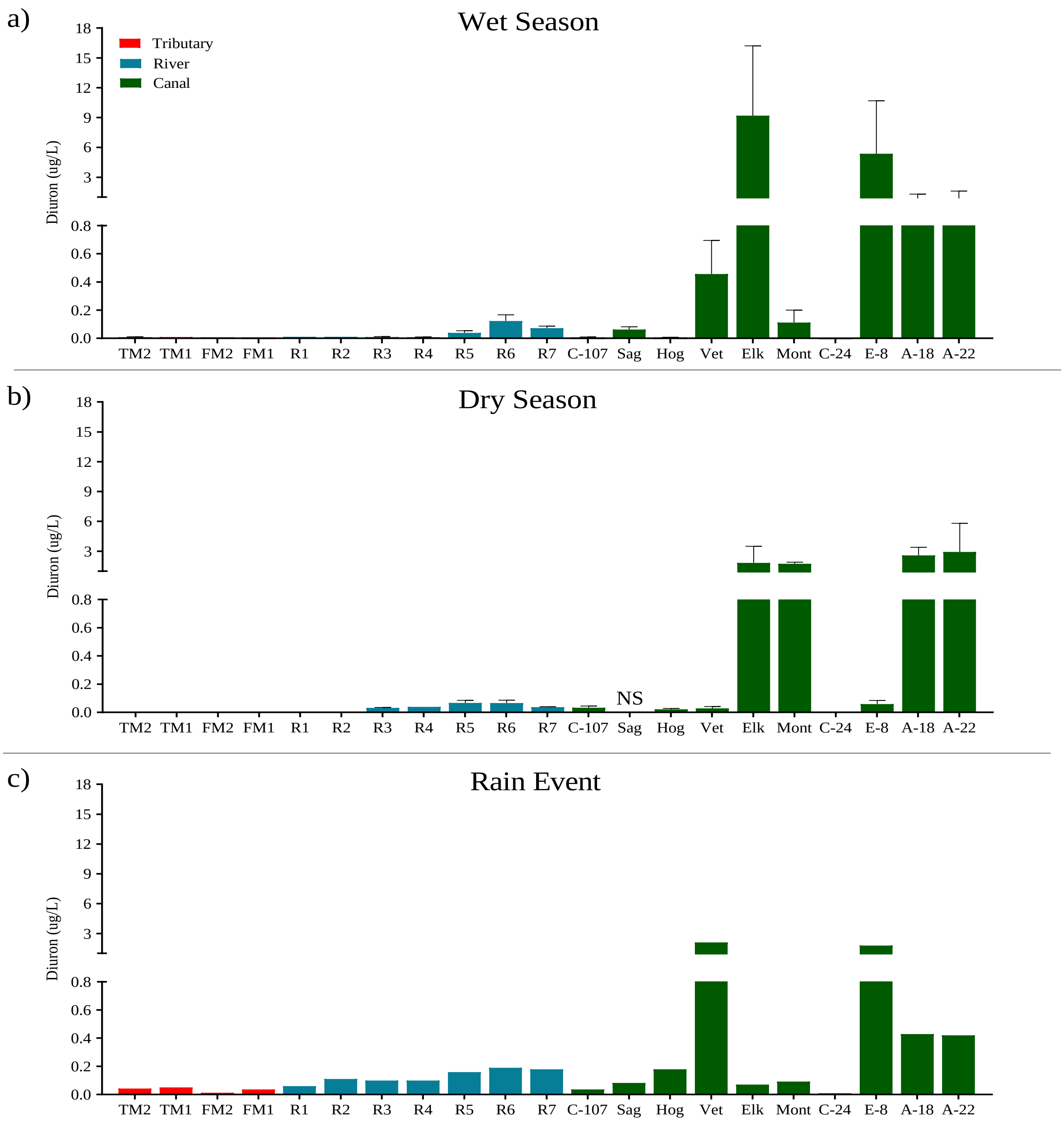
Type	Site	Rain Event 2017					
		Count	Ammonium (mg/L)	Nitrate +Nitrite (mg/L)	Soluble Reactive Phosphorus (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	DIN:SRP
Tributary	Tenmile 2 - upsteam	3	0.16±0.01	0.19±0.01	0.12±<0.01	0.35±0.01	6.21±0.20
	Tenmile 1 - downstream	3	0.09±<0.01	0.22±0.01	0.12±<0.01	0.31±0.01	5.92±0.18
	Fivemile 2 - upstream	3	0.08±<0.01	0.05±<0.01	0.08±0.02	0.13±<0.01	3.90±0.73
	Fivemile 1 - downstream	3	0.04±<0.01	0.05±<0.01	0.09±0.01	0.09±<0.01	2.41±0.17
River	R1 - upsteam	3	0.06±<0.01	0.11±0.01	0.17±0.03	0.17±0.01	2.43±0.31
	R2	3	0.08±<0.01	0.17±0.01	0.16±0.02	0.25±0.01	3.46±0.47
	R3	3	0.05±<0.01	0.16±<0.01	0.13±0.01	0.20±<0.01	3.50±0.21
	R4	3	0.03±<0.01	0.15±<0.01	0.13±0.02	0.19±<0.01	3.12±0.34
	R5	3	0.04±<0.01	0.14±<0.01	0.06±<0.01	0.19±<0.01	6.86±0.15
	R6	3	0.04±<0.01	0.17±0.01	0.06±0.01	0.21±0.01	7.62±1.20
	R7 - downstream	3	0.04±<0.01	0.19±<0.01	0.03±<0.01	0.23±<0.01	15.24±0.76
Canal	C-107	3	0.05±<0.01	<0.01±<0.01	0.02±0.01	0.05±<0.01	9.67±3.53
	Sagamore	3	0.03±0.01	0.01±<0.01	0.10±0.05	0.03±<0.01	1.03±0.42
	Hogpen Slough	3	0.01±<0.01	0.02±<0.01	0.07±0.06	0.03±<0.01	5.23±2.48
	Veterans Memorial	3	0.01±<0.01	<0.01±<0.01	0.03±0.02	0.01±<0.01	4.38±2.33
	Elkcam	3	0.01±<0.01	<0.01±<0.01	0.01±0.01	0.01±<0.01	4.31±1.89
	Monterrey	3	0.04±<0.01	0.01±<0.01	0.08±<0.01	0.04±<0.01	1.25±0.09
	C-24	3	0.05±<0.01	0.11±<0.01	0.14±0.08	0.16±0.01	4.15±1.52
	E-8	3	0.07±<0.01	<0.01±<0.01	0.11±0.11	0.07±<0.01	13.57±6.70
	Horseshoe A-18	3	0.01±<0.01	0.02±<0.01	<0.01±<0.01	0.02±<0.01	14.80±2.38
	Southbend/Horseshoe A-22	3	0.09±0.01	0.03±0.01	0.04±0.01	0.12±0.01	8.14±1.89

Type	Site	Wet Season 2017					
		Count	Ammonium (mg/L)	Nitrate +Nitrite (mg/L)	Soluble Reactive Phosphorus (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	DIN:SRP
Tributary	Tenmile 2 - upsteam	3	0.23±0.01	0.12±<0.01	0.27±0.02	0.35±0.01	2.87±0.20
	Tenmile 1 - downstream	3	0.28±0.01	0.17±<0.01	0.21±<0.01	0.45±<0.01	4.61±0.04
	Fivemile 2 - upstream	3	0.26±0.01	0.08±<0.01	0.38±0.01	0.34±0.01	1.99±0.07
	Fivemile 1 - downstream	3	0.23±0.01	0.14±<0.01	0.31±<0.01	0.37±<0.01	2.61±0.01
River	R1 - upsteam	3	0.30±0.01	0.15±<0.01	0.25±<0.01	0.44±<0.01	3.88±0.02
	R2	3	0.20±0.01	0.12±<0.01	0.31±0.01	0.32±0.01	2.28±0.03
	R3	3	0.18±0.01	0.13±<0.01	0.31±<0.01	0.31±0.01	2.23±0.03
	R4	3	0.13±0.01	0.12±<0.01	0.31±0.01	0.25±0.01	1.80±0.01
	R5	3	0.09±<0.01	0.11±<0.01	0.31±0.01	0.21±<0.01	1.45±0.04
	R6	3	0.04±0.01	0.18±0.01	0.06±<0.01	0.22±0.01	7.93±0.44
	R7 - downstream	3	0.08±<0.01	0.10±<0.01	0.13±0.01	0.18±0.01	3.04±0.16
Canal	C-107	3	0.14±<0.01	0.11±0.01	0.02±<0.01	0.24±0.01	22.61±2.44
	Sagamore	3	0.13±0.01	0.24±0.01	0.05±0.01	0.37±0.01	15.49±1.28
	Hogpen Slough	3	0.05±<0.01	0.05±0.01	0.03±<0.01	0.10±0.02	6.35±0.98
	Veterans Memorial	3	0.06±0.01	0.03±<0.01	0.01±<0.01	0.09±<0.01	36.28±0.48
	Elkcam	3	0.15±<0.01	0.15±<0.01	0.02±<0.01	0.30±<0.01	35.18±3.61
	Monterrey	3	0.23±0.01	0.18±<0.01	0.03±<0.01	0.41±<0.01	36.52±1.47
	C-24	3	0.12±<0.01	0.04±<0.01	0.30±<0.01	0.16±<0.01	1.17±0.01
	E-8	3	0.25±0.01	0.17±<0.01	0.01±<0.01	0.43±0.01	79.36±6.55
	Horseshoe A-18	3	0.11±<0.01	0.17±0.04	0.02±<0.01	0.27±0.04	27.16±2.41
	Southbend/Horseshoe A-22	3	0.14±0.01	0.18±<0.01	0.04±<0.01	0.32±<0.01	20.24±0.50

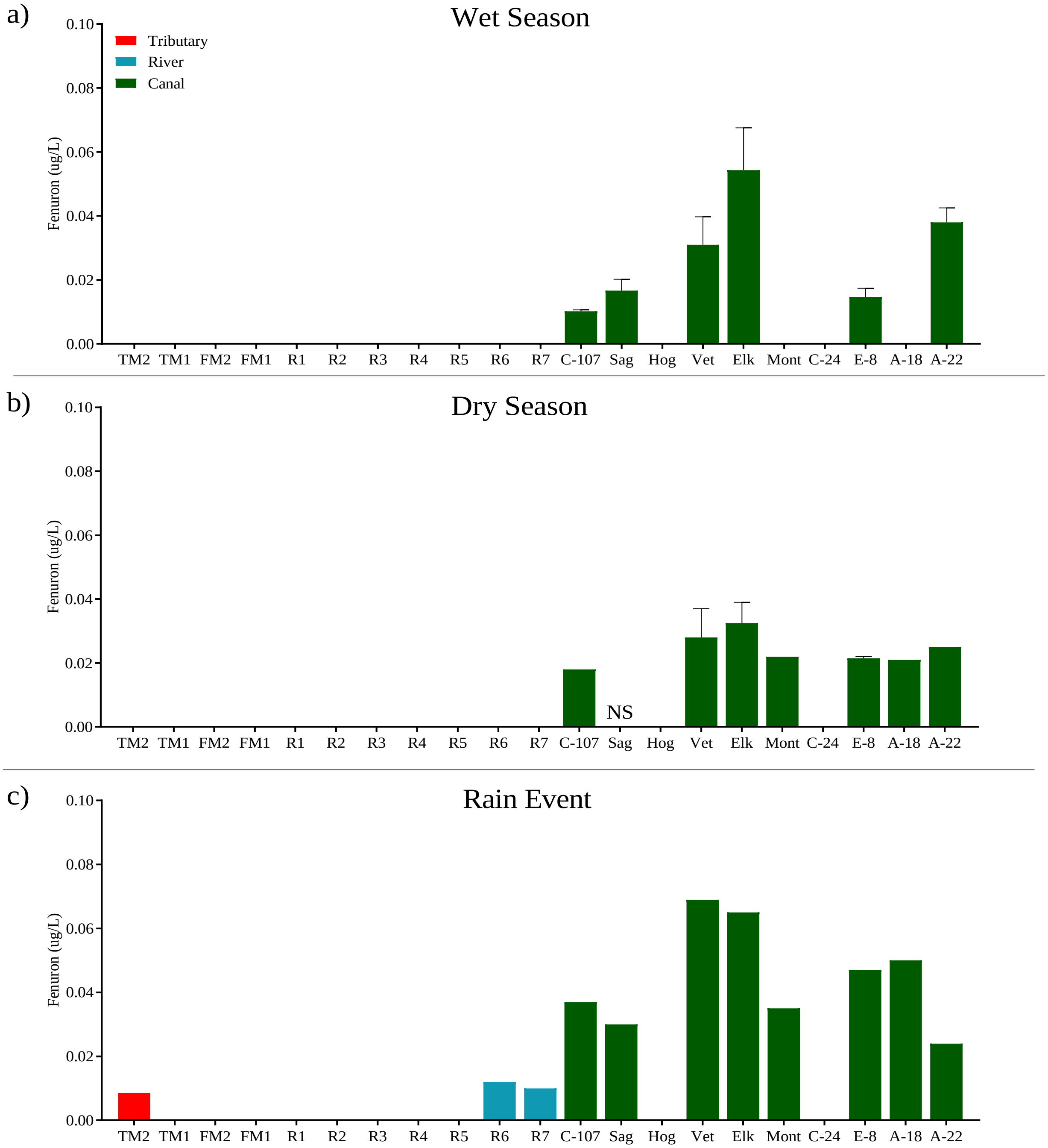
Appendix 8. Carbamazepine (pharmaceutical) concentrations (average ± standard error, except where only 1 detection occurred) collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore, Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.



Appendix 9. Diuron (herbicide for roadside vegetation and agricultural crops) concentrations (average ± standard error, except where only 1 detection occurred) observed in surface water collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore, Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C 24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

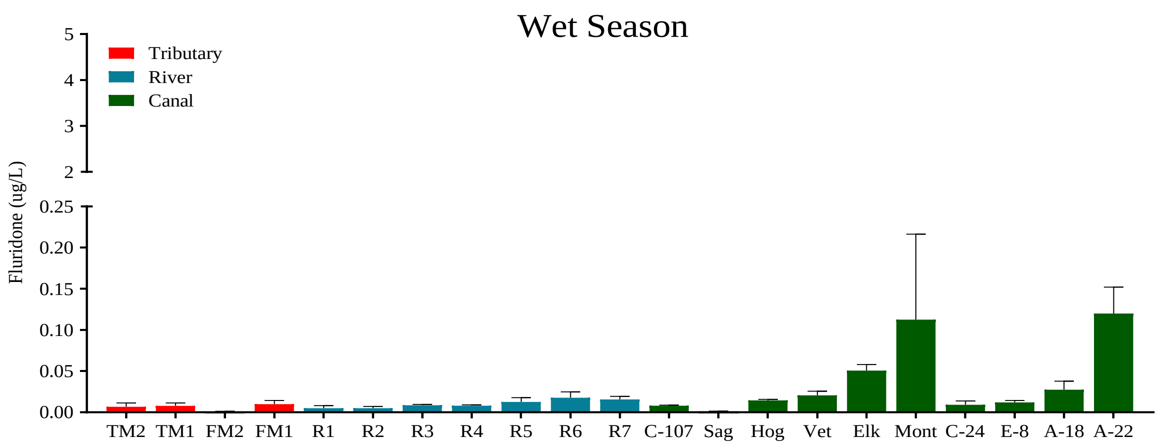


Appendix 10. Fenuron (herbicide for weeds and brush on non-crop land) concentrations (average ± standard error, except where only 1 detection occurred) observed in surface water collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore, Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

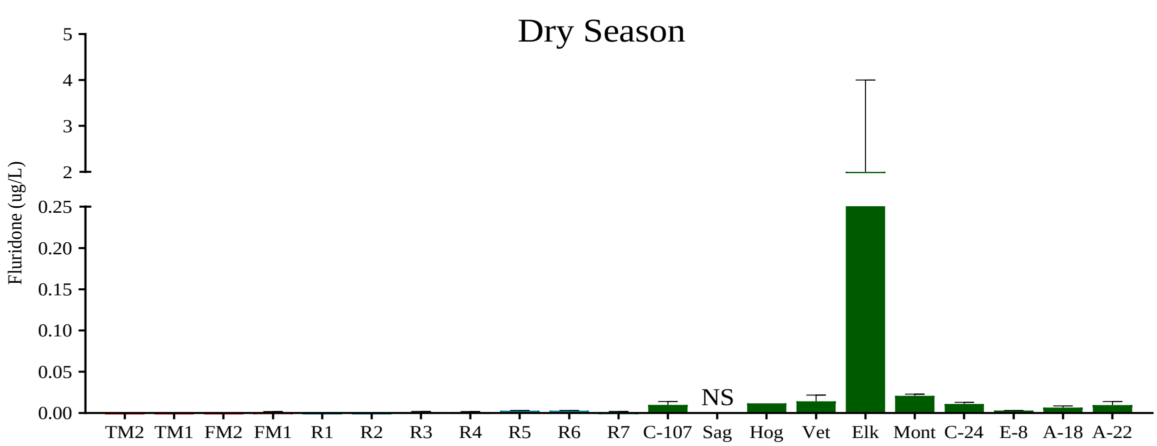


Appendix 11. Fluridone (herbicide used to control aquatic weeds) concentrations (average ± standard error, except where only 1 detection occurred) observed in surface water collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore, Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

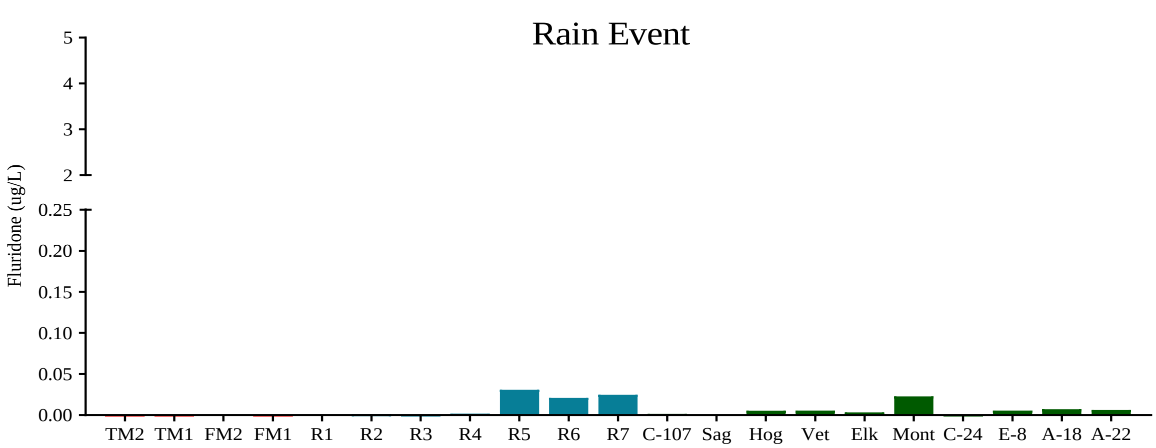
a)



b)



c)

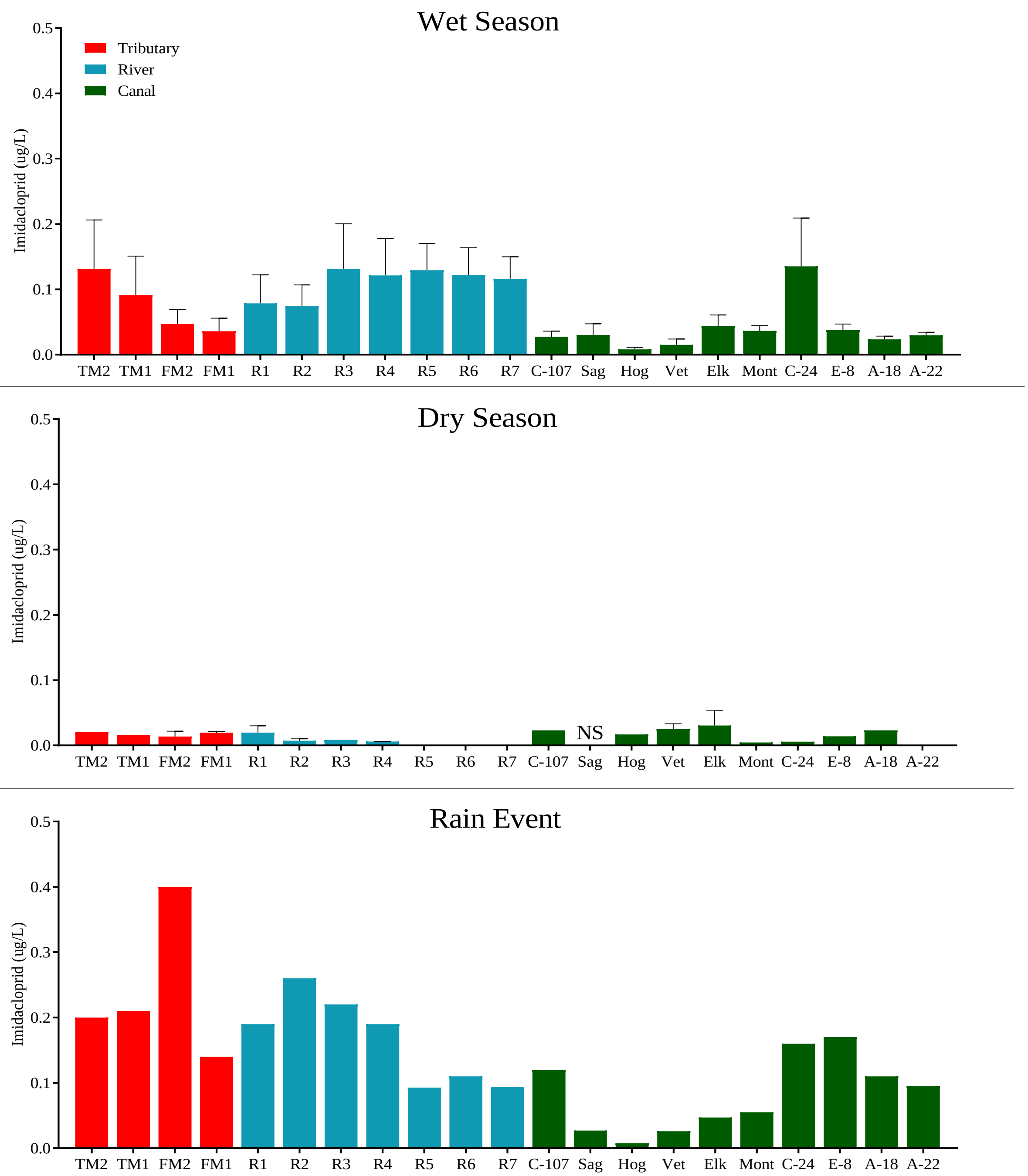


Appendix 12. Imidacloprid (insecticide widely used in agriculture and for termite control) concentrations (average ± standard error, except where only 1 detection occurred) observed in surface water collected during the a) 2016 wet season, b) 2017 dry season, and a c) rain event on July 19, 2017 from tributaries draining into the North Fork of the St. Lucie Estuary (Tributary), including Tenmile Creek (TM2 upstream and TM1 downstream) and Fivemile Creek (FM2 upstream and FM1 downstream); river sites within the North Fork (R1 upstream – R7 downstream); and canals draining into the North Fork at sites, C-107, Sagamore, Hogpen Slough (Hog), Veterans Memorial (Vet), Elkcam (Elk), Monterrey (Mont), C-24, E-8, Horseshoe A-18 (A-18), and Southbend/Horseshoe A-22 (A-22), NS = not sampled due to no flow.

a)

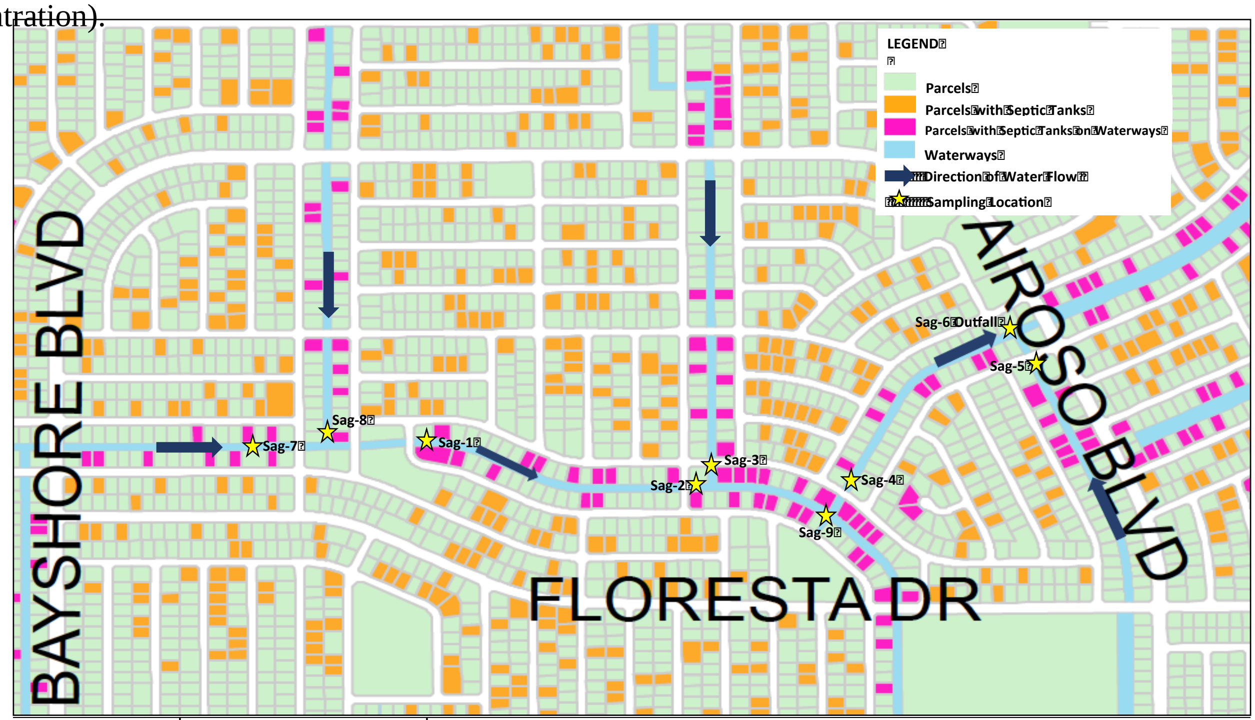
b)

c)



Appendix 13. Sagamore (Sag) study site area targeted, fine-scale sampling for *E. coli* concentrations of surface water showing a) sampling locations, septic systems, waterways, and flow direction of water within the system, as well as b) bacterial concentrations observed and rainfall prior to sample collection; cells highlighted in light red reflect surface water criteria exceedances, NS = not sampled due to low flow, J = estimated value (exceeded laboratory quality control limits due to bacterial high concentration).

a)

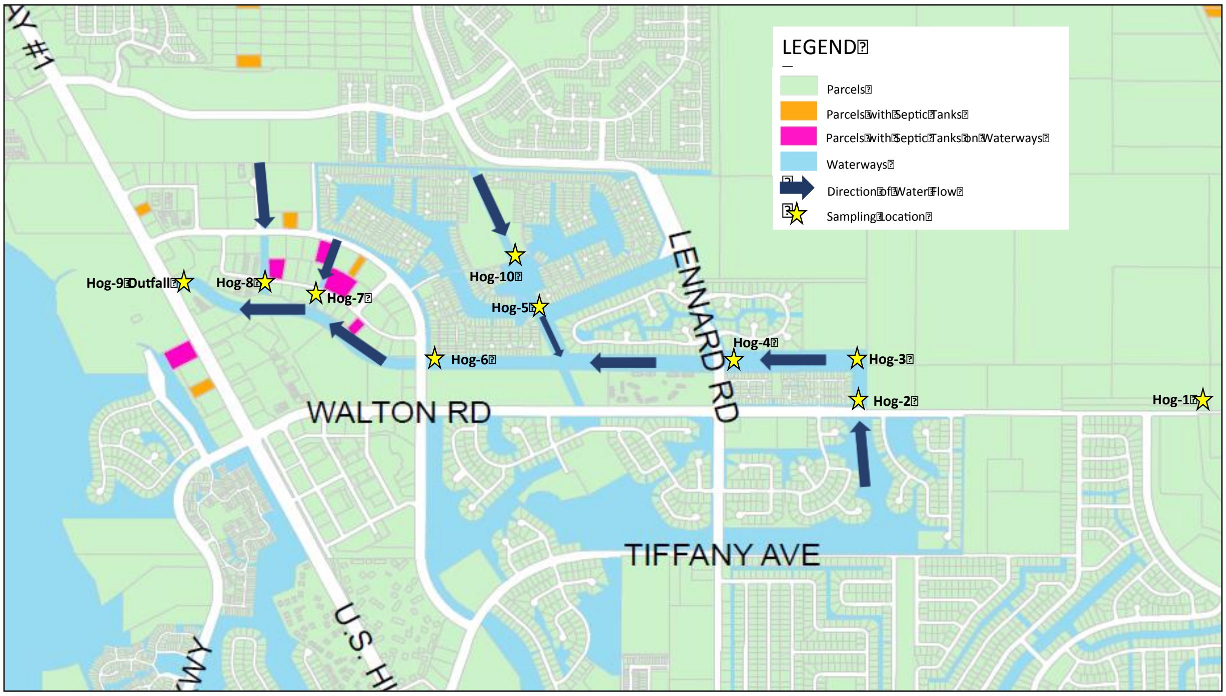


b)

Date	Site	Flow	*Escherichia coli* (MPN)	Rainfall, 7 days prior to sampling
6/7/17	Sag-1	Yes	2420	4.84"
	Sag-2	Yes	687	
	Sag-3	Yes	613	
	Sag-4	Yes	1120	
	Sag-5	No	NS	
	Sag-6 Outfall	Yes	1120	
6/19/17	Sag-1	Yes	1120	2.02"
	Sag-2	Yes	921	
	Sag-3	Yes	68	
	Sag-4	Yes	1200	
	Sag-5	Yes	2420J	
	Sag-6 Outfall	Yes	2420 J	
7/13/17	Sag-1	Yes	2420	3.00"
	Sag-2	Yes	2420	
	Sag-3	Yes	1050	
	Sag-4	Yes	2420 J	
	Sag-5	Yes	2420	
	Sag-6 Outfall	Yes	1300	
	Sag-7	Yes	1730	
	Sag-8	Yes	2420 J	
	Sag-9	Yes	1990	
9/19/17	Sag-1	Yes	98	0.43" (~12" Hurricane Irma 9/10/2017)
	Sag-2	Yes	243	
	Sag-3	Yes	10	
	Sag-4	Yes	650	
	Sag-5	Yes	9210	
	Sag-6 Outfall	Yes	399	
	Sag-7	Yes	158	
	Sag-8	Yes	243	
	Sag-9	Yes	155	

Appendix 14. Hogpen Slough (Hog) study site area targeted, fine-scale sampling for *E. coli* concentrations of surface water showing a) sampling locations, septic systems, waterways, and flow direction of water within the system, as well as b) bacterial concentrations observed and rainfall prior to sample collection; cells highlighted in light red reflect surface water criteria exceedances, NS = not sampled due to low flow, ND = no data available, J = estimated value (exceeded laboratory quality control limits due to bacterial high concentration).

a)

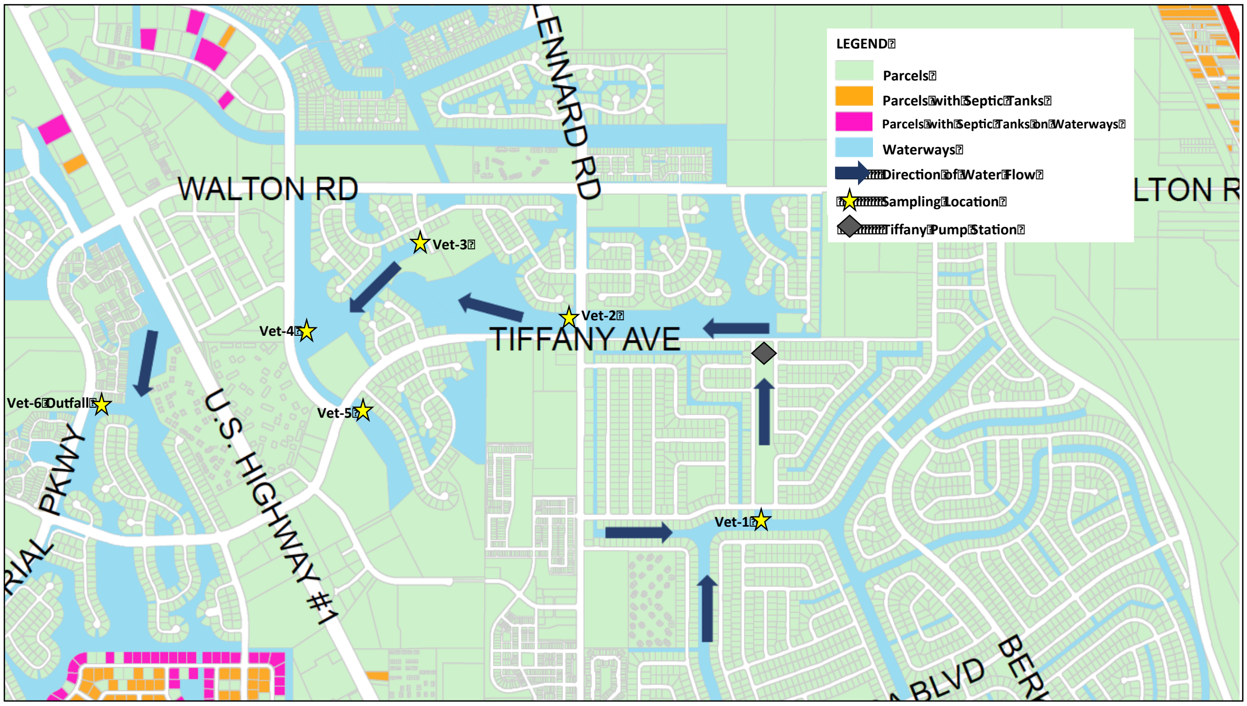


b)

Date	Site	Flow	*Escherichia coli* (MPN)	Rainfall, 7 days prior to sampling
5/4/17	Hog-9 Outfall	Yes	91	ND
	Hog-1	ND	291	
	Hog-2	No	NS	
	Hog-3	No	NS	
	Hog-4	No	NS	
6/5/17	Hog-5	Yes	613	2.25"
	Hog-6	Yes	23	
	Hog-7	No	NS	
	Hog-8	Yes	179	
	Hog-9 Outfall	Yes	345	
	Hog-1	ND	210	
	Hog-2	Yes	38	
	Hog-3	No	NS	
	Hog-4	No	NS	
6/19/17	Hog-5	Yes	727	2.02"
	Hog-6	Yes	35	
	Hog-7	Yes	96	
	Hog-8	Yes	96	
	Hog-9 Outfall	Yes	77	
	Hog-1	ND	308	
	Hog-2	No	NS	
	Hog-3	No	NS	
	Hog-4	No (From Pool)	28	
7/13/17	Hog-5	Yes	1550	3.00"
	Hog-6	Yes	111	
	Hog-7	Yes	178	
	Hog-8	Yes	579	
	Hog-9 Outfall	Yes	548	
	Hog-10	Yes	2420 J	
	Hog-1	ND	20	
	Hog-2	Backflowing	30	
	Hog-3	Backflowing	85	
	Hog-4	Yes	20	
9/19/17	Hog-5	Yes	10	0.43" (~12" Hurricane Irma 9/10/2017)
	Hog-6	Yes	10	
	Hog-7	No (From Pool)	332	
	Hog-8	Yes	144	
	Hog-9 Outfall	Yes	197 (Gates Open)	
	Hog-10	Yes	10	

Appendix 15. Veterans Memorial (Vet) study site area targeted, fine-scale sampling for *E. coli* concentrations of surface water showing a) sampling locations, septic systems, waterways, and flow direction of water within the system, as well as b) bacterial concentrations observed and rainfall prior to sample collection; cells highlighted in light red reflect surface water criteria exceedances.

a)



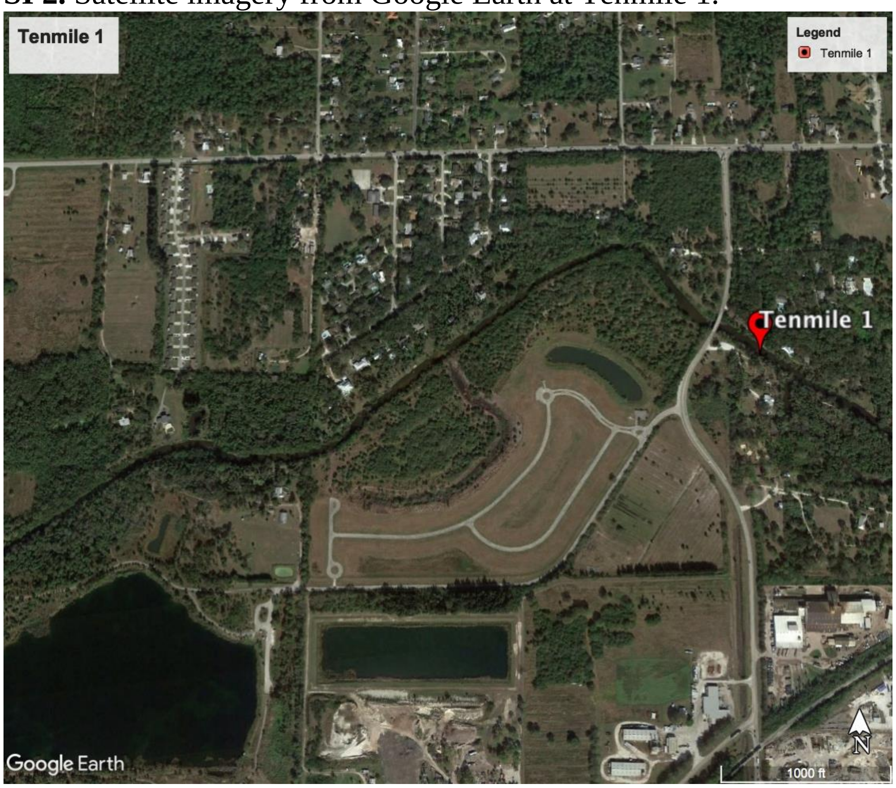
b)

Date	Site	Flow	*Escherichia coli* (MPN)	Rainfall, 7 days prior to sampling
6/5/17	Vet-1	Yes	20	2.25"
	Vet-2	Yes	75	
	Vet-3	Yes	272	
	Vet-4	Yes	179	
	Vet-5	Backflowing	1120	
	Vet-6 Outfall	Yes	461	
6/19/17	Vet-1	Yes	7	2.02"
	Vet-2	Yes	68	
	Vet-3	Yes	66	
	Vet-4	Yes	17	
	Vet-5	Yes	52	
	Vet-6 Outfall	Yes	111	
7/13/17	Vet-1	Yes	1990	3.00"
	Vet-2	Yes	1730	
	Vet-3	Yes	2420	
	Vet-4	Yes	118	
	Vet-5	Backflowing	157	
	Vet-6 Outfall	Yes	1050	
9/19/17	Vet-1	Yes	20	0.43" (~12" Hurricane Irma 9/10/2017)
	Vet-2	Yes	20	
	Vet-3	Yes	909	
	Vet-4	Yes	31	
	Vet-5	Yes	10	
	Vet-6 Outfall	Yes	185	

10. Supplemental Information. Satellite imagery of sites.

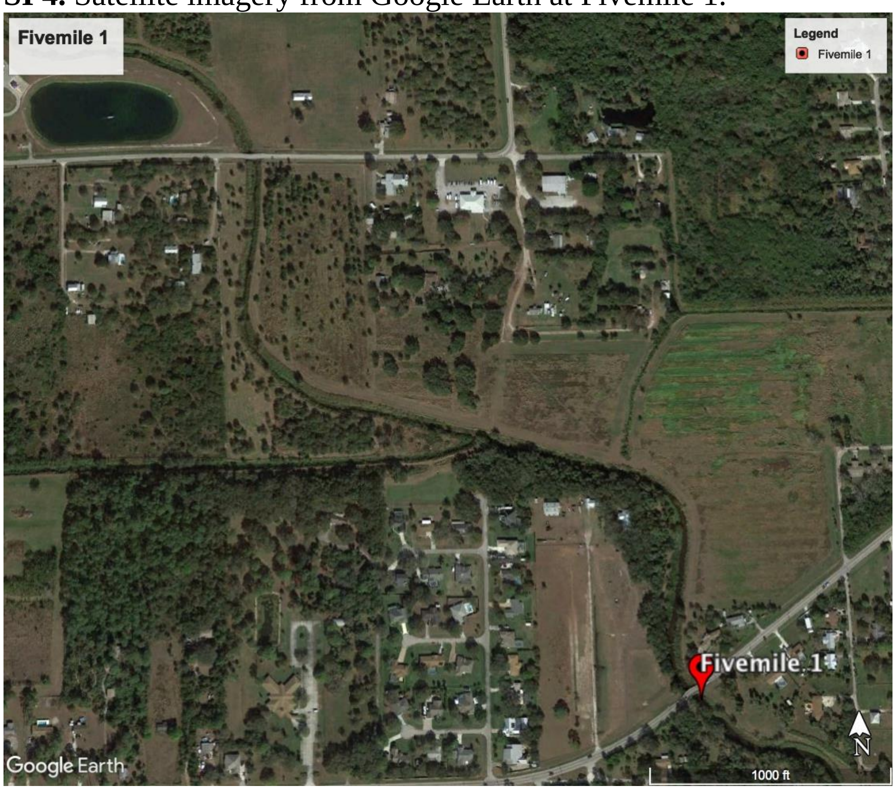
SI 1. Satellite imagery from Google Earth at Tenmile 2.

SI 2. Satellite imagery from Google Earth at Tenmile 1.

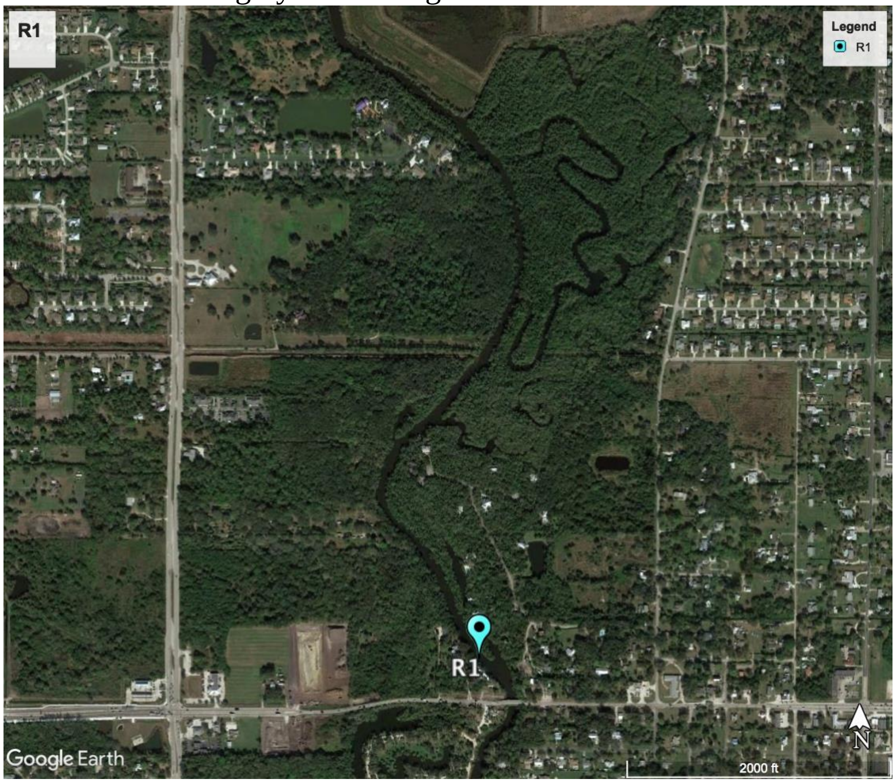


SI 3. Satellite imagery from Google Earth at Fivemile 2.

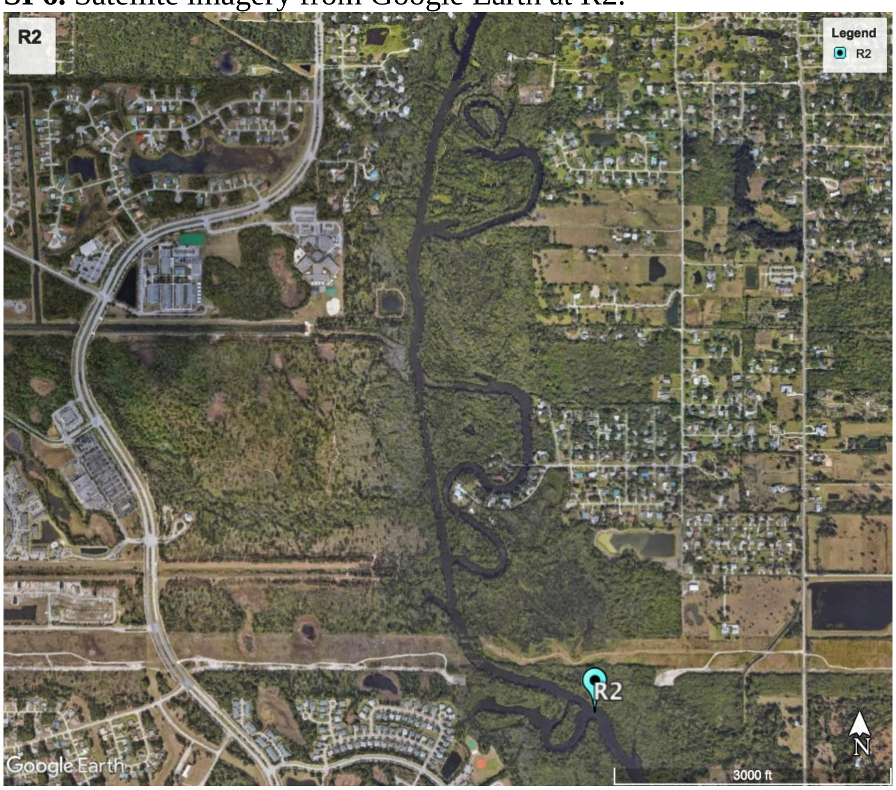
SI 4. Satellite imagery from Google Earth at Fivemile 1.



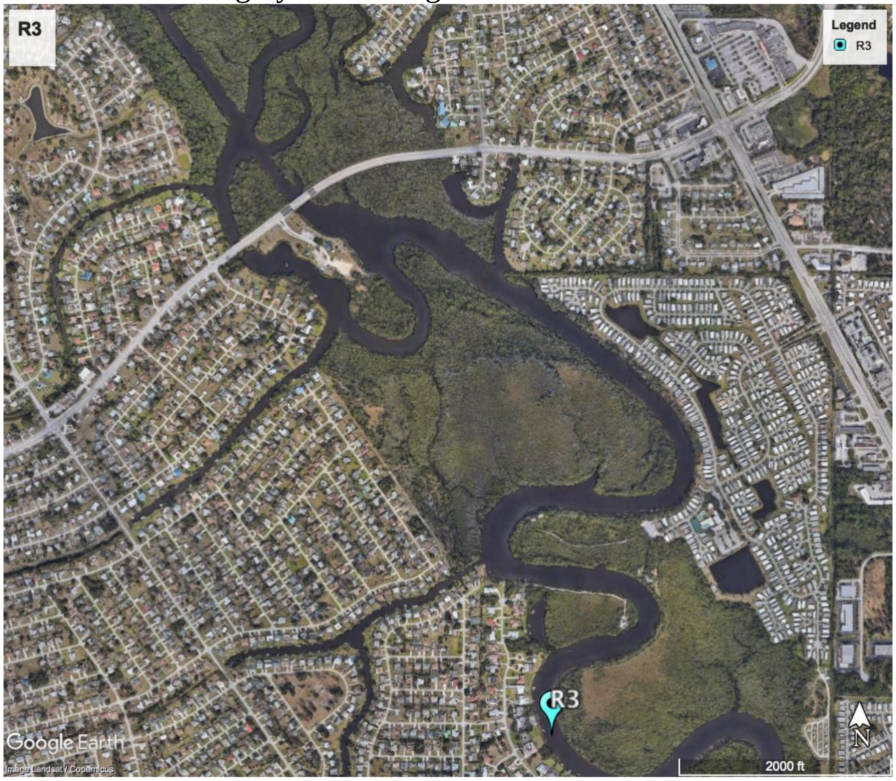
SI 5. Satellite imagery from Google Earth at R1.



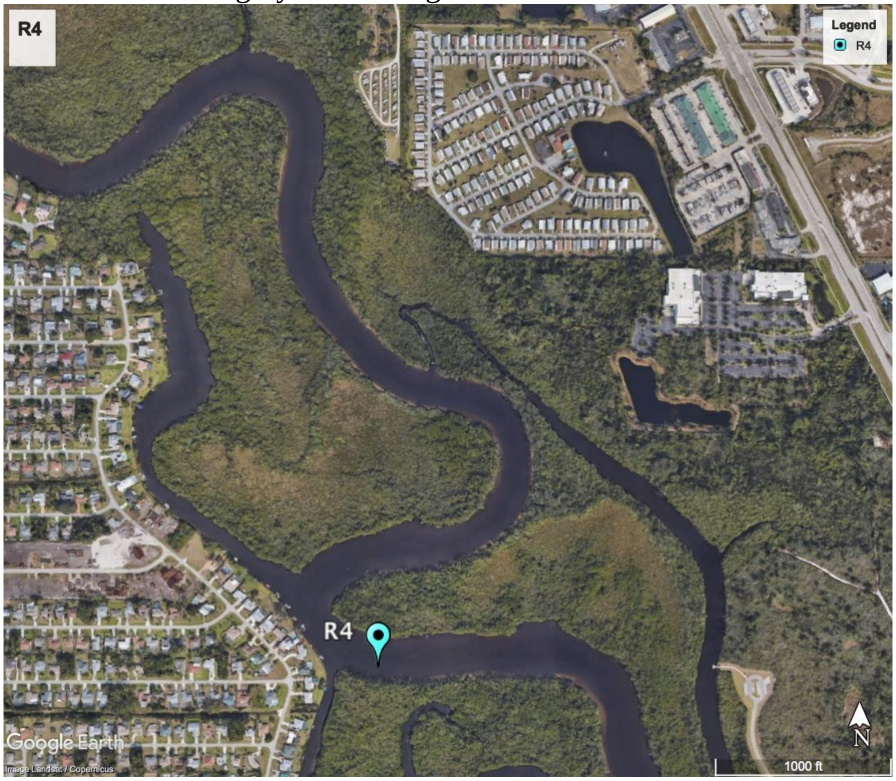
SI 6. Satellite imagery from Google Earth at R2.



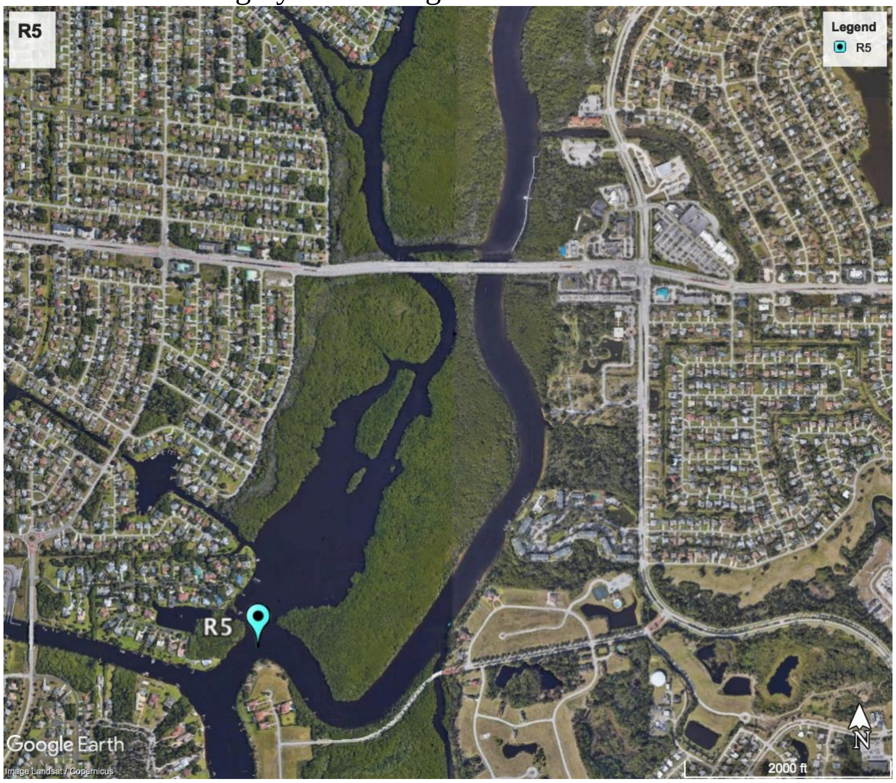
SI 7. Satellite imagery from Google Earth at R3.



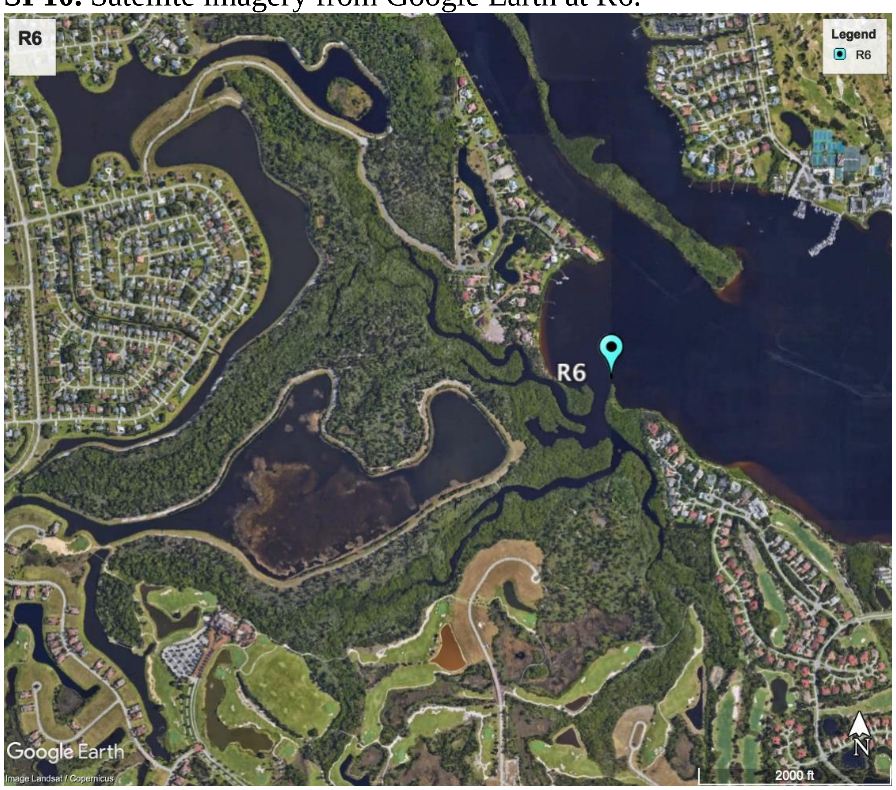
SI 8. Satellite imagery from Google Earth at R4.



SI 9. Satellite imagery from Google Earth at R5.

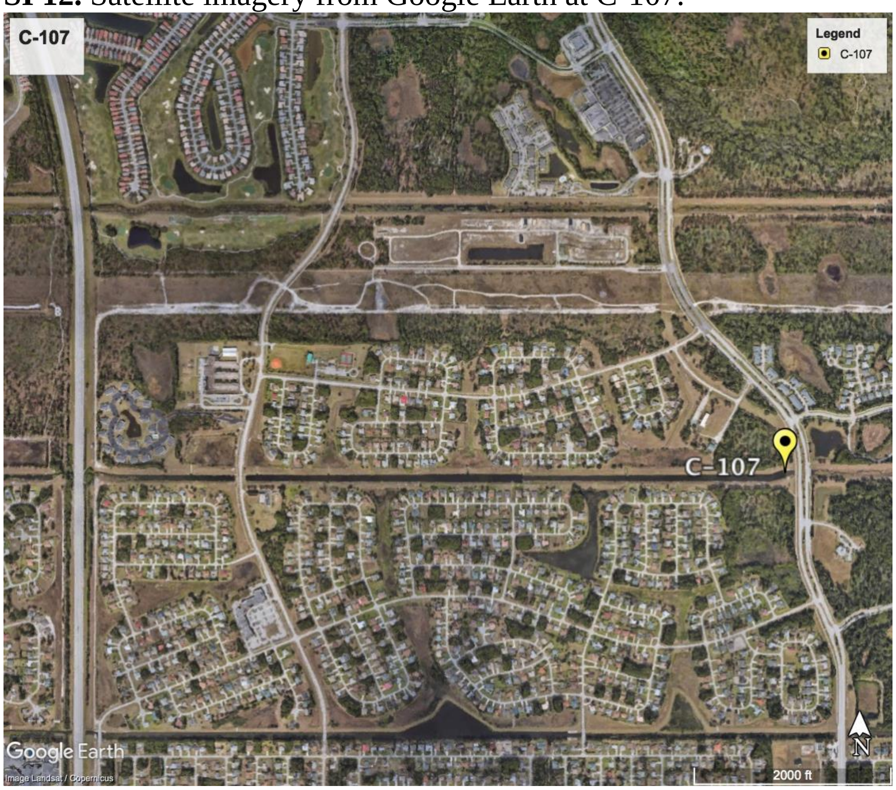


SI 10. Satellite imagery from Google Earth at R6.



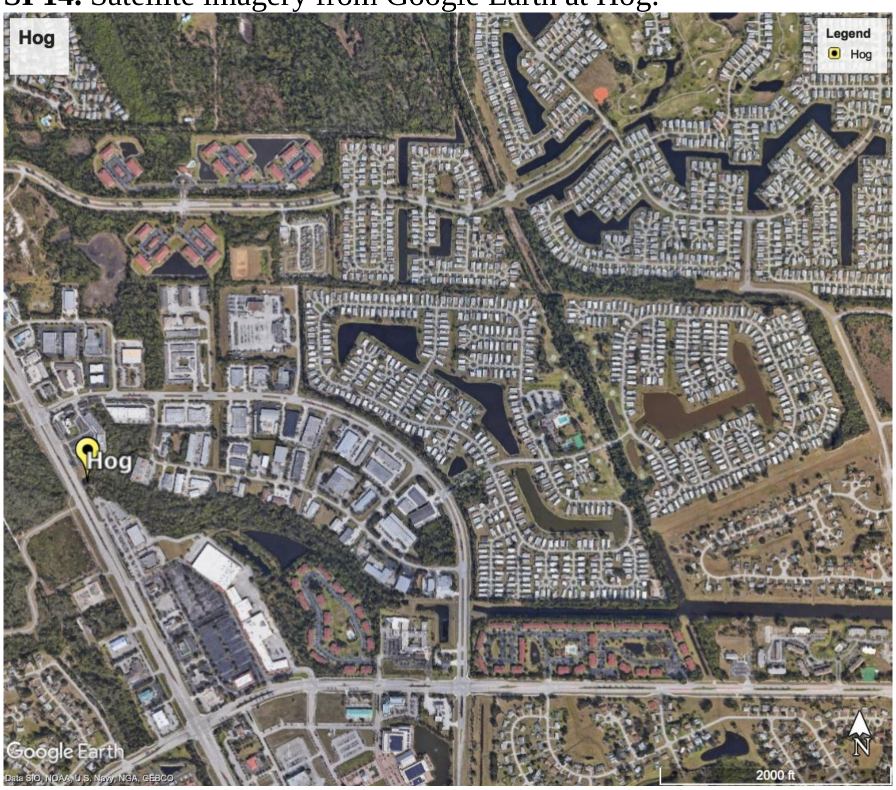
SI 11. Satellite imagery from Google Earth at R7.

SI 12. Satellite imagery from Google Earth at C-107.



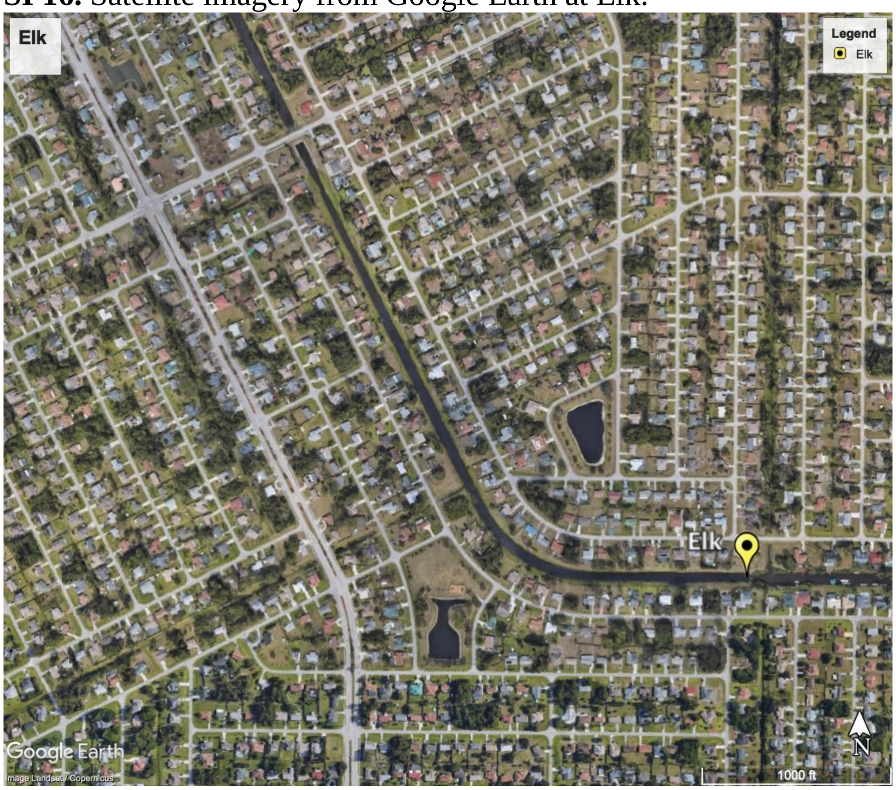
SI 13. Satellite imagery from Google Earth at Sag.

SI 14. Satellite imagery from Google Earth at Hog.

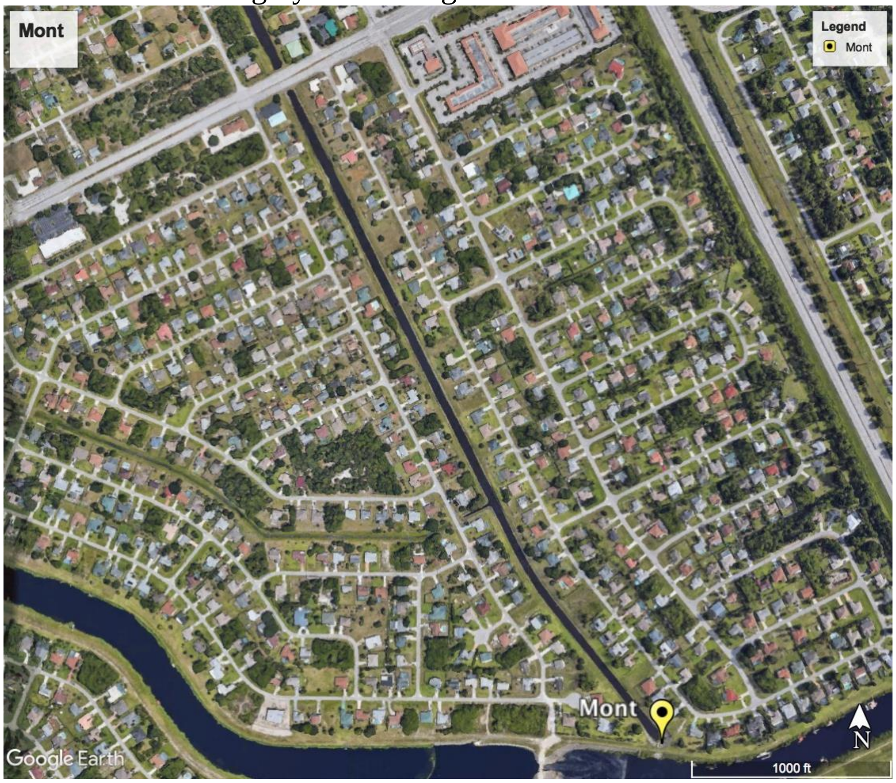


SI 15. Satellite imagery from Google Earth at Vet.

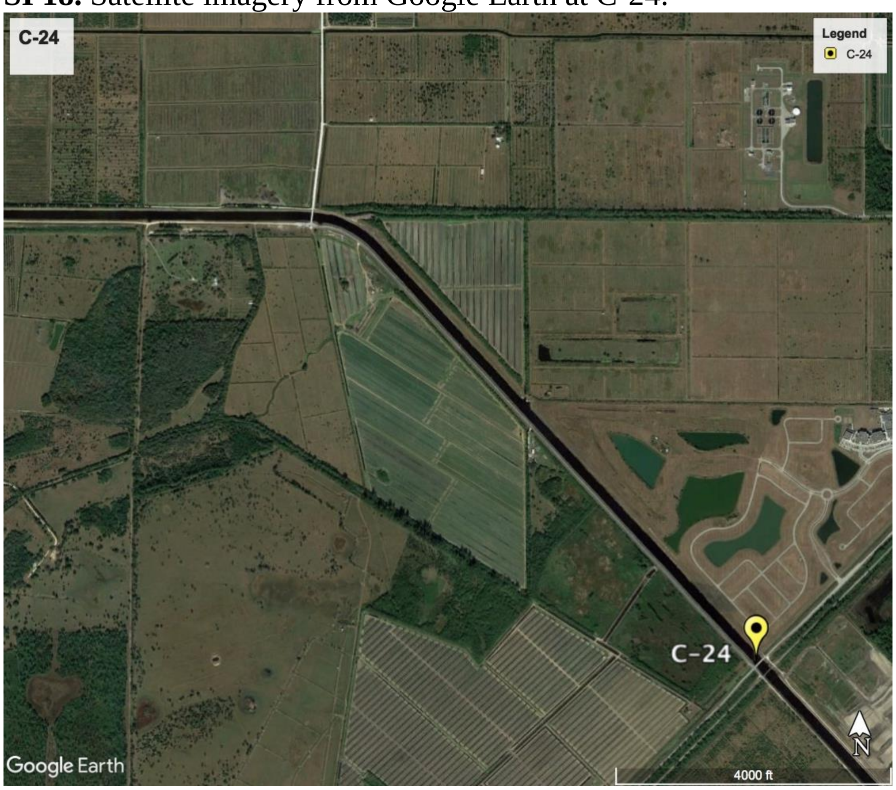
SI 16. Satellite imagery from Google Earth at Elk.



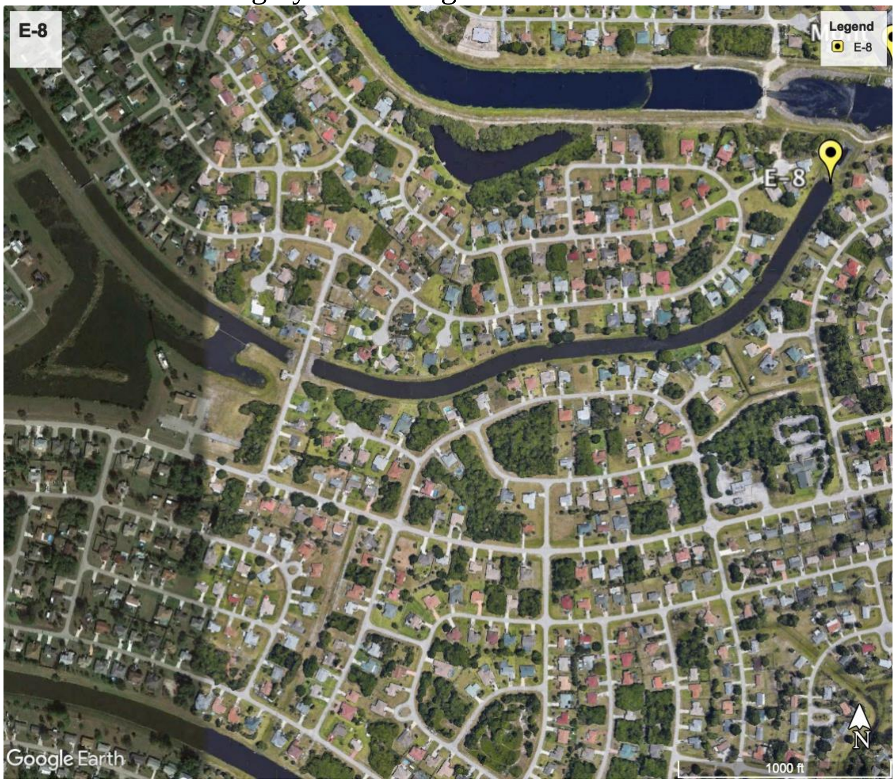
SI 17. Satellite imagery from Google Earth at Mont.



SI 18. Satellite imagery from Google Earth at C-24.

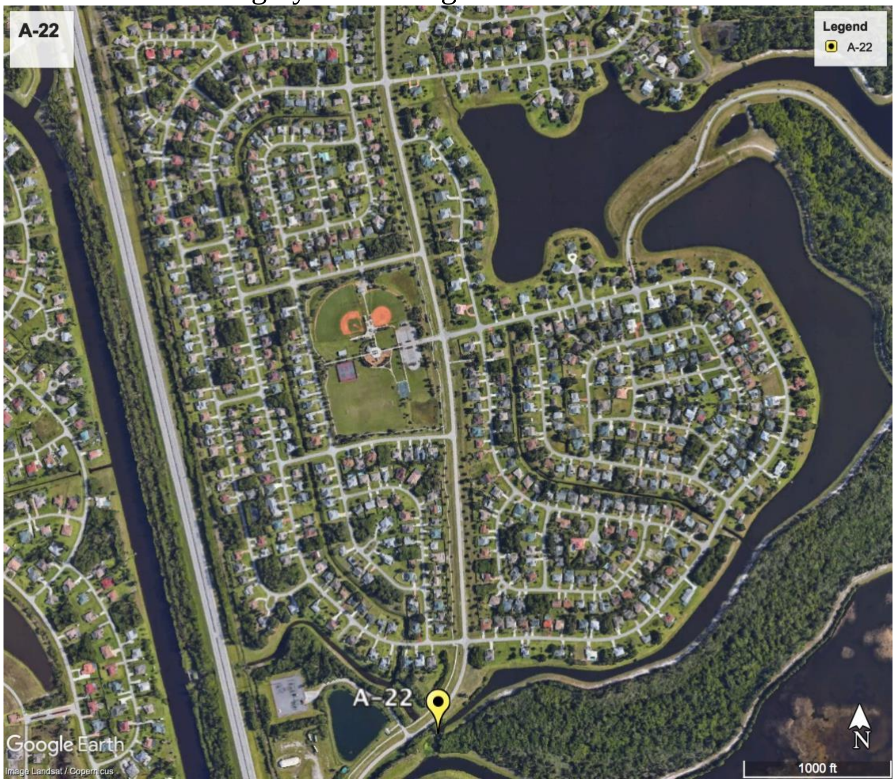


SI 19. Satellite imagery from Google Earth at E-8.



SI 20. Satellite imagery from Google Earth at A-18.

SI 21. Satellite imagery from Google Earth at A-22.



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