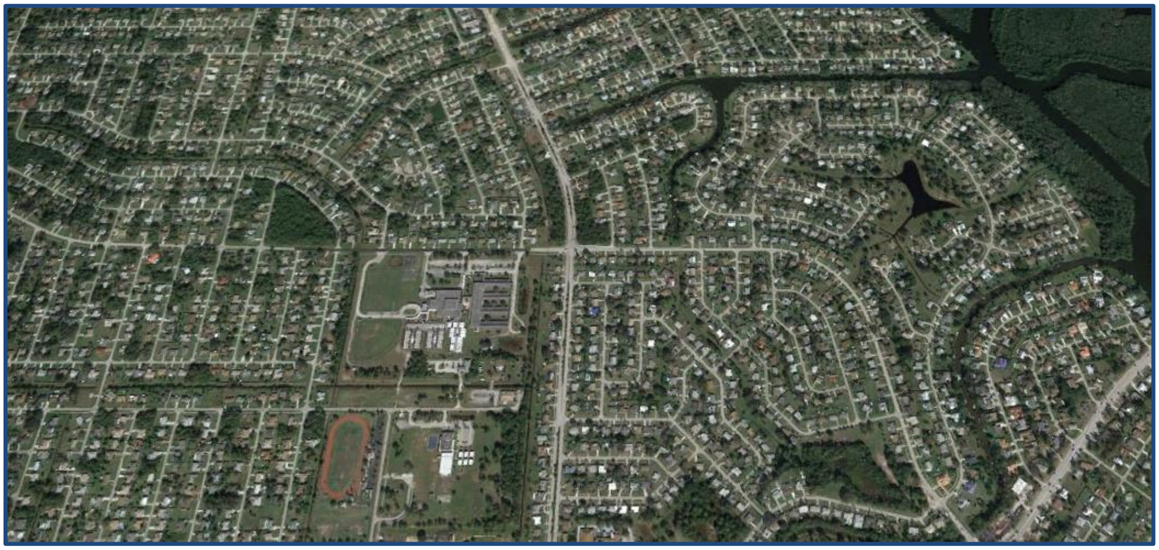
Septic System – Groundwater – Surface Water Couplings in the North Fork of the St. Lucie River: MST Phase II



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Executive Summary

The City of Port St. Lucie (CPSL) supported a one-year study to continue investigating sources contributing to the bacterial and nutrient impairment of the North Fork of the St. Lucie River (North Fork). Specifically, this research had two goals: **1) to better understand septic system - groundwater - surface water couplings within the Sagamore residential area of CPSL and 2) to continue long-term monitoring within canals throughout the North Fork drainage basin with the addition of stable nitrogen isotope ($\delta^{15}N$) analyses for the purpose of identifying nitrogen sources**. The results of the Phase II study are synthesized here to help the CPSL City Council guide their water quality improvement efforts for the North Fork.

Objective 1) Septic System - Groundwater - Surface Water Couplings

Eight groundwater monitoring wells were installed in CPSL right of way in the Sagamore residential area and one well was installed in the Floresta Pines Basin. Seven wells were shallow surficial wells (~8-10'), one represented a medium depth (~25'), and one was deeper (~50'). Of the nine wells, eight were installed in canal right of ways behind homes serviced by septic systems (non-sewered in the Sagamore basin) and one shallow well was installed in a sewered area that never had any septic systems as a "reference" site to provide background information (Floresta Pines Basin). Depth to water table in the monitoring wells was measured weekly by CPSL staff. The monitoring wells were also sampled twice seasonally (wet and dry) to determine bacterial counts and nutrient concentrations in the groundwater. During these four sampling events, groundwater samples were also collected for analysis of dissolved $\delta^{15}N$ and chemical tracers of wastewater, including artificial sweeteners (sucralose) and pharmaceuticals (ibuprofen, acetaminophen, carbamazepine, etc.) to help further discriminate the sources of nitrogen and bacteria within the Sagamore basin and the downstream North Fork. Additionally, environmental parameters, such as salinity, conductivity, dissolved oxygen (DO), pH, and temperature, were also measured.

In addition to groundwater, surface water samples were collected for determination of dissolved nutrient concentrations and bacterial counts in small drainage canals (D2, D2B, and D3) adjacent to the groundwater sites, when enough water (> 3 in. depth) was present. Further, samples of phytoplankton, and/or freshwater macrophytes, such as water lettuce or hydrilla, were also collected at these sites. These samples were analyzed to determine $\delta^{15}N$ values, as well as elemental composition (N:P ratios) to further distinguish between what nitrogen sources (e.g. rainfall, wastewater, stormwater runoff, inorganic fertilizers, etc.) are available and driving primary production in these surface waters.

Despite limitations of installation locations, the groundwater monitoring wells were informative regarding contamination of groundwater in the Sagamore area by septic tank effluent. The non-sewered wells generally had higher ammonium + nitrate + nitrite (= dissolved inorganic nitrogen; DIN) than the sewered well. Further, total dissolved nitrogen (TDN) and the ratios of nitrogen to phosphorus, including DIN:soluble reactive phosphorus (SRP) and TDN:total dissolved phosphorus (TDP) in the groundwater were much higher near septic systems than in the sewered areas. Two monitoring wells in the non-sewered area had consistently elevated fecal indicator bacteria (fecal coliforms and *E. coli*). Sucralose was detected at all wells, but the sewered well had the lowest concentration, which was between the Minimum Detection Limit (MDL) and the Practical Quantitation Limit (PQL). Dissolved $\delta^{15}N$ in the non-sewered area were all enriched and within the range of septic tank effluent, while the sewered well had a more depleted $\delta^{15}N$ value, indicating a different nitrogen source, such as rainfall or inorganic fertilizers. These data indicated the presence of wastewater in the groundwater of the area serviced by septic systems. The extent of this issue is demonstrated by all three well depths (10', 25', and 50') showing signs of wastewater contamination.

Sampling of the canals adjacent to the groundwater monitoring wells was useful in connecting the upstream contamination to the downstream surface waters of the North Fork. The non-sewered groundwater wells generally had higher DIN than the adjacent canals, which would result from attenuation due to dilution or uptake by macrophytes. These canals had consistently high fecal indicator bacteria, exceeding the FAC Statistical Threshold Value for *E. coli* (410 MPN/100 mL) in the wet and the dry seasons. It is not certain if the elevated bacteria in these adjacent canals is sourced directly from groundwater or if the growth is enhanced with the increased nutrient concentrations. Sucralose was detected at all three canals at similar levels in both the wet and dry seasons. The $\delta^{15}N$ values of macrophytes collected in these canals had enriched values indicative of a wastewater signal. Phytoplankton collected from these canals had slightly lower $\delta^{15}N$ values that were still in the range expected for wastewater. An exception was the D2B canal, which had the lowest $\delta^{15}N$ value, indicative of a more depleted nitrogen source, such as inorganic fertilizer or rainfall. The data from the adjacent canals confirmed the presence of wastewater in surface waters in the Sagamore basin.

The results of this sampling indicate that septic systems in the Sagamore basin are contributing to the impairment of the North Fork through groundwater contamination that discharges into adjacent canals, which ultimately flow into the North Fork. Further, there is evidence that indicates stormwater runoff also contributes nutrients and bacteria to these canals. Management actions, such as septic-to-sewer conversions and stormwater treatment areas (STAs), should continue to be prioritized by CPSL to mitigate these effects and reduce nutrient and bacterial loadings. This study establishes a baseline by which water quality improvements resulting from infrastructure improvements can be monitored.

Objective 2) Long-term Monitoring Sites

Bimonthly sampling was continued at nine long-term surface water monitoring sites established in the Phase 1 MST study of the North Fork. At these sites, measurements were made to determine salinity, conductivity, DO, pH, and temperature. Surface water samples were collected and analyzed to determine bacterial counts, biochemical oxygen demand (BOD), and nutrient concentrations.

Phytoplankton and macrophytes were also collected for analysis of $\delta^{15}N$ values for the purpose of nitrogen source identification.

Water quality was variable between canal sites and these differences may be useful in determining what management actions could be taken at locations in need of improvement. For example, the average concentrations at all canal sites exceeded the FAC standards for TDN (0.72 mg/L) and BOD (2.0 mg/L), confirming a basin-wide need to decrease nitrogen and bacterial loadings. TDP only exceeded the FAC standard (0.081 mg/L) at a few canal sites, including Sagamore, Monterrey, and A-22. All sites had elevated TDN:TDP ratios (> 16), indicating a tendency towards nitrogen enrichment and phosphorus limitation of plant growth.

The $\delta^{15}N$ values in macrophytes and phytoplankton at long-term canal sites were useful in identifying nitrogen sources. At some sites, the enriched $\delta^{15}N$ values in macrophytes indicated wastewater as a primary nitrogen source, including Sagamore, Elkcam, Monterrey, E-8, A-18, and A-22. Other sites, like C-107, had lower $\delta^{15}N$ values, which indicate a more depleted nitrogen source, such as inorganic fertilizers or rainfall. Some sites displayed variability in the macrophyte $\delta^{15}N$ values, which indicates multiple nitrogen sources were available for primary producers. For example, at Hogpen Slough and Veterans Memorial macrophyte samples had depleted $\delta^{15}N$ values in the dry season, but enriched in the wet season, which confirms the influence of increased rainfall on wastewater nitrogen transport. However, phytoplankton samples from Hogpen Slough and Veterans Memorial had similarly enriched $\delta^{15}N$ values in both seasons. These $\delta^{15}N$ data confirm findings from the Phase I MST Study, which indicated a widespread presence of wastewater throughout the North Fork.

These data allowed for a longer-term assessment of water quality within canals that drain into the North Fork and provided information regarding likely sources of nutrients and bacteria. Likewise, these data allow for CPSL to better understand results from infrastructure improvements in these areas, such as septic-to-sewer conversions and construction of STAs.

Summary Table: Septic System - Groundwater - Surface Water Couplings

Compilation of all data collected during the Phase 2 Study showing relative levels of analytes, summarized for groundwater wells (GW) and adjacent surface water (ASW); a dash (-) indicates the substance was below detection limits, "NA" indicates the substance was not analyzed at that site, green shading indicates trace concentrations or a low value relative to applicable standards (not all analytes have numerical standards), 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. There are no numerical standards for reactive nutrients, so classifications were based on an estimated percent contribution of the FDEP surface water standard for the North Fork (TDN=0.72 mg/L and TDP=0.081 mg/L): ammonium and nitrate were considered elevated at 10% of the total nitrogen (TN) standard, DIN was considered elevated at 20% of the TN standard, and phosphate (SRP) was considered elevated at 20% of the total phosphorus standard; the numerical classifications and units for each parameter are listed in the legend. Stable nitrogen isotope ($\delta^{15}N$) values were considered "Significant" when > +3 ‰, "Moderate" when > +2 ‰, and "Low" when < +2‰.

Septic System – Groundwater – Surface Water Couplings

Type	Site	Bacteria		Wastewater Tracer	Dissolved Nutrients						Nitrogen Isotopes		
		Fecal	*E. coli*	Sucralose	NH4	NO3	DIN	SRP	TDN	TDP	Groundwater	Macrophytes	Phytoplankton
Groundwater	GW 2											NA	NA
	GW 1											NA	NA
	GW 4	-										NA	NA
	GW 5	-										NA	NA
	GW 6	-										NA	NA
	GW 3 -10'											NA	NA
	GW 3 -25'	-										NA	NA
	GW 3 -50'	-										NA	NA
	Sewered	-										NA	NA
Adjacent Surface Water	D2	842	1114								NA		
	D2B	369	417								NA		
	D3	805	2203								NA		
Legend	Low	<99	<99	<0.1	<0.02	<0.02	<0.1	<0.005	<0.2	<0.02	<2.0	<2.0	<2.0
	Moderate	100-399	100-399	0.1-0.99	0.02-0.071	0.02-0.071	0.1-0.15	0.005-0.015	0.2-0.71	.02-0.079	2.0-3.0	2.0-3.0	2.0-3.0
	Significant	≥400	≥400	≥1.0	≥0.072	≥0.072	≥0.14	≥0.016	≥0.72	≥0.08	≥3.0	≥3.0	≥3.0
	Parameter Units	cfu/100mL	MPN/100mL	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	δ15N	δ15N	δ15N

Summary Table: Long-term Monitoring

Compilation of all data collected during the Phase 1 and Phase 2 studies showing relative levels of analytes, summarized for long-term surface water monitoring sites; a dash (-) indicates the substance was below detection limits or did not amplify, “NA” indicates the substance was not analyzed at that site, green shading indicates trace concentrations or a low value relative to applicable standards (not all analytes have numerical standards), 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. There are no numerical standards for reactive nutrients, so classifications were based on an estimated percent contribution of the FDEP surface water standard for the North Fork (TDN=0.72 mg/L and TDP=0.081 mg/L): ammonium and nitrate were considered elevated at 10% of the total nitrogen (TN) standard, DIN was considered elevated at 20% of the TN standard, and phosphate (SRP) was considered elevated at 20% of the total phosphorus standard; the numerical classifications and units for each parameter are listed in the legend. Stable nitrogen isotope ($\delta^{15}N$) values were considered “Significant” when > +3 ‰, “Moderate” when > +2 ‰, and “Low” when < +2‰.

Long-term Monitoring

Type	Site	Molecular		Wastewater Tracers			Stormwater Tracers			Macrophyte Control	Dissolved Nutrients						Bacteria			Isotopes	
		GULL2	HF183	Sucra-lose	Acetam-inophen	Carbama-zepine	Diuron	Fenuron	Imida-cloprid	Fluridone	NH4	NO3	DIN	SRP	TDN	TDP	Fecal	*E. coli*	BOD	Macro-phytes	Phyto-plankton
Surface Water	C-107	-	-			-															
	Sagamore	-																			
	Hogpen Slough	-	-					-													
	Vet Memorial	-	-			-															
	Elkcam	-	-																		
	Monterrey	-	-		-																
	E-8	-	-		-																
	A-18	-	-		-																
	A-22	-	-																		
Legend	Low	<10,000	<10,000	<0.09	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.02	<0.02	<0.04	<0.005	<0.2	<.02	0-99	0-99	<1	<2	<2
	Moderate	10,000-99,999	10,000-99,999	0.1-0.99	0.01-0.04	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.02-0.071	0.02-0.071	0.04-0.13	0.005-0.015	0.4-0.71	.02-0.07	100-399	100-399	1-1.9	2.0-3.0	2.0-3.0
	Significant	≥100,000	≥100,000	≥1.0	≥0.05	≥1.0	≥1.0	≥1.0	≥1.0	≥1.0	≥0.072	≥0.072	≥0.14	≥0.016	≥0.72	≥0.08	≥400	≥400	≥2.0	≥3.0	≥3.0
	Parameter Units	TSC/100mL	GEU/100mL	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	cfu/100mL	MPN/100mL	mg/L	δ15N	δ15N

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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
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
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus
TSC	target sequence copies
µg/L	micrograms per liter
USEPA	United States Environmental Protection Agency
WBID	water body identification number

Introduction

1.1 Problem Statement and Project Objective

The North Fork of the St. Lucie River (North Fork hereafter; WBID 3194) is a Class III waterbody with designated uses of recreation, propagation, and maintenance of healthy, well balanced populations of fish and wildlife. This waterbody has been classified as impaired on the United States Environmental Protection Agency (US EPA) 303d list for dissolved oxygen (DO), total dissolved phosphorus (TDP), total dissolved nitrogen (TDN), and fecal coliforms with a Total Maximum Daily Load (TMDL) in place (SFWMD et al. 2009, White and Turner 2012). Further, high bacterial counts have resulted in recurring closures of the North Fork for recreational use (Lapointe et al. 2018). The North Fork ultimately terminates into the St. Lucie River, where it comprises 23.2% of the watershed, 11.3% of the flow, 11.6% of the total phosphorus (TP) load, and 8.4% of the total nitrogen (TN) load (SFWMD et al. 2009).

In a Microbial Source Tracking (MST) Phase I study, that was supported by Florida Department of Environmental Protection (FDEP) and analyzed by Lapointe et al. (2018) from Harbor Branch Ocean Oceanographic Institute- Florida Atlantic University (HBOI), several recommendations were made to better understand causative factors degrading local water quality and leading to this impairment of the North Fork. For these purposes, a one-year study was commissioned by the City of Port St. Lucie (CPSL). **This study had two objectives: 1) to better understand septic system - groundwater - surface water couplings within the Sagamore drainage basin of CPSL and 2) to continue long-term monitoring within canals throughout the North Fork drainage basin with the addition of stable nitrogen isotope ($\delta^{15}N$) analyses for the purpose of identifying nitrogen sources.**

1.2 Study site

The North Fork roughly flows through the center of CPSL and drains approximately 119,680 acres with major land uses of residential/urban (45 %), agricultural (31 %) and natural (21 %; SFWMD et al. 2009). CPSL covers 120 square miles and is the 8th largest city in Florida with 195,000 residents in 2018 (US Census), making it the most populous municipality in St. Lucie County. CPSL was founded by the General Development Corporation (GDC) in 1958 and officially incorporated in 1961. Unfortunately, GDC did not plan CPSL with sufficient wastewater infrastructure to allow for sustainable development, which has led to degradation of surface water quality as the city has grown. For example, CPSL contains many dense residential areas near the North Fork that are reliant on septic systems for on-site wastewater treatment. Similar issues regarding degraded water quality can also be found in other Florida cities founded by GDC, such as Palm Bay (Port Malabar; Arnade 1999) and Port Charlotte (Lapointe et al. 2016).

A septic-to-sewer program was developed by CPSL in 1999 to support new growth sustainably, as well as alleviate water quality issues. At present, over 8,662 septic systems have been converted to a low-pressure, centralized sewer system. This infrastructure improvement plan is ambitious and is crucial for achieving their long-term development and water quality goals. CPSL remains one of the only coastal cities in Florida to execute such a plan. Further, more than 24,871 newly built homes have been immediately connected to the low-pressure sewer system. In other areas, many homes have been connected to a gravity sewer system, such as Tesoro, Viscona, Tradition, Southern Grove, Copper Creek, Verano, Veranda Gardens, Viscaya, and others. Recent estimates indicate that there are approximately 59,126 houses connected to a sewer system and roughly 17,052 on septic systems in the CPSL Utility service area. These numbers are ever changing but are based on the best available information and demonstrate that CPSL is proactively decreasing their reliance on septic systems in dense urban areas and increasing the availability of sewer connections to its residents.

1.3 Groundwater contamination

Septic systems have been demonstrated to affect the quality of groundwater (Bicki et al. 1984, Yates 1985, Lapointe and Krupa 1995, Lapointe et al. 2017).The issue of groundwater contamination by septic systems is worsened in high density areas, which can lead to the waterborne spread of pathogens (Yates 1985, Verhougstraete et al. 2015). This contamination can be evidenced by high concentrations of fecal indicator bacteria, elevated water color, increased biochemical oxygen demand, and high dissolved nutrient concentrations (Yates 1985, Lapointe and Krupa 1995). For example, in Jupiter, FL groundwater monitoring wells immediately adjacent to septic system drain fields had fecal coliform bacterial counts as high as 30,000 CFU/100 mL, ammonium concentrations up to 17.9 mg/L and nitrate + nitrite concentrations up to 21.6 mg/L with combined DIN ranging up to 21.8 mg/L (Lapointe and Krupa 1995). Wastewater can be confirmed as the source of the groundwater contamination using chemical tracers, such as sucralose, and $\delta^{15}N$ values of groundwater, phytoplankton, and macrophytes (Cole et al. 2006, Lapointe et al. 2017).

Objective 1: Sagamore basin septic system - groundwater - surface water couplings

2. Methods

The Sagamore basin of CPSL was identified as a poor water quality “hotspot” in the Phase I study (Lapointe et al. 2018). In other similar locations, such as Martin County, FL and Jupiter, FL, detailed studies of septic system - groundwater - surface water couplings have illustrated how septic systems can negatively influence nearby surface waters (Lapointe and Krupa 1995, Lapointe et al. 2017). Thus, a study was designed to assess these couplings in the Sagamore basin hotspot.

2.1 Site selection and monitoring well installation

In the Sagamore basin, intensive preliminary sampling was conducted by HBOI-FAU and CPSL staff to locate potential well sites in right of way areas owned by

CPSL near drainage canals that were also close to septic systems. The goal of site selection was to locate sites that seemed to be indicative of influence by septic contamination, so that sampling could help to characterize the influence of septic systems on local groundwater. After locating potential sites, groundwater samples were initially obtained with a portable well point sampler. The well point sampler was driven into the ground, which created a temporary well that could quickly be sampled and then returned to its previous state (Fig. 1A).

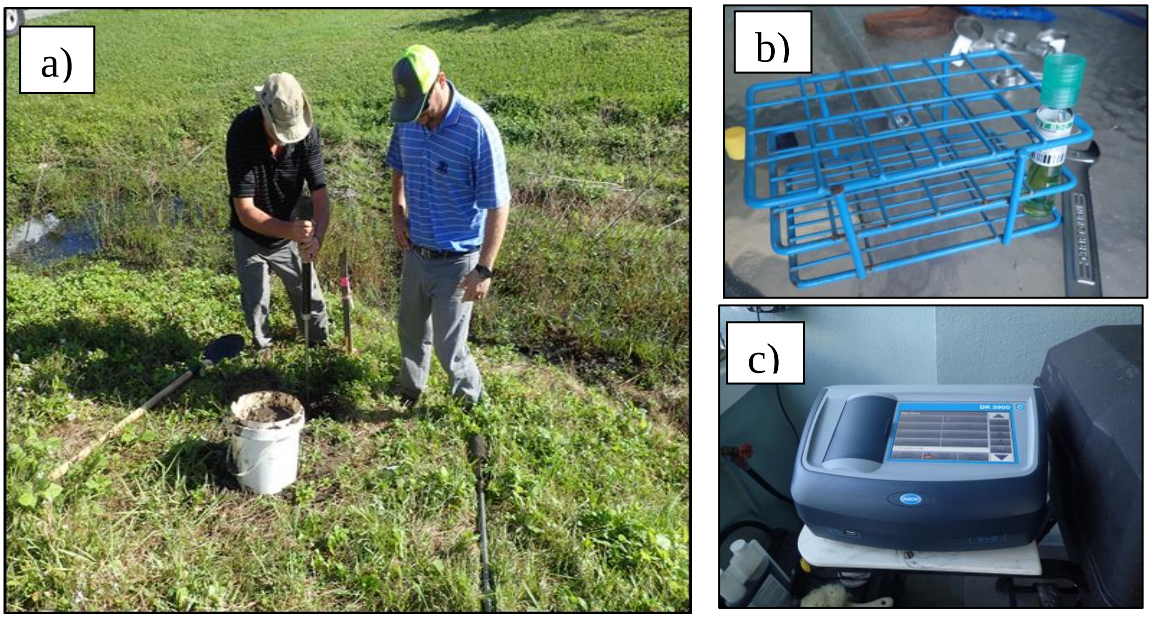

Figure 1. Preliminary sampling conducted in the Sagamore basin of CPSL was conducted a) with a well point sampler, b) using TNTplus vial test kits, and c) a Hach DR 3900 Spectrophotometer.

Once collected, these water samples were analyzed in the field to determine estimates of nitrate and ammonium concentrations using TNTplus vial tests with a Hach DR 3900 Spectrophotometer (Fig. 1b,c). Sites with the highest detectable nitrogen concentrations in the groundwater were selected for well installation, as it was assumed these would reflect the greatest influence of nearby septic systems. Due to the method used to locate septic plumes and the spatial limitations of available right of way, site selection was limited by a few factors, including the hardness of the substrate, depth to water table, and availability of CPSL right of way near septic systems. Therefore, the sites chosen were representative of the diffuse exterior of a septic system effluent plume, rather than the center of the plume.

After site selection, nine groundwater monitoring wells with 2-inch diameter PVC well casings were installed by Ardaman and Associates, Inc. on 02/18/2019 (Fig. 2). Each well included a screened section with 0.01-inch slotted pipe with sand packed between the well bore and the well casing along with a 1-foot bentonite cap on top of the packed sand. Wells were completed with two-by-two concrete well pads and flush mounted vaults with locking lids.

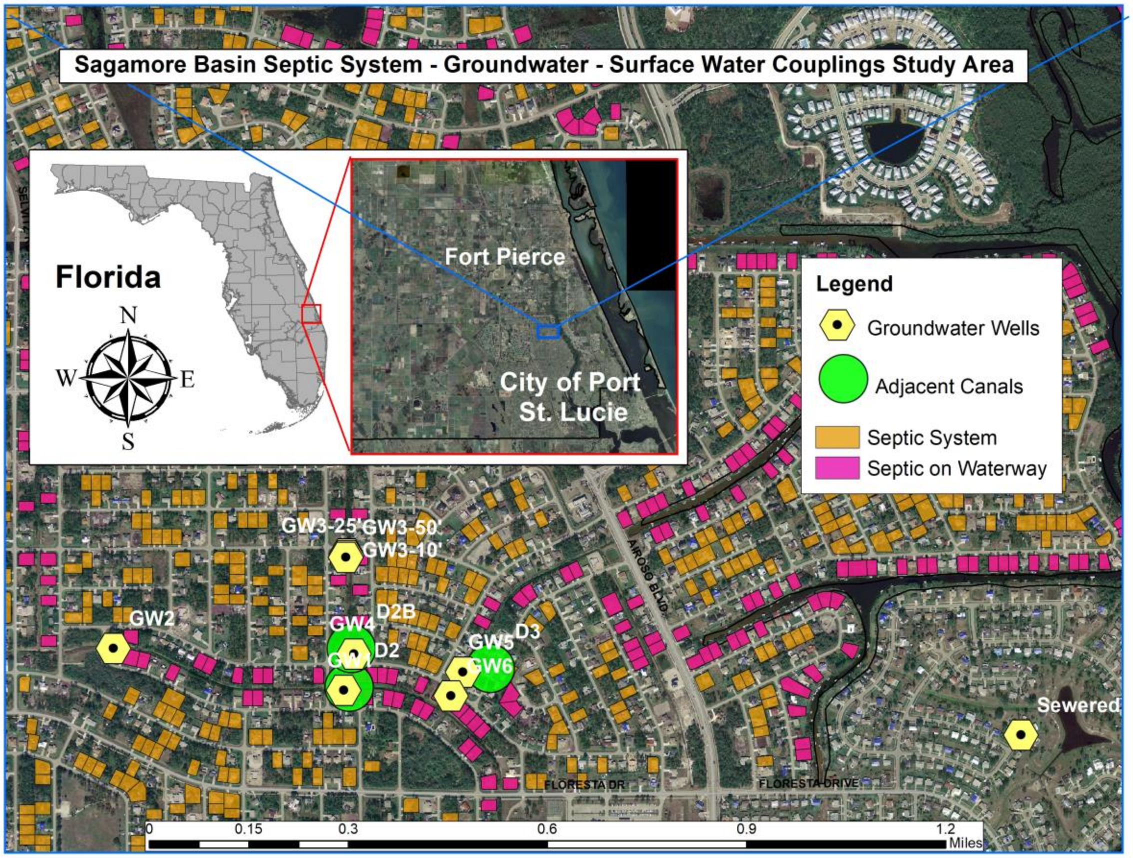

Figure 2. Sampling sites for the City of Port St. Lucie septic system - groundwater - surface water coupling study in the Sagamore drainage basin, showing locations of groundwater wells (yellow), adjacent canal sites (green circles), septic systems (orange shading) and septic systems on waterways (pink shading).

Seven of the wells were installed at a depth of ≤10’, including six in an area serviced by septic systems (GW 1-6) and one to serve as a “reference” site in a sewered area that had never had any septic systems (GW 9). To better understand the vertical mixing of septic effluent influence in the basin, two deeper wells were also installed near GW 3, one at 25’ and one at 50’. Three drainage canals that are adjacent to Groundwater Wells 1, 4, and 5 were also monitored during this study (D2, D2B, and D3).

2.2 Site descriptions

GW 1 – Located on the D2 canal, east of NW Grenada Street between NW Ferris Drive and NW Concord.

GW 2 – Located on the D2 canal, south east of NW Concord Street and NW Ferris Drive intersection.

GW 3 (10’, 25’, and 50’)– Located on the D2B Canal, south of NW Placid Drive between NW Grenada Street and NW Avens Street. Note: in the original lab data, GW 3 -25’ is named “GW 7” and GW 3 -50’ as “GW 8”.

GW 4 – Located on the D2B canal, north of NW Ferris Drive between NW Grenada Street and NW Avens Street.

GW 5 – Located on the D-3 canal, north of NW Ferris Drive between NW Twylite Terrace and NW Sagamore Terrace.

GW 6 - Located on the D-3 canal, south of NW Ferris Drive between NW Twylite Terrace and NW Sagamore Terrace.

GW 9 – Background well, located in a sewered area with no septic systems at the end of NE Redrock Court

2.3 Sample collection

The depth to water table was monitored weekly by CPSL staff from well installation through the end of the project using a water level meter. The nine groundwater monitoring wells and three adjacent canals were sampled adhering to FDEP groundwater and surface water sampling standard operating protocols (SOPs) to the extent possible. Most of the shallow wells were not able to be purged until reaching stable environmental conditions (dissolved oxygen, pH, temperature, specific conductivity, and turbidity), despite purging considerably more than one well volume. Due to time constraints for sample shipment and low recharge rates, these wells were purged for at least one well volume or as long as determined to be possible without draining the well dry. Prior to the sample collection for groundwater and after samples were collected for surface water, a calibrated YSI ProPlus sonde was used to measure pH, salinity, temperature, conductivity, and DO at each site (Fig. 3).

Figure 3. CPSL staff sampling groundwater monitoring wells.

At every site, water samples for determination of dissolved nutrient concentrations were collected in duplicate into acid-washed 250 mL high-density polyethylene (HDPE) bottles. These water samples were filtered in the lab at HBOI-FAU through 0.7 µm GF/F filters and then frozen at -20°C. The frozen samples were shipped to the Nutrient Analytical Services Laboratory at the Chesapeake Biological Laboratory (NASL-CBL) to be analyzed for dissolved nutrient concentrations following standard methods (http://nasl.cbl.umces.edu/methods/WCC.html). At NASL-CBL the following analytes were measured, including ammonium (NH_4, MDL = 0.013 mg/L), nitrate + nitrite (NO_x, MDL = 0.0007 mg/L), soluble reactive phosphorus (SRP, MDL = 0.0034 mg/L), total dissolved nitrogen (TDN, MDL = 0.05 mg/L), and total dissolved phosphorus (TDP, MDL = 0.0015 mg/L).

At all sites water was collected into a sterile bottle for microbial analyses. These samples were immediately stored on ice and transported to Flowers Chemical Laboratories, Inc., Port St. Lucie, FL within six hours. At the lab, these samples were analyzed to determine fecal coliform and *E. coli* counts. The fecal coliform analysis was conducted by following EPA standard method (SM) 9222D and the *E. coli* was analyzed following SM 9223B. Bacteria data were compared to the FAC Statistical Threshold Value for *E. coli* (410 MPN/100 mL).

Chemical tracer samples were also collected at all groundwater and adjacent canal surface water sites (Fig. 4). The samples were collected in 1 L amber glass bottles and immediately preserved on ice, prior to being shipped overnight to FDEP for analysis of chemical tracer concentrations. The analysis was performed following standard methods (https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops). Chemical tracer samples were analyzed for sucralose (artificial sweetener); acetaminophen, hydrocodone, ibuprofen, and naproxen (pain relievers); and carbamazepine and primidone (anticonvulsants). Additionally, one set of samples was also analyzed for imazapyr (herbicide). Any samples that were marked as below the detection limit (U) were changed to zero to indicate a non-detect prior to analyses. Samples marked as between the MDL and PQL (I) or less than the criterion of detection (T) were included in analyses.

Figure 4. Sampling groundwater for chemical tracers.

At surface water sites, aquatic macrophytes were collected into clean plastic bags and stored on top of a towel in an ice chest. In the lab, these samples were rinsed briefly in DI water and cleaned of any extraneous items. The cleaned macrophytes were placed in plastic weigh boats and dried at 65°C in a laboratory oven for ~48 h. The dried samples were homogenized with a mortar and pestle and split into two vials. One vial was sent to the University of South Florida Marine Environmental Chemistry Laboratory (USF-MECL) and one was sent to the University of Missouri Soil and Plant Testing Laboratory (UM-SPTL). At USF-MECL, samples were analyzed for $\delta^{15}N$ and %N were measured by Continuous Flow Elemental Analyzer Isotope Ratio Mass Spectrometry on a ThermoFinnigan DeltaV+ IRMS - FlashIRMS Fast Oven EA - ConFLo IV system. Secondary reference materials (NIST 8574 $\delta^{15}N$ = +47.57 ± 0.22 ‰, N = 9.52%, $\delta^{15}N$ = -4.52 ± 0.12‰ N = 9.52%) are used to normalize raw measurements to the AT-Air ($\delta^{15}N$) scales (Werner et al 2002, Qi et al 2002) and to calibrate elemental N, C, and C:N. At UM-SPTL, %P was determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (Viso and Zachariadis 2018). These data were used to calculate N:P ratios and P limitation was indicated by N:P ratios > 16 (Atkinson and Smith 1983, Lapointe 1987, Lapointe et al. 2015).

Particulate organic matter (POM) was also collected from surface water sites as a proxy for phytoplankton. For POM collections, surface water was collected with a clean secondary vessel. Samples were coarse filtered into a 1 L HDPE bottle at the site through a 200 µm nylon netting to remove macrodetritus and microzooplankton, as per Savoye et al. (2003). The filtered samples were immediately placed on ice in a cooler. Upon return to the lab (within 6 hours), POM samples were filtered through 47 mm glass fiber filters (GF/F) using a vacuum pump. The volume filtered was recorded and the filter was dried at 65°C until analyses. Once dry, filters were cut in half with sterile scissors and each half was folded with the phytoplankton on the inside and wrapped separately in foil. The POM filters were then analyzed similarly to macrophyte samples at USF-MECL and UM-SPTL to determine $\delta^{15}N$ values, %N, and %P.

Groundwater was also collected for analysis of nitrogen isotopic composition through determination of $\delta^{15}N$-NH_4 and $\delta^{15}N$-NO_3 aqueous $\delta^{15}N$ values. These samples were collected into 1 L HDPE bottles and immediately acidified to < 2 in the field by the addition of sulfuric acid. pH was confirmed upon return to the laboratory and samples were stored in the dark until shipment shipped to the Boston University Stable Isotope Laboratory (BU-SIL) for analysis. At BU-SIL, the water samples were run through ammonia diffusion. This involved increasing the pH of the dissolved sample to convert ammonium to gaseous ammonia, which was captured on an acidified filter in the bottle headspace. NO_3-specific N was quantified by first boiling-off the volatile ammonia, adding a reducing agent to convert oxidized N to NH_4, followed by standard diffusion and ammonia capture on an acidified filter. The filter was then analyzed as a typical solid sample on a mass spectrometer for $\delta^{15}N$-NH_4 and $\delta^{15}N$-NO_3.

2.4 Data analyses

Well sites are organized from west to east in figures and tables. Averages and standard error (SE) were calculated for nutrient, bacteria, chemical tracer, and $\delta^{15}N$ data, and then compared by site and season. For a point of reference, FAC water quality standards and Statistical Threshold Levels were compared to results. Values used in comparisons include 0.72 mg/L for TDN, 0.081 mg/L for TDP, 2 mg/L for BOD, and 410 MPN/100 mL for *E. coli*. After which, relationships between concentrations of various parameters were then examined to make inferences regarding sources contributing to water quality degradation. Local knowledge from CPSL and site visits provided land-use data, which was used to ground truth interpretation.

Results

3.1 Rainfall

All sampling events were conducted on days with little to no precipitation (0.0 – 0.1" rain; Fig. 5). Sites were sampled twice in 2019 during the dry season (February 27, March 27), and twice during the wet season (August 8, September 18; Fig. 5). As expected, during the dry season, less rainfall was observed. In the 30 days prior to the February event, there was approximately 3.4" of rainfall and there was 0.07" the week prior. In March, 30 days prior to sampling, approximately 2.1" of rain was recorded with 0.96" the week prior to sampling. In the wet season, 6.4" of rain was recorded prior to the August sampling event with 3.3" of rain in the seven days prior to sampling. Similarly, in September 6.5" of precipitation was recorded prior to sampling with 1.3" in the week immediately prior.

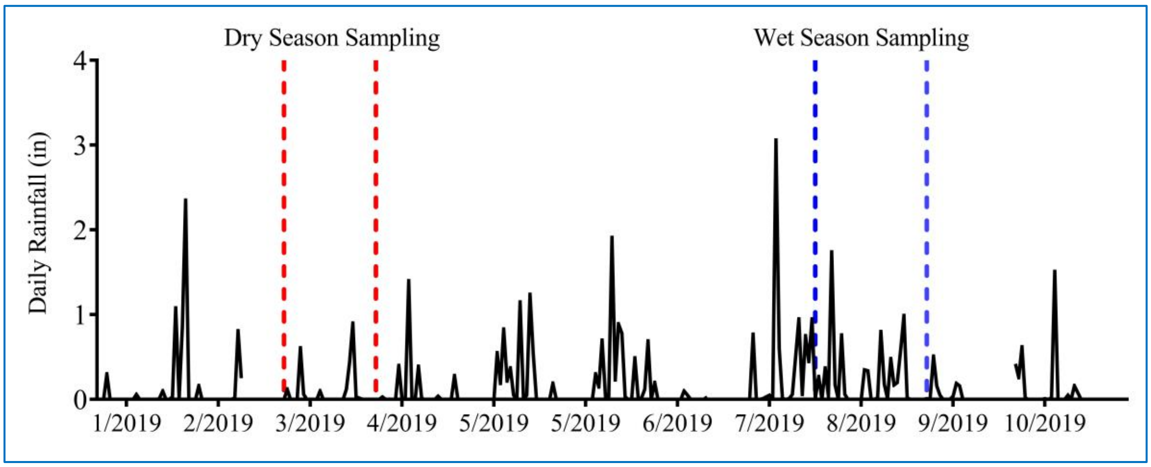

Figure 5. Daily precipitation (inches) in central CPSL over duration of the study period; dashed bars represent sampling events.

3.2 Depth to water table

Weekly measurements of the depth to water table revealed that many wells had water levels too high to support properly functioning septic systems (Fig. 6). Overall, there was a trend of increasing water levels as rainfall increased in the wet season. Monitoring wells GW 1, GW 2, GW 4, and GW 5 had consistently high water levels (~3 – 4' depth) year round with many measurements not meeting the minimum separation recommended by the Florida Administrative Code (FAC) for proper septic system functioning (3.5' separation between ground surface and the septic system drain field; Fig. 6). These sites had even higher water levels in the wet season (~1 – 2' depth). Other monitoring wells, such as GW 3, GW 6, and the sewered site, had lower water levels in the dry season, but the water levels also increased to non-compliant levels during the wet season (Fig. 6). The deeper wells had an offset from the shallow wells, with greater depth to groundwater (~8' in the dry season). These also showed the upward trend in the wet season (~4 – 5' depth; Fig. 6).

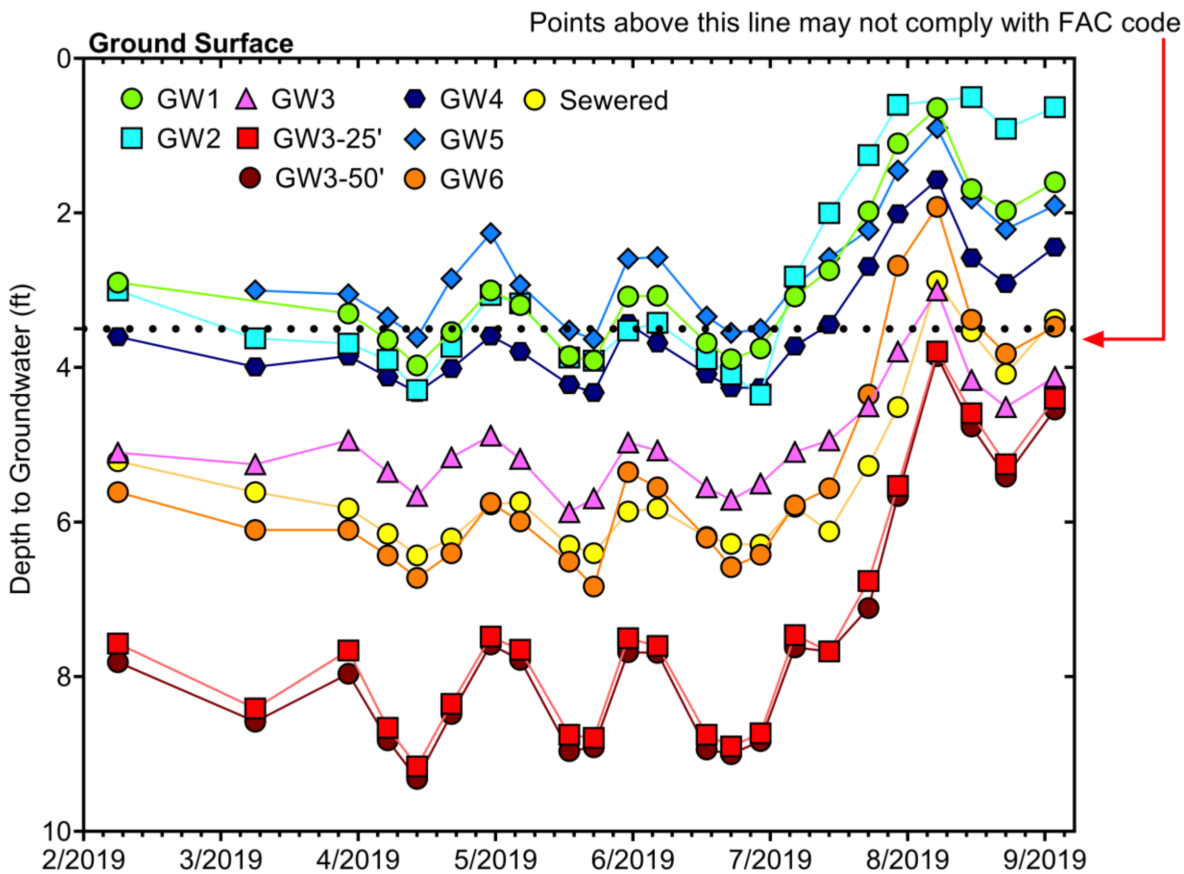

Figure 6. Depth to water table observed at groundwater monitoring well sites in the Sagamore basin of CPSL, with a black dotted line that indicates the approximate minimum separation required from the ground surface (at zero) to the water table required by FAC Rule 62E-6. Values above this line indicate septic systems in this area may not be compliant with current requirements for new septic systems. General required separation is 6" of cover, a 1' drain field (can be less in some sediments), and 2' from the bottom of the drain field to the high water table; for a total of 3.5' required separation from ground to high water table. Effects of these high water table levels may be mitigated somewhat when mounding has been used to increase separation.

3.3 Environmental parameters

Variability in environmental parameters was observed between groundwater monitoring well sites (Table 1). For example, pH at GW 5 was very low (3.6 ± 1.2) compared to most other wells, which averaged between 5 and 6. Conductivity was also variable between wells ranging from 212 µS at GW 6 to 1,212 µS at GW 3 -25' (Table 1). There was also variability observed in DO, however for many of the shallow wells accurate readings were difficult to obtain due to low flow rates.

Table 1. Environmental parameters (project average ± standard error) observed during sampling events in the Sagamore basin.

Type	Site	Count	pH	Temperature (°C)	Count	Salinity
Groundwater Wells	GW2	4	6.9±0.2	24.5±1.0	1	0.55
	GW1	4	6.6±0.1	25.2±1.4	3	0.41±<0.01
	GW4	4	6.5±0.2	26.9±1.1	2	0.60±0.30
	GW5	4	3.6±1.2	26.8±1.2	4	0.09±0.07
	GW6	4	5.1±0.3	26.5±1.6	2	0.12±0.01
	GW3-10'	4	5.8±0.1	26.0±1.3	4	0.15±0.05
	GW3-25'	4	6.6±0.1	25.2±0.4	4	0.60±0.03
	GW3-50'	4	6.8±0.1	25.1±0.1	4	0.51±0.01
	Sewered	4	5.6±0.4	28.7±1.5	4	0.10±0.01
Adjacent Surface Water Canals	D2	4	7.4±0.1	27.4±1.6	4	0.29±0.05
	D2B	4	6.6±0.2	27.6±1.3	4	0.26±0.01
	D3	4	7.0±0.1	25.3±1.8	4	0.31±0.01

Type	Site	Count	Conductivity	Count	DO (mg/L)	Count	DO (%)
Groundwater Wells	GW2	3	1,105±44	3	1.34±0.21	4	22.3±5.3
	GW1	4	803±25	4	0.91±0.21	3	11.4±3.9
	GW4	4	833±369	4	3.18±0.72	2	24.0
	GW5	4	53.5±3.1	4	1.71±0.65	4	22.2±8.8
	GW6	4	212±38	4	2.83±0.69	2	15.7
	GW3-10'	4	311±98	4	0.87±0.12	4	10.7±1.7
	GW3-25'	4	1,212±54	4	0.37±0.28	2	8.30±6.1
	GW3-50'	4	1,042±18	3	0.19±0.06	3	1.99±0.99
	Sewered	4	240±36	3	1.98±0.95	3	10.2±8.8
Adjacent Surface Water Canals	D2	4	718±31	4	6.88±0.39	4	66.0±19
	D2B	4	542±19	4	7.07±2.1	4	90.8±27
	D3	4	640±33	4	6.41±1.3	4	79.4±17

The adjacent surface water canals also showed slight variability between sites. D2B had the lowest pH (6.6 ± 0.2) compared to D3 (7.0 ± 0.1) and D2 (7.4 ± 0.1; Table 1). Salinity and conductivity were similar between the sites. Dissolved oxygen was highest at D2B (7.07 ± 2.1 mg/L), compared to D2 (6.88 ± 0.39 mg/L) and D3 (6.41 ± 1.3 mg/L; Table 1).

3.4 Dissolved nutrient concentrations

Although concentrations were variable among groundwater monitoring wells, DIN was consistently higher near septic systems than the sewered site. GW 2 had the highest average ammonium of all wells (2.08 ± 0.17 mg/L; Table 2). These concentrations were similarly high in both the wet and dry seasons (Fig. 7a). This and other groundwater wells had ammonium levels higher than the sewered site, including GW 1 (0.61 ± 0.01), GW 3 -25' (0.60 ± 0.02 mg/L), and GW 3 -50' (0.28 ± 0.01 mg/L; Table 2). The highest average nitrate + nitrite was observed at GW 4

(4.61 ± 1.3 mg/L), followed by GW 3 -50' (1.36 ± 0.89 mg/L) and GW 3 -10' (1.30 ± 0.56 mg/L; Table 2). Seasonally, nitrate + nitrite was higher during the wet season than the dry season at most wells where the concentrations were detectable, including at GW 2, GW 4, GW 6, GW 3 -10', and GW 3 -50' (Fig. 7b). As such, the highest average DIN was observed at GW 4 (4.70 ± 1.4 mg/L), followed by GW 2 (2.09 ± 0.17), and GW 3- 50' (1.64 ± 0.89; Table 2). Seasonally, DIN was generally higher in the wet season than the dry season (Fig. 7c).

Table 2. Dissolved nutrient concentrations and ratios (project average ± standard error) observed in the Sagamore basin of CPSL.

Type	Site	Count	Ammonium (mg/L)	Nitrate + Nitrite (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	Soluble Reactive Phosphorus (mg/L)
Groundwater Wells	GW2	8	2.08±0.17	0.02±0.01	2.09±0.17	0.002±<0.001
	GW1	8	0.61±0.01	0.01±0.01	0.62±0.01	0.002±<0.001
	GW4	8	0.09±0.04	4.61±1.3	4.70±1.4	0.015±<0.001
	GW5	8	0.11±0.02	0.02±0.01	0.13±0.02	0.083±0.01
	GW6	8	0.06±0.01	0.87±0.41	0.92±0.41	0.042±0.02
	GW3-10'	8	0.05±0.01	1.30±0.56	1.34±0.56	0.031±0.01
	GW3-25'	8	0.60±0.02	0.11±0.05	0.71±0.04	0.002±<0.001
	GW3-50'	8	0.28±0.01	1.36±0.89	1.64±0.89	0.002±<0.001
	Sewered	8	0.03	0.04±0.01	0.06±0.01	0.016±<0.001
Adjacent Surface Water Canals	D2	8	0.03±0.01	0.10±0.05	0.13±0.06	0.006±<0.001
	D2B	8	0.25±0.10	0.01±0.01	0.26±0.10	0.020±0.01
	D3	8	0.07±0.02	0.08±0.04	0.15±0.05	0.006±<0.001

Type	Site	Count	DIN:SRP	Total Dissolved Nitrogen (mg/L)	Total Dissolved Phosphorus (mg/L)	TDN:TDP
Groundwater Wells	GW2	8	2,251±509	2.96±0.14	0.003±<0.001	2,119±290
	GW1	8	1,318±346	1.14±0.02	0.004±<0.001	603±35
	GW4	8	610±176	6.94±1.1	0.033±<0.001	552±111
	GW5	8	5.07±2.2	1.17±0.15	0.107±0.02	29.9±6.00
	GW6	8	647±292	1.53±0.43	0.070±0.03	76.0±14
	GW3-10'	8	217±217	2.58±0.53	0.060±0.02	154±44
	GW3-25'	8	780±113	0.97±0.03	0.005±<0.001	493±54
	GW3-50'	8	4,935±138	0.59±0.02	0.004±<0.001	405±45
	Sewered	7	16.65±2,960	0.40±0.04	0.026±0.00	38.6±6.4
Adjacent Surface Water Canals	D2	8	43.0±17.8	1.08±0.12	0.042±0.01	69.1±11
	D2B	8	24.0±8.4	1.63±0.16	0.150±0.02	25.9±1.8
	D3	8	43.7±8.5	1.10±0.13	0.050±0.01	67.3±12

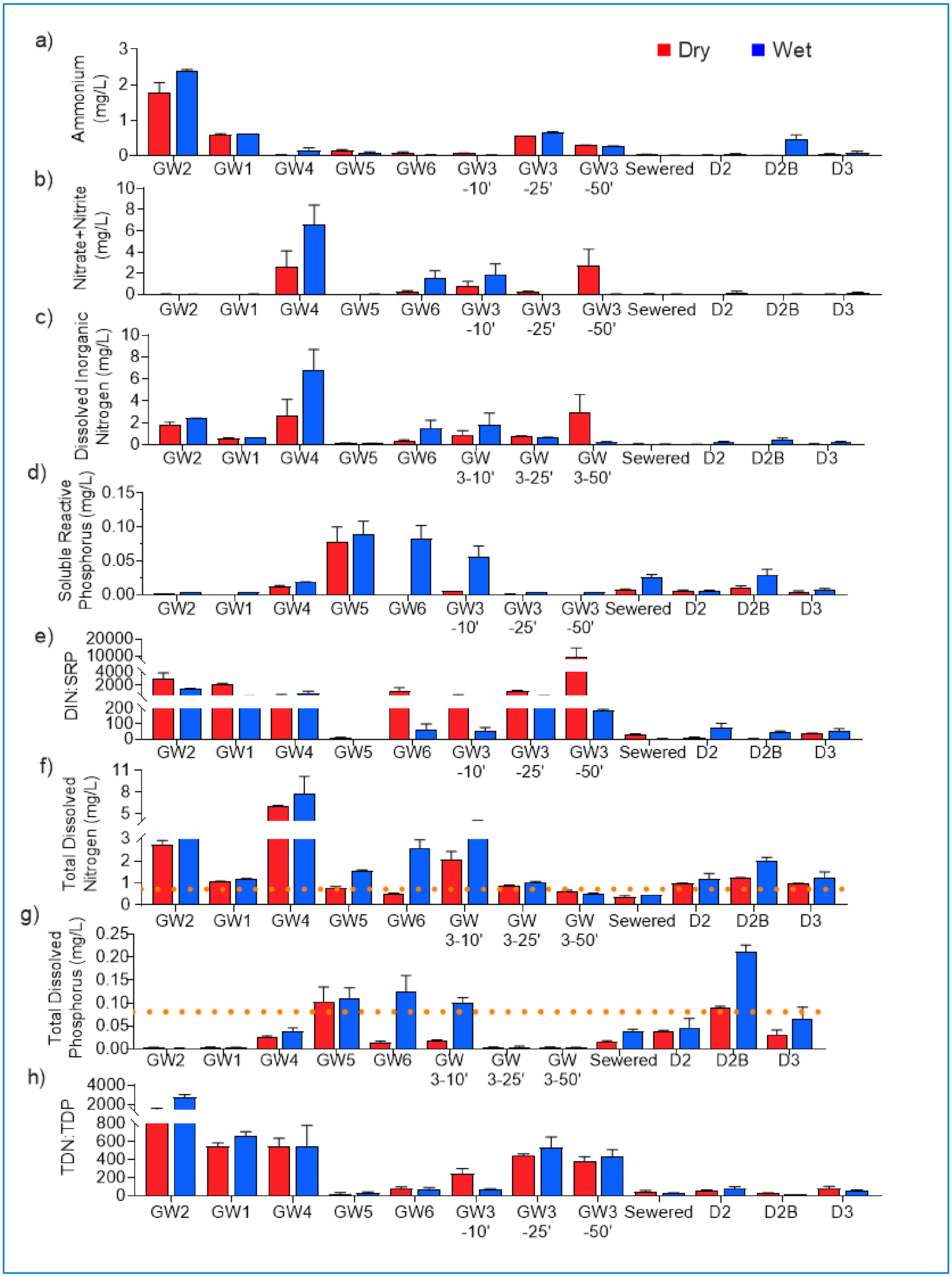

Figure 7. Seasonal dissolved nutrient concentrations and ratios (average ± standard error) observed in the Sagamore basin of CPSL.

DIN was lower in adjacent canals than in the groundwater (Table 2). The highest ammonium was at D2B (0.25 ± 0.10 mg/L), followed by D3 (0.07 ± 0.02 mg/L), and D2 (0.03 ± 0.01 mg/L; Table 2). Interestingly, nitrate + nitrite was lowest at D2B (0.01 ± 0.01 mg/L) with higher concentrations observed at D2 (0.10 ± 0.05 mg/L) and D3 (0.08 ± 0.04 mg/L; Fig. 7b). Overall DIN concentrations were highest at D2B (0.26 ± 0.10 mg/L), followed by D3 (0.15 ± 0.05 mg/L), and D2 (0.13 ± 0.06 mg/L; Table 2).

Some of the monitoring wells near septic systems also had high SRP concentrations. For example, elevated SRP was observed at GW 5 (0.083 ± 0.01 mg/L), GW 6 (0.042 ± 0.02 mg/L), and GW 3- 10' (0.031 ± 0.01 mg/L; Table 2). Seasonally, SRP was higher in the wet season, than the dry season (Fig. 7d).

SRP in the adjacent canals was relatively low. The highest SRP concentration for the adjacent canals was observed at D2B (0.020 ± 0.01 mg/L) with both D2 and D3 having a project average of 0.006 ± <0.001 mg/L). SRP varied seasonally in the adjacent canals with a higher concentration at D2B in the wet season (Fig. 7d).

The DIN:SRP ratio was higher at the groundwater monitoring wells near septic systems (overall average = 1,345), compared to the sewered site (average = 16.65; Table 2). In particular, GW3 -50' (4,935 ± 138), GW2 (2,251 ± 509) and GW1 (1,318 ± 346) had very high DIN:SRP (<1,000). DIN:SRP was higher in the dry season (average = 4,415), than the wet season (average = 526; Fig. 7e).

Average DIN:SRP in the adjacent canals (overall average = 36.9) was lower than in the groundwater near septic systems in the Sagamore basin (overall average 1,345). All the adjacent canals exceeded the Redfield ratio (16), indicating high nitrogen availability. Seasonally, DIN:SRP was higher in the wet season (average = 57.4) than the dry season (average = 16.4).

TDN was high at many of the wells near septic systems (overall average = 2.23 mg/L) and lower at the sewered well (average = 0.40 mg/L). For example, TDN was variable by location with the highest average values at GW 4 (6.94 ± 1.1 mg/L), GW 2 (2.96 ± 0.14 mg/L), and GW 3- 10' (2.58 ± 0.53 mg/L; Table 2). TDN exceeded the FAC standard at all sites, except the sewered site. Seasonally, at the wells near septic systems TDN was higher in the wet season (2.63 mg/L) than the dry season (1.83 mg/L; Fig. 7f).

Average TDN in adjacent canals (1.27 mg/L) was lower than in the wells near septic systems (2.23 mg/L), but higher than the sewered well (0.40 mg/L). D2B had the highest TDN (1.63 ± 0.16 mg/L), followed by D3 (1.10 ± 0.13 mg/L), and D2 (1.08 ± 0.12 mg/L; Table 2). TDN was higher in the wet season (1.48 mg/L) than the dry season (1.05 mg/L) at most of the wells (Fig. 7f).

Some of the groundwater monitoring wells also had elevated TDP concentrations. For example, average TDP was above the FAC standard for the North Fork (0.081

mg/L) at GW 5 (0.107 ± 0.02 mg/L). Average TDP concentrations approached the FAC standard at GW 3- 10’ (0.060 ± 0.02 mg/L) and GW 6 (0.070 ± 0.03 mg/L; Table 2). Seasonally, TDP was higher in the wet season (0.049 mg/L) than the dry season (0.022 mg/L; Fig. 7g).

Average TDP in canals (0.081 mg/L) was higher than the average for the wells near septic systems (0.036 mg/L). The highest TDP was at D2B (0.150 mg/L), followed by D3 (0.050 mg/L), and D2 (0.042 mg/L; Table 2). Seasonally, TDP was higher in the wet season (0.108 mg/L) than the dry season (0.053 mg/L; Fig. 7g).

TDN:TDP ratios were highly variable between wells, but all wells exceeded the Redfield ratio (16), indicating high N loading. The highest average TDN:TDP was at GW 2 (2,119 ± 290), followed by GW1 (603 ± 35) and GW4 (552 ± 111; Table 2). Seasonally, average TDN:TDP ratios were higher in the wet season (636) than in the dry season (472; Fig. 7h).

Average TDN:TDP in the adjacent canals (54.1) was lower than in the groundwater wells near septic systems (554). The highest TDN:TDP was at D2 (69.1), followed by D3 (67.3), and D2B (25.9; Table 2). In the adjacent canals, seasonal average TDN:TDP was similar in the wet (51.1) and dry (57.1) seasons (Fig. 7h).

3.5 Bacterial Prevalence

Overall fecal indicator bacteria counts were relatively low in most groundwater wells (Table 3). However, GW 1 and GW 2 had higher counts of both fecal coliforms and *E. coli* than the other well sites (Table 3, Fig. 8). There were not clear seasonal patterns observed in groundwater bacterial counts (Fig. 9a,b). The FAC Statistical Threshold Value for *E. coli* was not exceeded at any of the groundwater monitoring wells (Fig. 8).

Table 3. Fecal indicator bacteria counts observed in the Sagamore basin study (project average ± standard error).

Type	Site	Count	Fecal Coliform (CFU/100 mL)	*Escherichia coli* (MPN/100 mL)
Groundwater Wells	GW2	4	76.0±46	177±103
	GW1	4	106±61	126±73
	GW4	4	ND	ND
	GW5	4	ND	ND
	GW6	4	ND	0.75±0.75
	GW3-10'	4	1.00	9.5±9.5
	GW3-25'	4	ND	ND
	GW3-50'	4	ND	ND
	Sewered	4	ND	9.00±8.8
Adjacent Surface Water Canals	D2	4	842±530	1,114±446
	D2B	4	369±172	417±157
	D3	4	805±540	2,203±218

The adjacent surface water canals had higher counts than the groundwater monitoring wells (Table 3). At these sites, *E. coli* exceeded the FAC Statistical Threshold Value and fecal coliform counts were also elevated. Seasonally, bacterial counts in the adjacent surface water canals were higher in the wet season, than the dry season (Fig. 9a,b).

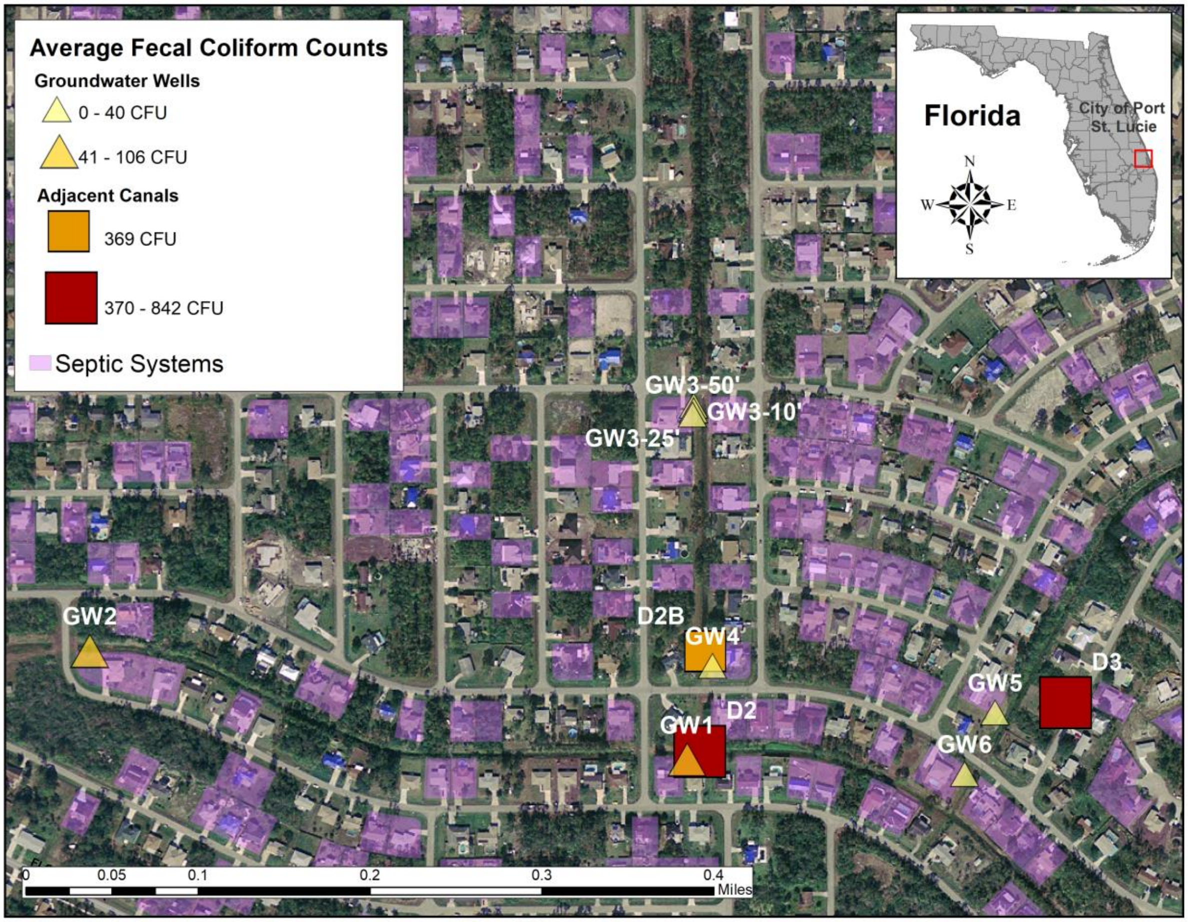

Figure 8. Fecal coliform counts (project average) observed in the Sagamore study area, showing locations of known septic systems (purple shading).

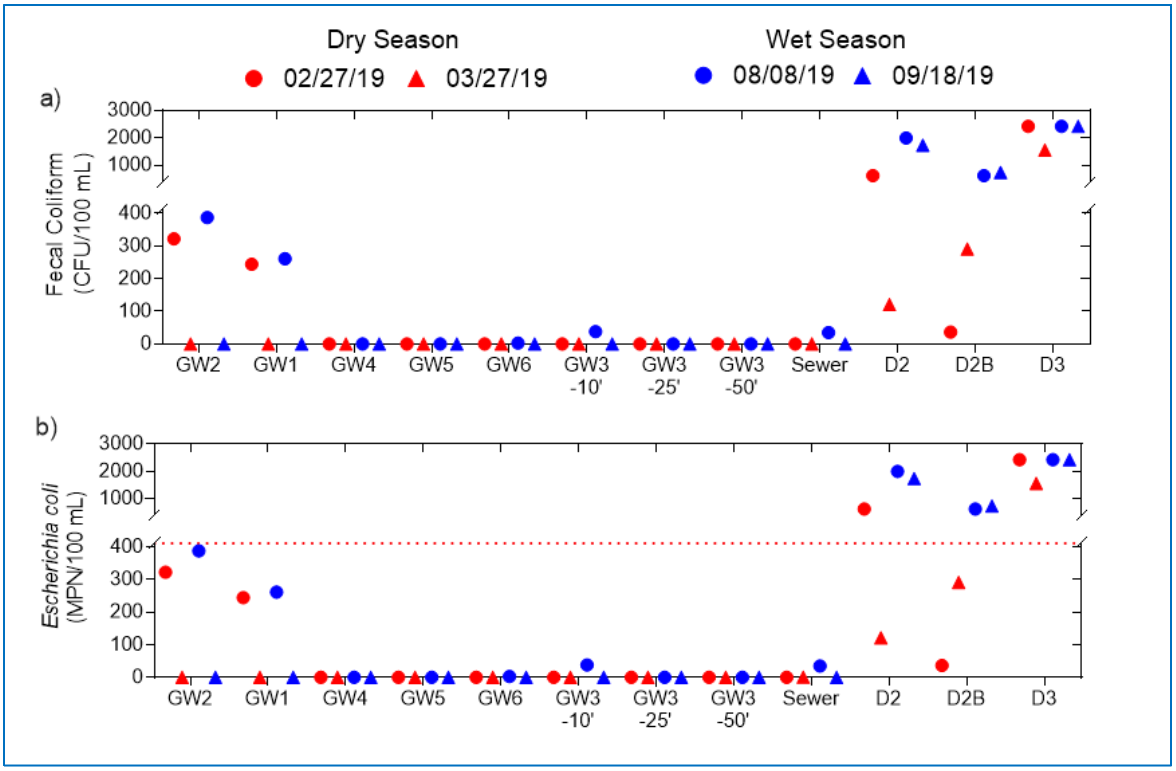

Figure 9. Seasonal fecal indicator bacteria counts by sampling date observed in the Sagamore basin of CPSL for a) fecal coliforms and b) *E. coli* with a dotted line representing the FAC Statistical Threshold Value.

3.6 Chemical tracers

Chemical tracers observed in the groundwater monitoring wells indicated a widespread wastewater presence in the Sagamore basin (Table 4). The artificial sweetener, sucralose, was detected at all the groundwater monitoring wells, though at many locations these detections were "I" flagged, which means the concentrations detected were between the MDL and PQL (Table 4; Fig. 10a). Groundwater monitoring wells with particularly high concentrations of sucralose included GW 6 (6.98 ± 3.3 µg/L), followed by GW 3- 25' (1.10 ± 0.23 µg/L). Other wells with noteworthy sucralose concentrations include GW 1 (0.31 ± 0.06 µg/L), GW 5 (0.21 ± 0.15 µg/L), and GW 3 – 50' (0.40 ± 0.06 µg/L; Table 4). Sucralose was relatively consistent in the groundwater monitoring wells between seasons (Fig. 10a). All acetaminophen detections were very low concentrations and were "I" flagged (Table 4, Fig. 10b). Interestingly, one of these low acetaminophen detections was at the sewered groundwater monitoring well (Fig. 10b). Carbamazepine was detected only at GW 2 in low levels ("I" flagged), while ibuprofen, naproxen, primidone, imazapyr, and hydrocodone were not detected at any of the groundwater monitoring wells during the project (Table 4).

Table 4. Chemical tracer concentrations (project average ± standard error) observed in the Sagamore basin of CPSL; sites with asterisks include “I-flagged” samples that were between the MDL and the PQL.

Type	Site	Count	Sucralose (ug/L)	Acetaminophen (ug/L)	Ibuprofen (ug/L)	Naproxen (ug/L)
Groundwater Wells	GW2	4	0.10±0.04	0.003±0.003*	ND	ND
	GW1	4	0.31±0.06	ND	ND	ND
	GW4	4	0.08±0.05*	0.003±0.003*	ND	ND
	GW5	4	0.21±0.15*	0.005±0.01*	ND	ND
	GW6	4	6.98±3.3	0.008±0.01*	ND	ND
	GW3-10'	4	0.15±0.14*	ND	ND	ND
	GW3-25'	4	1.10±0.23	0.002±0.002*	ND	ND
	GW3-50'	4	0.40±0.06	ND	ND	ND
	Sewered	4	0.01±0.004*	0.003±0.003*	ND	ND
Adjacent Surface Water Canals	D2	4	1.32±0.31	ND	ND	ND
	D2B	4	0.81±0.15	0.003±0.003*	0.01±0.01*	ND
	D3	4	2.55±0.43	ND	ND	ND

Type	Site	Count	Primidone (ug/L)	Count	Imazapyr (ug/L)	Carbamazepine (ug/L)	Hydrocodone (ug/L)
Groundwater Wells	GW2	4	ND	1	ND	0.001±<0.001*	ND
	GW1	4	ND	1	ND	ND	ND
	GW4	4	ND	1	ND	ND	ND
	GW5	4	ND	1	ND	ND	ND
	GW6	4	ND	1	ND	ND	ND
	GW3-10'	4	ND	1	ND	ND	ND
	GW3-25'	4	ND	1	ND	ND	ND
	GW3-50'	4	ND	1	ND	ND	ND
	Sewered	4	ND	1	ND	ND	ND
Adjacent Surface Water Canals	D2	4	ND	1	2.1	0.004±0.002*	ND
	D2B	4	ND	1	51	ND	ND
	D3	4	ND	1	8.3	0.002±<0.001*	ND

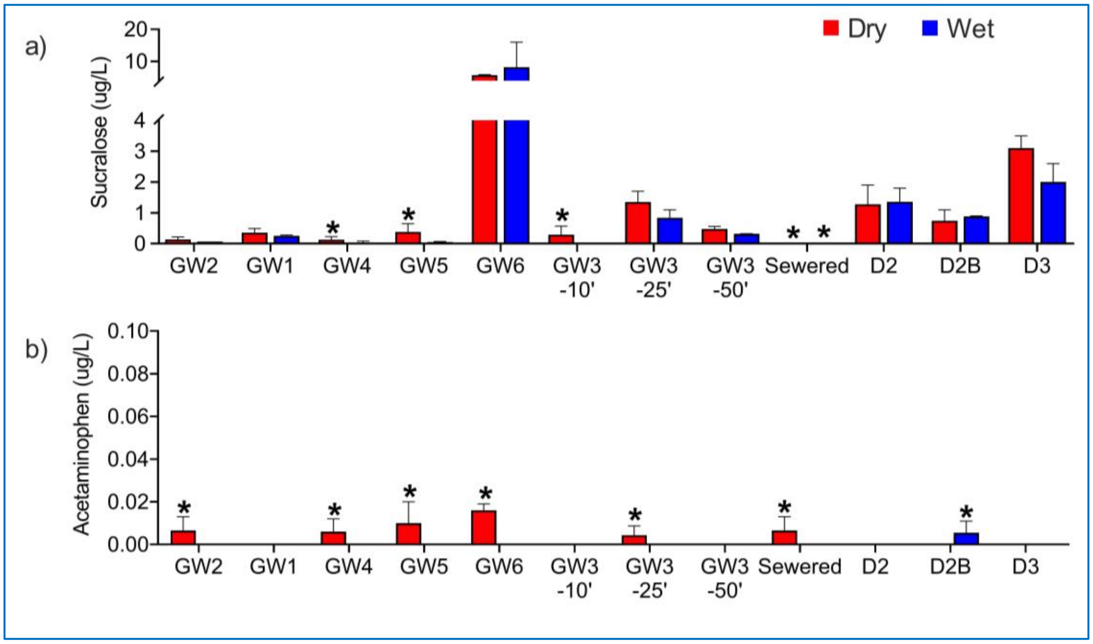

Figure 10. Seasonal human wastewater chemical source tracer concentrations (average ± standard error) observed in the Sagamore basin of CPSL; sites with asterisks include “I-flagged” samples that were between the MDL and the PQL.

The adjacent canal sites also had chemical tracers that indicated the presence of wastewater (Table 4). Sucralose was detected at significant levels in all three sites in both seasons (Fig. 10a, Fig. 11), while acetaminophen was only detected at D2B in the wet season at an “I-flagged” concentration (Fig. 10b). Ibuprofen was also detected at D2B, while carbamazepine and imazapyr were detected at all three adjacent canal sites Table 4). Naproxen, primidone, and hydrocodone were not detected at any of the adjacent canal sites (Table 4).

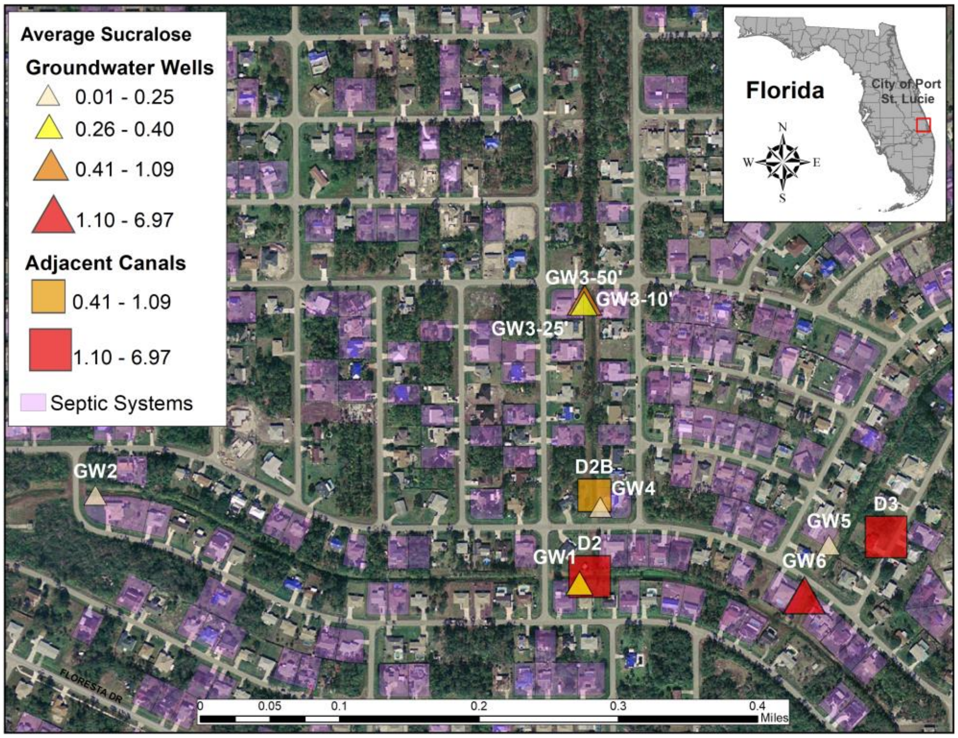

Figure 11. Sucralose concentrations (project average ± standard error) observed in the Sagamore basin of CPSL, showing locations of known septic systems (purple shading).

3.7 Stable nitrogen isotopes

The dissolved $\delta^{15}N$ data are still preliminary and only represent the wet season. The BU-SIL lab plans to rerun these samples to obtain more accurate values and to provide the dry season data. Unfortunately, the BU-SIL lab was shut down because of coronavirus concerns before the analyses were completed and the data will now not be available until later in spring 2020. At that time the report will be updated with an addendum to include the new data.

Despite this, the dissolved $\delta^{15}N$ of groundwater from monitoring wells near septic systems were enriched compared to the groundwater in the sewered area, indicating the influence of septic tank effluent. In particular, these enriched $\delta^{15}N$ values were observed at GW 2, GW 4, GW 3 -10’, and GW 3 -25’ (Fig. 12). GW 1, GW 5, and GW 6 had lower values with enough variability in the measurements that it may indicate a mixed nitrogen source, such as inorganic fertilizers and septic tank effluent. In the sewered area, the $\delta^{15}N$ values of the groundwater were lower and depleted, which is indicative of an isotopically depleted nitrogen source, such as inorganic fertilizers or rainfall (Fig. 12).

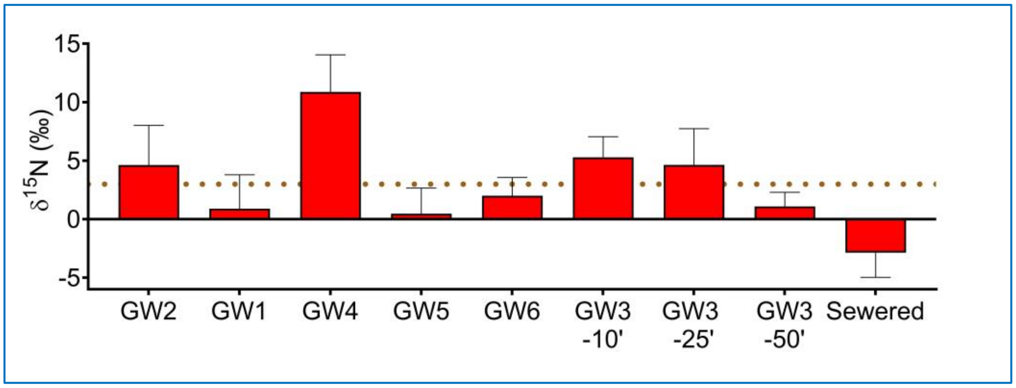

Figure 12. Dissolved nitrogen isotope ($\delta^{15}N$) values for groundwater (average ± standard error) collected from monitoring well sites in the dry season 2019.

Further, the $\delta^{15}N$ values of macrophytes and phytoplankton from the adjacent canal sites supported the availability of wastewater as a nitrogen source available for primary producers. For example, during both the dry and wet seasons, the relatively high average $\delta^{15}N$ values for all macrophytes was indicative of a wastewater nitrogen source (Fig. 13a). Different macrophytes were collected at the adjacent canal sites based on availability during the sampling events (Table 5). Despite these differences, the various species had $\delta^{15}N$ values that were all enriched above +3 ‰, indicating there is a wastewater nitrogen source in these drainage canals (Table 5). Particulate organic matter samples collected as a proxy for phytoplankton at the adjacent canal sites were more variable in $\delta^{15}N$ values, however the averages for D2 and D3 were still above +3 ‰ (Fig. 13b). The average $\delta^{15}N$ value at D2B was slightly below this value, which may indicate a more mixed nutrient source of both septic tank effluent and inorganic fertilizers (Fig. 13b).

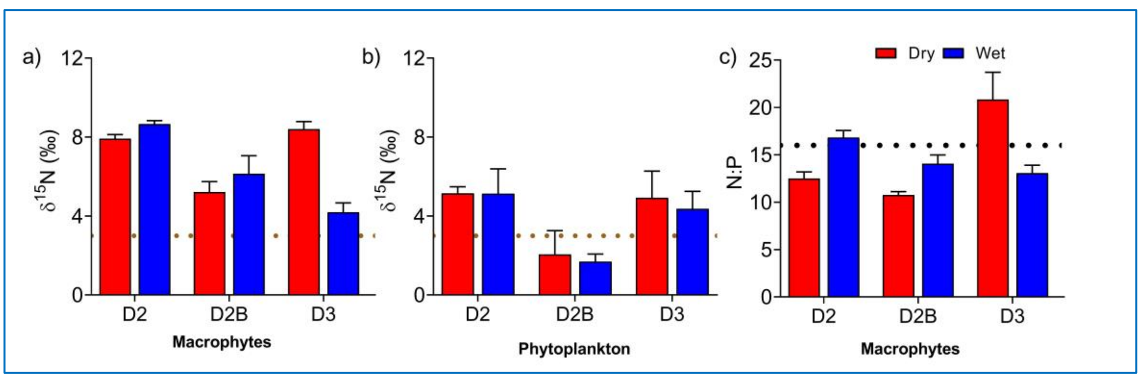

Figure 13. Seasonal stable nitrogen isotope ($\delta^{15}N$) values of primary producers (average ± standard error) observed in the Sagamore basin of CPSL, including a) macrophytes and b) particulate organic matter, a proxy for phytoplankton, as well as c) N:P ratios of macrophytes, showing the Redfield ratio (16).

Table 5. Macrophytes collected by site and season, showing species, stable nitrogen isotope ($\delta^{15}N$) values and N:P ratios (project average ± standard error).

Site	Season	Species	n	δ15N (‰)	N:P
D2			12	+8.29 ± 0.17	14.7 ± 0.8
	Dry	*Hydrilla verticillata*	**6**	**+7.92 ± 0.21**	**12.5 ± 0.7**
	Wet	*Hydrilla verticillata*	**6**	**+8.66 ± 0.17**	**16.8 ± 0.7**
D2B			10	+5.86 ± 0.66	13.1 ± 0.8
	Dry	*Spirodela polyrhiza*	**3**	**+5.22 ± 0.53**	**10.8 ± 0.4**
	Wet		**7**	**+6.14 ± 0.92**	**14.1 ± 0.9**
		Spirodela polyrhiza	6	+5.98 ± 1.07	13.6 ± 0.9
		Hydrilla verticillata	1	+7.09	17.03
D3			17	+6.42 ± 0.60	17.2 ± 1.8
	Dry		**9**	**+8.40 ± 0.39**	**20.8 ± 2.9**
		Spirodela polyrhiza	3	+9.56 ± 0.05	32.0 ± 2.3
		Hydrilla verticillata	6	+7.82 ± 0.40	15.3 ± 0.4
	Wet		**8**	**+4.19 ± 0.47**	**13.1 ± 0.8**
		Unk. Green Algae	1	+6.46	17.31
		Hydrilla verticillata	6	+3.50 ± 0.19	13.0 ± 0.5
		Bacopa sp.	1	+6.03	9.38
Overall Average			**39**	**+6.85 ± 0.35**	**15.4 ± 5.5**

3.8 Objective 1 Discussion

Though it was not possible to install monitoring wells in septic system plumes on private property to capture the full effect of septic systems, the groundwater and adjacent canals were still indicative of wastewater contamination. Qualities of wastewater observed in the groundwater and adjacent canals included, high nitrogen concentrations, elevated N:P ratios, the presence of sucralose, and enriched $\delta^{15}N$ values. These data combined strongly indicate that wastewater is contaminating the Sagamore area of CPSL and contributing to poor water quality downstream in the North Fork.

Nitrogen was higher near septic systems than in the sewered area. For example, while DIN was not as high as has been observed directly in septic system plumes in other areas of South Florida, like Jupiter (21.8 mg/L; Lapointe and Krupa 1995), it was higher in the area near septic systems than in the sewered well. Similarly, on Captiva Island, FL higher nitrate was observed in the groundwater in areas with septic systems compared to sewered areas (Thompson et al. 2012). Further, in this study TDN was also higher near septic systems than in the sewered well, particularly in the wet season. Finally, DIN:SRP and TDN:TDP were very high at monitoring wells near septic systems compared with the sewered well.

This study confirms that the local basin of CPSL is contributing nutrients to the North Fork that can support blooms of harmful algae, such as the blue-green alga *Microcystis aeruginosa* that created a state of emergency for the St. Lucie Estuary in 2016. A similar study of septic system - groundwater - surface water interactions in nearby Martin County, FL found that septic systems were contaminating groundwater and downstream surface waters with sucralose and DIN (Lapointe et al. 2017). Nitrogen from the watershed supports *Microcystis aeruginosa* growth and toxicity, thus reductions of this loading have been identified as an important management need (Kramer et al. 2018). Further, in the St. Lucie watershed chlorophyll *a* concentrations and enterococci counts are positively correlated to dissolved nutrient concentrations (Kelly et al. 2020).

Bacterial counts were higher at GW 1 and GW 2 than in the sewered area. The other groundwater wells in the area near septic systems did not have high concentrations of fecal indicator bacteria. This may indicate that the septic systems in the Sagamore area were able to remove enteric bacteria from septic tank effluent. A similar relationship was observed on Captiva Island by Thompson et al. (2012), which they attributed to a low density of septic systems (1.8 units / hectare). Alternatively, in this study the well locations may have been located too far from the source in diffuse septic system plumes, and thus were not able to capture this signal. For example, in Jupiter when groundwater monitoring wells were placed directly in a septic system plume very high fecal coliform counts were observed (Lapointe and Krupa 1995). However, during that study heavy rainfall occurred, including Tropical Storm Gordon, which may have mobilized more bacteria, whereas this study was conducted during a relatively dry year. Other studies conducted in the North Fork watershed have observed a positive relationship between rainfall and fecal indicator bacteria (Liang et al. 2013).

The adjacent canal sites were consistently high in fecal indicator bacteria, which could indicate that groundwater contaminated with fecal bacteria is flowing into the surface waters from multiple sources, such as septic systems, that have an additive effect. This notion is supported by the presence of human wastewater tracers in these canals, as well as by the enriched $\delta^{15}N$ values (>+3 ‰) observed in most of the macrophyte and phytoplankton samples. Further, the bacteria in adjacent canals may also be sourced from stormwater runoff, sediment disturbances, or other natural sources. Additionally, the high phosphorus concentrations in the adjacent canals and downstream may foster bacterial growth (Mallin and Cahoon 2020). In the study area, microbial contamination correlates with salinity (Ortega et al. 2009; Lapointe et al. 2012), reinforcing the importance of the local watershed.

This study suggests a complicated relationship between septic systems and fecal bacteria. Similar coupled studies conducted in Virginia's coastal plain (Reay 2004) and on Captiva Island (Thompson et al. 2012) found similarly low fecal coliform counts in groundwater near septic systems with higher counts in downstream surface waters. Regardless, a landscape level study of Michigan's lower peninsula found septic systems to be the primary driver of fecal bacteria in surface waters (Verhougstraete et al. 2015). This relationship is supported by a significant reduction in fecal indicator bacteria exceedances at Monroe County, FL beaches following recent upgrades in wastewater infrastructure (i.e. septic to sewer conversions, vacuum sewer, and Advanced Wastewater Treatment; Barreras et al. 2019).

Sucralose is a conservative tracer of human sewage and confirmed the presence of wastewater in the Sagamore basin. For example, sucralose concentrations were higher at the sites near septic systems than the sewered site, indicating the presence of wastewater in the groundwater. Further, the adjacent canals all had relatively high concentrations of sucralose indicating that there was wastewater moving into surface waters in the Sagamore basin. There was little seasonal variability observed in sucralose, indicating the source was not dependent on rainfall. It is not known why very low levels of sucralose and acetaminophen were detected at the sewered site. There have never been septic systems at this site and no sewer leaks or spills have been reported. This detection did not persist or increase to measurable levels throughout the study and thus does not appear to be an issue requiring response by CPSL utilities.

Finally, the $\delta^{15}N$ values of groundwater at the non-sewered sites were all enriched compared to the sewered site, which indicates different nitrogen sources were available in these areas. For example, the sewered site had a depleted $\delta^{15}N$ value more indicative of inorganic fertilizers and rainfall, while the area near septic systems generally had enriched $\delta^{15}N$ values, indicative of a wastewater signal. Enriched groundwater with similar $\delta^{15}N$ values were observed in monitoring wells near septic systems in Jupiter (Lapointe and Krupa 1995) and Martin County (Lapointe et al. 2017).

These data provide multiple lines of evidence supporting the hypothesis that septic tank effluent has negatively affected the quality of groundwater and surface water in the Sagamore area of CPSL. As this neighborhood drains into the North Fork, it is reasonable to conclude that decreasing reliance on septic systems for wastewater treatment in this area, would improve downstream water quality in the North Fork and St. Lucie Estuary. Further, these data also support that continued stormwater improvements would also be beneficial for water quality in the Sagamore drainage basin.

Objective 2: North Fork Long-term Monitoring

2. Methods

2.1 Site descriptions

Surface water sampling was conducted bimonthly from 01/25/2018 – 09/19/2019 at nine canal sites which drain into the North Fork that were previously described by Lapointe et al. (2018).

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.

Sagamore is 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 Sagamore were collected upstream of a concrete outfall structure. Previous sampling by CPSL found high fecal coliform and nutrient concentrations at this site.

Hogpen Slough is located at the H-60 structure. The Hogpen site is primarily serviced by CPSL centralized sewer, with only seven septic systems permitted in this basin. In this basin, there are several industrial lift stations that are not the responsibility of CPSL. Hogpen receives water flow from the Savannas Preserve State Park area, where there are houses located outside of the city limits with septic systems. Samples were collected upstream of a concrete outfall structure. Previously, high fecal coliform counts were observed by CPSL.

Veterans Memorial 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. The water is pumped and drains through ~50 acres of vegetated area before discharging to the river. During the Phase I MST Study, 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 and the Phase I MST Study. After the Phase I MST Study concluded, a control structure and stormwater pond were installed at this location, which stabilized water levels to form a permanent water body. Thus, following installation of this structure water samples have been collected from a deeper water body, therefore any previous influence of sediment disturbance due to shallow waters has been minimized.

Elkcam 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 Elkcam were collected upstream of a concrete outfall structure.

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

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 a concrete structure located on 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 the concrete outfall structure.

A-22 is a concrete structure and 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. Samples were collected upstream of the concrete outfall structure.

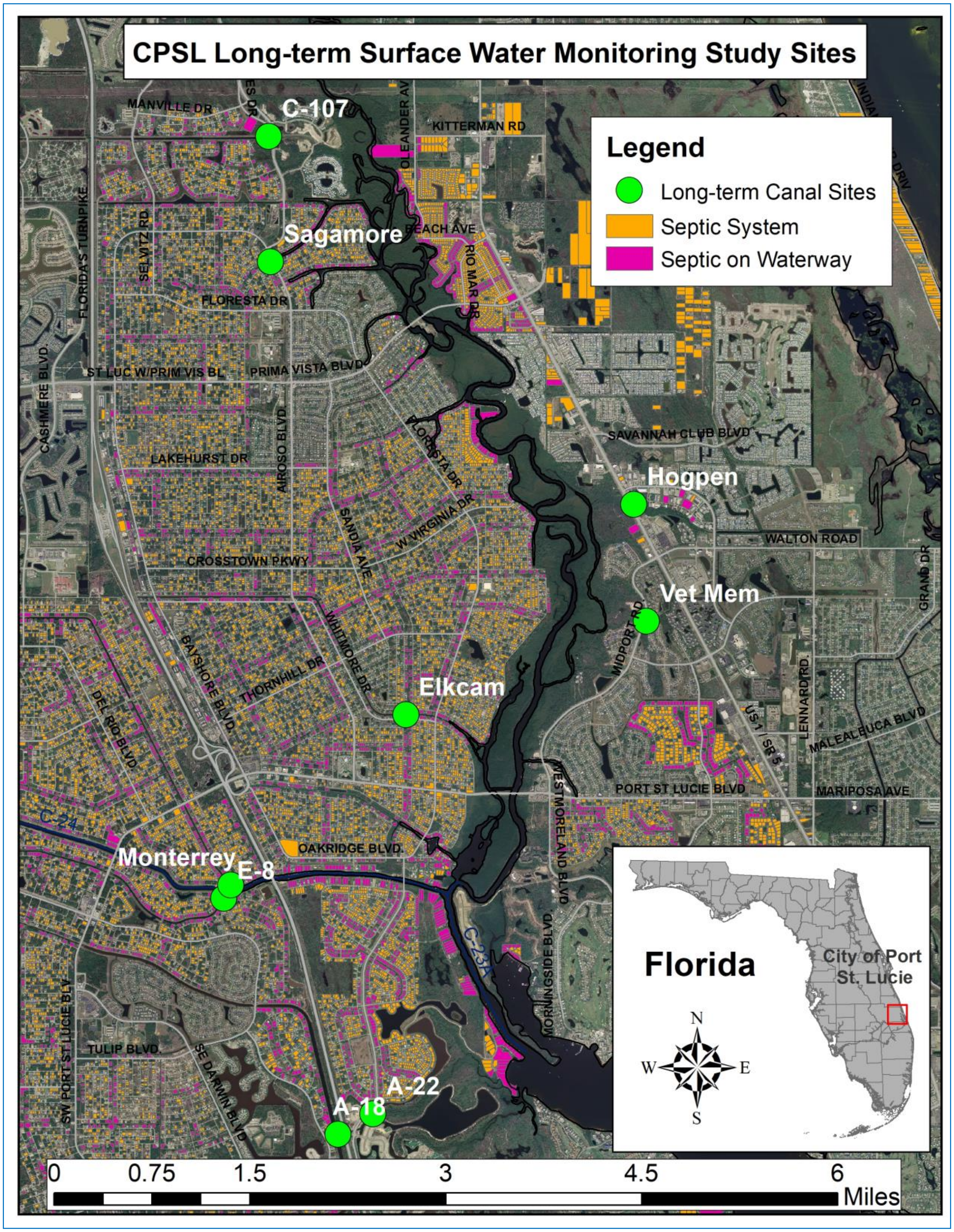

Figure 14. Long-term sampling sites of canals in CPSL that drain into the North Fork (green circles), showing locations of septic systems (orange shading) and septic systems on waterways (pink shading).

2.2 Data collection

Surface water samples were collected bimonthly by CPSL staff following FDEP standard operating procedures and stored on ice in a dark cooler. Water samples were then shipped to Pace Analytical Labs, Ormond Beach, FL for analysis following EPA approved standard methods for the following analytes: turbidity, alkalinity, total suspended solids, BOD (5-day), chlorophyll, and dissolved nutrient concentrations. Nutrient analyses (with MDLs) included ammonium (0.035 mg/L), nitrate (0.025 mg/L), nitrite (0.025 mg/L), soluble reactive phosphate (orthophosphate; 0.0038 mg/L), total Kjedahl nitrogen (0.086 mg/L), total dissolved nitrogen (0.086 mg/L), and total dissolved phosphorus (0.0028 mg/L).

Microbial analyses were conducted bimonthly and handled as in Objective 1. Further, at Hogpen Slough and Veterans Memorial, targeted microbial sampling was conducted at additional sites with a higher frequency to identify the movement of bacteria though these drainage basins.

Macrophytes and phytoplankton were collected for $\delta^{15}N$ analyses at all sites as described in Objective 1.

2.3 Data analyses

MST and nutrient data were compared by considering overall and seasonal site averages. Any trends between concentrations of various parameters were then examined for any relationships between variables. Local knowledge from CPSL and site visits provided land-use data, which was used for ground truth interpretation. These data were considered with the combined with MST and water quality data to make deductions regarding sources of nutrients and bacteria to the North Fork.

Results

3.1 Rainfall

Sampling was conducted approximately bimonthly between 01/2018 – 09/2019. During this period the rainfall was variable allowing for samples to be collected during both wet and dry seasons (Fig. 15). In total, eleven sampling events were conducted.

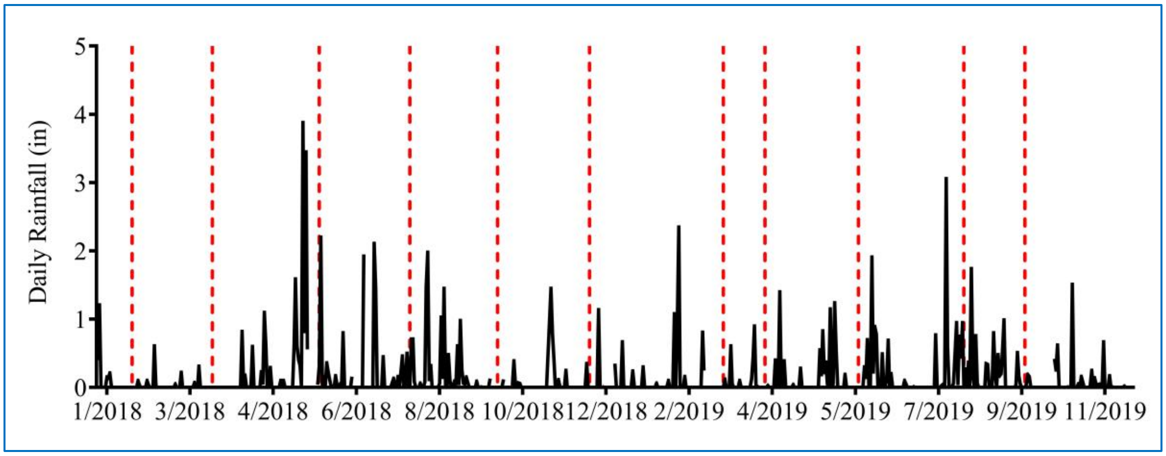

Figure 15. Rainfall as related to long-term surface water monitoring; sampling events represented by red dashed bars.

3.2 Environmental parameters

There was some variability observed in environmental parameters at the long-term monitoring canal sites. pH was similar between all sites ranging from 7.25 ± 0.19 at C-107 to 7.98 ± 0.09 at Veterans Memorial (Table 6). Turbidity was more variable between sites and ranged from an average of 2.50 ± 0.37 at Hogpen to 10.7 ± 1.4 at Sagamore (Table 6). Conductivity was also lowest at Hogpen (292 ± 21 mS), while the highest average conductivity was observed at E-8 (717 ± 27; Table 6). DO varied slightly between sites with the highest at Veterans Memorial (6.90 ± 0.45 mg/L) and the lowest at A-22 (4.83 ± 0.58 mg/L; Table 6).

Table 6. Environmental parameters (project average ± standard error) observed at long-term monitoring sites.

Site	Count	pH	Turbidity (NTU)	Conductivity (mS)	DO (mg/L)
C-107	11	7.25±0.19	3.22±0.49	463±55	5.81±0.53
Sagamore	6	7.40±0.10	10.7±1.4	596±36	5.68±0.87
Hogpen	11	7.91±0.06	2.50±0.37	292±21	5.22±0.51
VetMem	11	7.98±0.09	4.12±0.79	330±15	6.90±0.45
Elkcam	11	7.47±0.07	4.37±0.70	552±25	5.70±0.35
Monterrey	11	7.81±0.05	5.12±0.48	635±22	6.20±0.50
E-8	11	7.78±0.05	3.21±0.47	717±27	6.86±0.52
A-18	11	7.79±0.05	3.29±0.36	475±21	6.69±0.45
A-22	11	7.70±0.06	4.75±0.69	421±24	4.83±0.58

3.3 Bacteria prevalence

Bacterial counts varied by site and by season at the long-term monitoring sites with higher bacterial counts in the wet season and hot spots confirmed at Sagamore, Hogpen, and A-22. For example, average fecal coliforms and *E. coli* were highest at Sagamore, followed by Hogpen (Table 7). Seasonally, fecal coliform counts were also higher in the wet season at most sites, including C-107, Sagamore, Hogpen, Elkcam, Monterrey, E-8, and A-22 (Fig. 16a). Veterans Memorial and A-18 had lower fecal coliform counts in the wet season. Counts of *E. coli* were higher in the wet season than the dry season at Sagamore, C-107, Elkcam, Monterrey, E-8, and A-22 (Fig. 16a,b). Interestingly, A-18 and Hogpen had lower counts of fecal bacteria in the wet season, than the dry. The FAC Statistical Threshold Value for *E. coli* was exceeded during both seasons at Sagamore and during the wet season at Hogpen (Fig. 16a,b). The average BOD was similar between sites with an overall range of 0.43 mg/L between the highest and lowest concentrations; all sites slightly exceeded the FAC standard (2 mg/L; Table 7).

Table 7. Bacterial counts and biochemical oxygen demand (BOD; project average ± standard error) observed at long-term monitoring sites.

Site	Count	Fecal Coliform (CFU)	Count	*E. coli* (MPN)	Count	BOD (mg/L)
C-107	6	61.6±59	7	36.2±33	11	2.03±0.03
Sagamore	5	740±427	6	1,017±449	6	2.02±0.02
Hogpen	6	292±89	7	771±294	11	2.17±0.15
VetMem	6	73.7±29	7	80.7±36	11	2.30±0.17
Elkcam	6	23.8±14	7	46.3±28	11	2.23±0.13
Monterrey	6	65.4±17	7	71.6±15	11	2.35±0.22
E-8	6	87.7±52	7	70.3±37	11	2.31±0.13
A-18	6	42.4±30	7	20.6±11	11	2.45±0.23
A-22	6	171±116	7	120±95	11	2.10±0.08

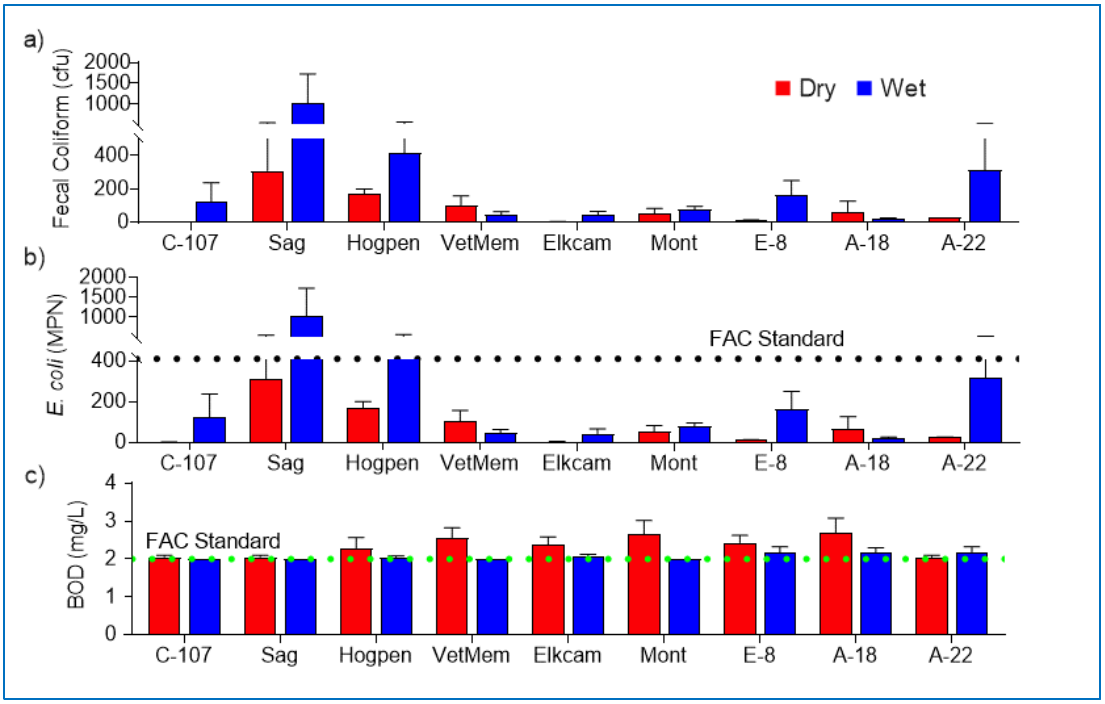

Figure 16. Seasonal bacterial counts (average ± standard error) observed at long-term monitoring sites for a) fecal coliforms and b) *E. coli* with a dotted line representing the FAC Statistical Threshold Value, which is shown for reference but is applicable to single sample points, not averages, as well as c) biochemical oxygen demand (BOD) with a dotted line representing the FAC surface water quality standard.

3.4 Dissolved nutrient concentrations

Variability between site and season was observed in dissolved reactive nutrient concentrations and ratios at the long-term monitoring canal sites, but there were hotspots with consistently high reactive nutrient concentrations at Sagamore and A-22. For example, average ammonium concentrations were highest at A-22 (0.13 ± <0.01 mg/L), followed by Sagamore (0.12 ± 0.04 mg/L; Table 8). Other sites ranged between 0.04 – 0.07 mg/L ammonium (Table 8). Ammonium was generally higher in the wet season, particularly at Sagamore and A-22 (Fig. 17a). Nitrate + nitrite was by far highest at Sagamore (0.16 ± 0.04 mg/L), followed by Hogpen (0.09 ± 0.02 mg/L), and Monterrey (0.08 ± <0.01 mg/L; Table 8). Seasonally, nitrate + nitrite was again highest at Sagamore in the wet season (Fig. 17b). Nitrate + nitrite was higher at Hogpen in the dry season than in the wet season (Fig. 17b). As such, DIN was highest at Sagamore (0.29 ± 0.06 mg/L), followed by A-22 (0.20 ± 0.03 mg/L), Monterrey (0.15 ± 0.03 mg/L), and Hogpen (0.13 ± 0.02 mg/L; Table 8). The greatest seasonal difference in DIN concentrations was observed at Sagamore, where the wet season was much higher than the dry season (Fig. 17c). SRP was also highest at Sagamore (0.05 ± 0.02 mg/L) and A-22 (0.05 ± <0.01 mg/L). Large seasonal differences in SRP were also evident at Sagamore (Fig. 17d). DIN:SRP had a range of about 10 between all sites (Table 8). C-107 and E-8 had higher DIN:SRP in the dry season, than in the wet season (Fig. 17e).

Total dissolved nutrient concentrations, TND:TDP ratios, and chlorophyll *a* concentrations were also variable by site and season with many sites exceeding FAC standards for TDN and TDP. For example, the overall average TDN at all sites, except E-8, exceeded the FAC standard (Table 8). Seasonally, TDN was higher at Sagamore in the wet season (Fig. 17f). TDP exceeded the FAC standard at Monterrey, Sagamore, A-22, and A-18 (Table 8). There were seasonal differences in TDP with all the sites, except Veterans Memorial having higher wet season TDP concentrations (Fig. 17g). TDN:TDP was highest at Veterans Memorial (65.6 ± 9.9), followed by C-107 (50.2 ± 7.3; Table 8). There were some seasonal differences in TDN:TDP with many sites having lower ratios in the wet season (Fig. 17h). Chlorophyll *a* was also variable between sites with the highest concentration observed at A-18 (11.2 ± 3.0 μg/L) and the lowest at Sagamore (2.87 ± 0.15 μg/L; Table 8). Small seasonal differences were observed in chlorophyll *a* with most sites having higher concentrations in the dry season (Fig. 17i).

Table 8. Dissolved nutrient and chlorophyll *a* concentrations, as well as nutrient ratios (project average ± standard error) observed at long-term monitoring sites.

Site	Count	Ammonium (mg/L)	Nitrate+Nitrite (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	Soluble Reactive Phosphorus (mg/L)
C-107	11	0.06±<0.01	0.03	0.09±<0.01	0.020±0.007
Sagamore	6	0.12±0.04	0.16±0.04	0.29±0.06	0.049±0.017
Hogpen	11	0.04	0.09±0.02	0.13±0.02	0.027±0.004
VetMem	11	0.04±0.00	0.03	0.07	0.006±0.001
Elkcam	11	0.07±0.02	0.06±0.02	0.12±0.03	0.024±0.007
Monterrey	11	0.07±<0.01	0.08±<0.01	0.15±0.03	0.041±0.008
E-8	11	0.05±<0.01	0.04±<0.01	0.09±0.02	0.021±0.008
A-18	11	0.04	0.04	0.08±<0.01	0.019±0.005
A-22	11	0.13±<0.01	0.07±0.02	0.20±0.03	0.045±0.007

Site	Count	DIN:SRP	Total Dissolved Nitrogen (mg/L)	Total Dissolved Phosphorus (mg/L)	TDN:TDP	Chl *a* (ugL)
C-107	11	8.6±1.8	0.77±0.05	0.045±0.009	50.2±7.3	3.62±0.54
Sagamore	6	7.3±1.6	1.17±0.16	0.097±0.019	28.3±2.2	2.87±0.15
Hogpen	11	6.2±1.2	0.86±0.04	0.061±0.008	35.8±3.9	4.52±0.91
VetMem	11	13.9±1.7	0.87±0.04	0.034±0.004	65.6±9.9	5.00±0.77
Elkcam	11	7.8±1.7	0.87±0.08	0.065±0.012	34.6±3.3	6.57±2.0
Monterrey	11	4.1±0.7	0.86±0.06	0.107±0.010	18.9±1.6	6.74±1.2
E-8	11	8.2±1.7	0.66±0.05	0.064±0.009	25.4±2.8	7.54±1.8
A-18	11	5.3±0.9	0.86±0.06	0.075±0.009	27.7±2.7	11.2±3.0
A-22	11	4.9±0.5	0.95±0.07	0.093±0.009	23.7±1.8	4.25±0.93

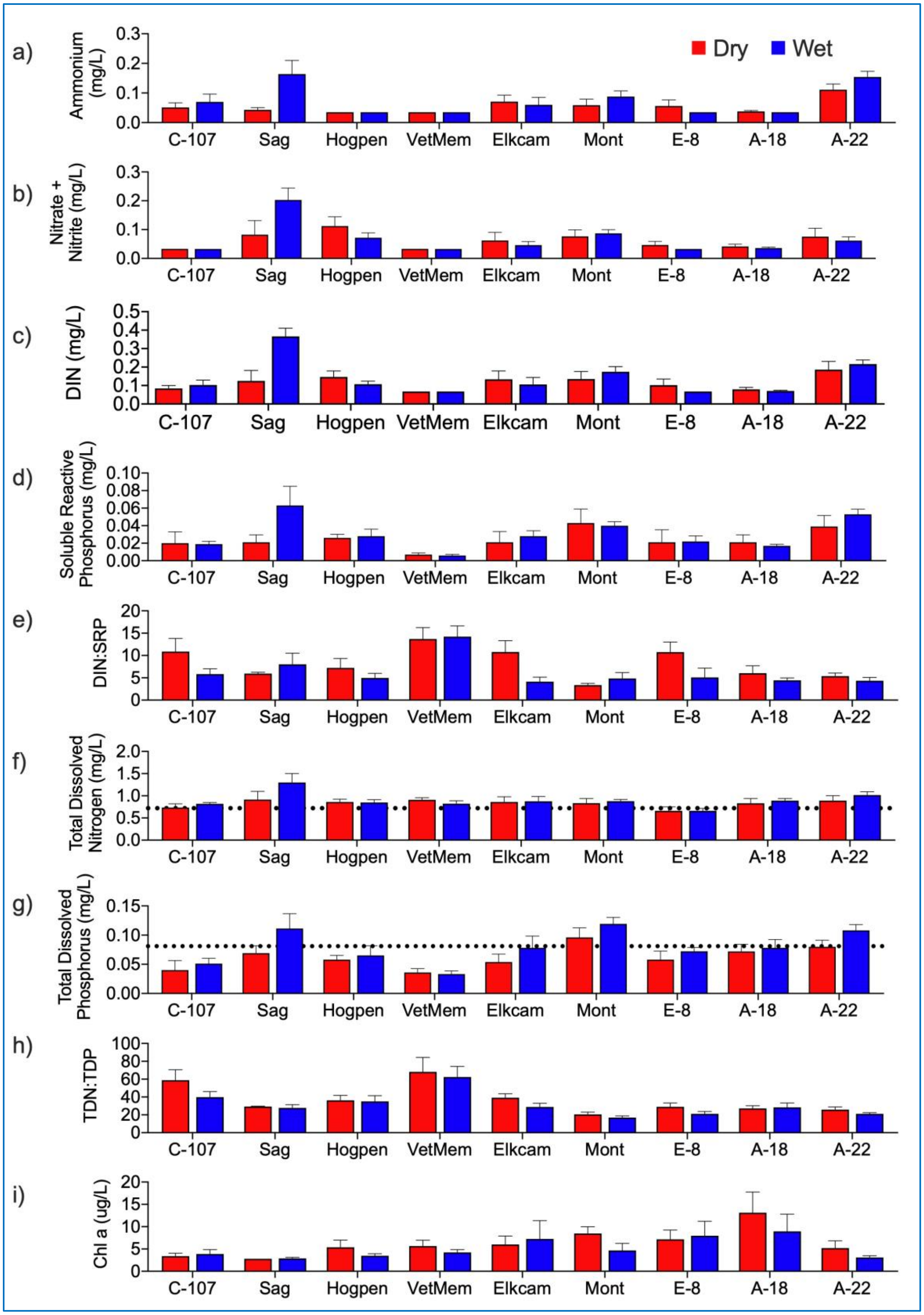

Figure 17. Seasonal dissolved nutrient and chlorophyll *a* concentrations, as well as nutrient ratios (average ± standard error) observed at long-term monitoring sites.

3.5 Stable nitrogen isotopes

The $\delta^{15}N$ values of macrophytes collected at the long-term monitoring canal sites were informative as to nitrogen sources available for primary producers, which included wastewater and inorganic fertilizer. For example, some sites had more depleted values, indicative of an inorganic fertilizer influence. These included C-107 and Hogpen (Table 9). Seasonally, the macrophytes from both sites had more enriched $\delta^{15}N$ values in the wet season, indicative of a wastewater signal (Fig. 18a). Despite this, the phytoplankton collected at C-107 was lower in the dry season and higher in the wet season (Fig. 18b). Interestingly, the phytoplankton from Hogpen had a more enriched $\delta^{15}N$ values than the macrophytes, and both seasons indicate a wastewater influence (Fig. 18b). Many of the other canal sites had macrophyte $\delta^{15}N$ values that indicated wastewater as a nitrogen source (Table 9). For example, the average values at Sagamore (+7.87 ± 0.41 ‰), Veterans Memorial (+3.29 ± 0.38 ‰), Elkcam (+4.06 ± 0.31 ‰), Monterrey (+9.30 ± 0.25 ‰), E-8 (+5.25 ± 0.65 ‰), A-18 (+6.40 ± 0.29 ‰), and A-22 (+5.80 ± 0.27 ‰) were all enriched above +3 ‰ (Table 9).

N:P ratios of macrophytes were close to the Redfield ratio (16), and generally indicated phosphorus limitation, particularly in the wet season (Table 9). Many sites, such as C-107, Sagamore, Monterrey, E-8, Veterans Memorial, and A-18, had higher N:P ratios in the wet season (Fig. 18c). This seasonal rise in N:P reflects the increased availability of N during the wet season at these sites. Macrophytes at Elkcam had similar N:P ratios during the wet and dry seasons (Fig. 18c). Hogpen Slough was the only site where there was a marked decrease in macrophyte N:P from the dry to the wet season, indicating an increase in P availability during the wet season at this site (Fig. 18c).

Table 9. Macrophytes collected at long-term monitoring sites by season and species, showing average (± standard error) stable nitrogen isotope values ($\delta^{15}N$) and nitrogen to phosphorus ratios (N:P).

Site	Season	Species	n	δ15N (‰)			N:P		
C-107			8	+2.34	±	0.35	16.91	±	2.56
	Dry		**5**	**+2.09**	**±**	**0.37**	**13.33**	**±**	**1.49**
		Unk. Green Algae	3	+2.53	±	0.33	12.63	±	2.61
		Hydrilla verticillata	2	+1.44	±	0.57	14.38	±	0.16
	Wet		**3**	**+2.74**	**±**	**0.76**	**25.88**	**±**	**1.66**
		Spirodela polyrhiza	2	+3.50	±	0.07	25.88	±	1.66
		Hydrilla verticillata	1		+1.22			NA	
Sagamore			10	+7.87	±	0.41	15.57	±	1.05
	Dry	*Hydrilla verticillata*	**5**	**+8.06**	**±**	**0.46**	**13.32**	**±**	**1.13**
	Wet		**5**	**+7.69**	**±**	**0.71**	**17.82**	**±**	**1.10**
		Hydrilla verticillata	3	+6.72	±	0.72	16.17	±	0.76
		Chara sp.	2	+9.14	±	0.07	20.29	±	0.39
Hogpen			2	+1.63	±	2.05	19.70	±	3.70
	Dry	*Spirodela polyrhiza*	**1**		**-0.41**			**23.40**	
	Wet	*Bacopa sp.*	**1**		**+3.68**			**16.01**	
Elkcam			13	+4.06	±	0.31	15.70	±	1.03
	Dry		**7**	**+4.80**	**±**	**0.23**	**15.95**	**±**	**1.84**
		Unk. Green Algae	2	+5.28	±	0.65	22.74	±	0.29
		Hydrilla verticillata	5	+4.61	±	0.19	13.24	±	0.79
	Wet	*Hydrilla verticillata*	**6**	**+3.20**	**±**	**0.39**	**15.40**	**±**	**0.85**
Monterrey			12	+9.30	±	0.25	22.09	±	2.70
	Dry	*Pistia stratiotes*	**6**	**+9.48**	**±**	**0.33**	**14.78**	**±**	**1.58**
	Wet	*Pistia stratiotes*	**6**	**+9.13**	**±**	**0.40**	**29.40**	**±**	**2.88**
E-8			11	+5.25	±	0.65	23.69	±	1.41
	Dry		**5**	**+3.55**	**±**	**0.63**	**21.31**	**±**	**1.24**
		Unk. Green Algae	2	+2.04	±	0.41	20.45	±	1.35
		Pistia stratiotes	3	+4.55	±	0.14	21.89	±	2.02
	Wet	*Pistia stratiotes*	**6**	**+6.67**	**±**	**0.61**	**25.68**	**±**	**2.14**
Veterans Memorial			12	+3.29	±	0.38	35.04	±	3.96
	Dry		**6**	**+2.62**	**±**	**0.38**	**22.32**	**±**	**1.38**
		Spirodela polyrhiza	3	+1.77	±	0.08	19.65	±	1.12
		Hydrilla verticillata	3	+3.47	±	0.08	25.00	±	1.05
	Wet		**6**	**+3.96**	**±**	**0.57**	**47.76**	**±**	**1.51**
		Hydrilla verticillata	3	+5.24	±	0.02	44.50	±	0.37
		Utricularia	3	+2.69	±	0.02	51.02	±	0.78
A-18			12	+6.40	±	0.29	23.51	±	2.50
	Dry	*Pistia stratiotes*	**6**	**+6.21**	**±**	**0.12**	**16.71**	**±**	**1.86**
	Wet	*Pistia stratiotes*	**6**	**+6.58**	**±**	**0.59**	**30.31**	**±**	**2.35**
A-22			14	+7.57	±	0.27	22.41	±	1.72
	Dry		**8**	**+8.04**	**±**	**0.28**	**23.71**	**±**	**2.25**
		Unk. Green Algae	2	+6.96	±	0.03	32.64	±	3.18
		Pistia stratiotes	6	+8.40	±	0.20	20.74	±	1.30
	Wet	*Pistia stratiotes*	**6**	**+6.94**	**±**	**0.39**	**20.66**	**±**	**2.71**
Overall Average			**94**	**+5.80**	**±**	**0.27**	**20.14**	**±**	**0.79**

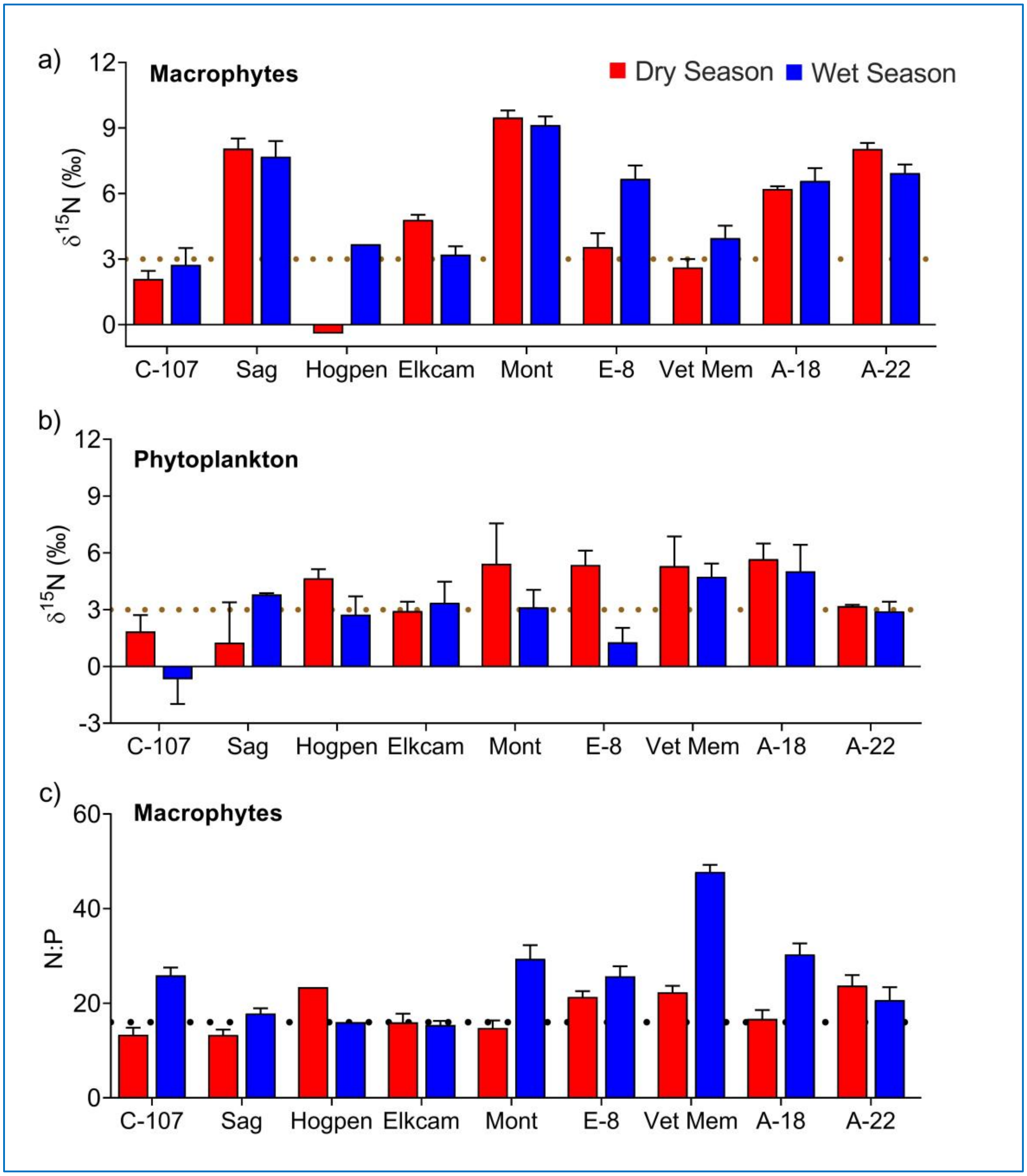

Figure 18. Seasonal stable nitrogen isotope values ($\delta^{15}N$) and nitrogen to phosphorus ratios (N:P) of primary producers (average ± standard error) observed in the long-term monitoring canal sites, including a,c) macrophytes and b) particulate organic matter, a proxy for phytoplankton.

3.6 Targeted bacterial sampling

The Hogpen area consists of mostly sewered parcels with only a few utilizing septic systems (Fig. 19). During the Phase I MST Study, the sites Hog-5, Hog-8, Hog-9, and Hog-10 had bacterial counts that exceeded the FAC Statistical Threshold Value for *E. coli* with counts ranging from 548 to 2,420 MPN/100mL (Fig. 20a;

Lapointe et al. 2018). During the long-term monitoring, multiple sites also exceeded the FAC Statistical Threshold Value for *E. coli*, including HP4, HP5, HP6, HP9, and HP10 (Fig. 20a,b). The sites with the most exceedances were HP5 and HP9.

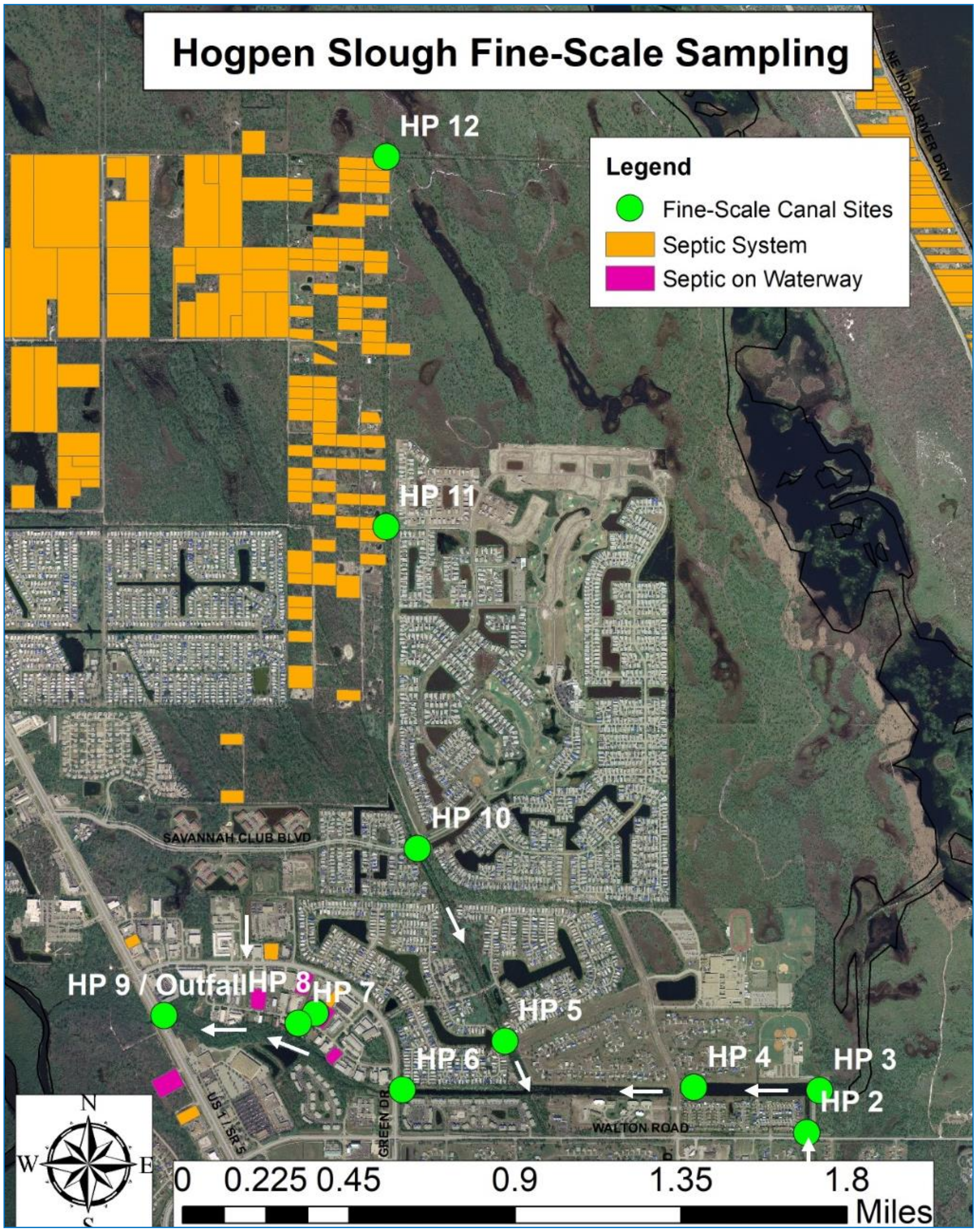

Figure 19. Hogpen Slough study site area targeted, fine-scale sampling for E. *coli* concentrations of surface water showing sampling sites of canals (green circles), septic systems (orange shading), septic systems on waterways (pink shading), and flow direction of water (white arrows) within the system.

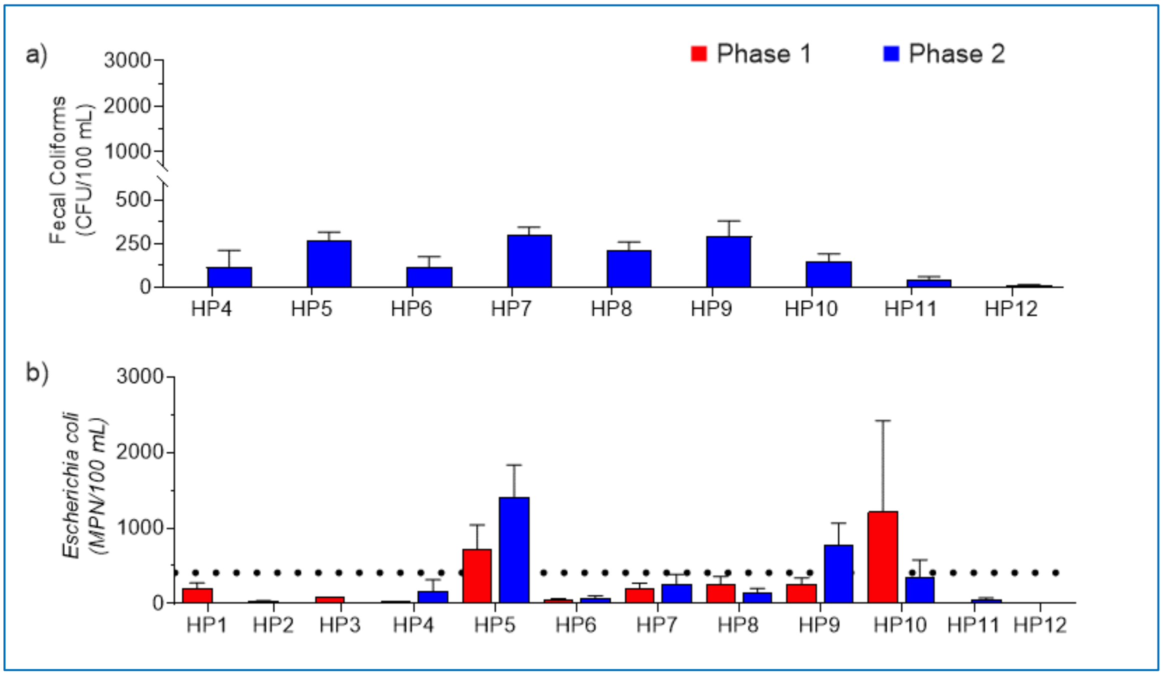

Figure 20. Bacterial counts for Hogpen Slough localized bacteria sampling (average ± standard error), separated by Phase 1 and Phase 2; showing a) fecal coliforms and b) *E. coli* with a dotted line representing the FAC Statistical Threshold Value, which is shown for reference but is applicable to single sample points, not averages.

The Veterans Memorial area is largely sewered having only a few parcels using septic systems (Fig. 21), which would not be expected to widely influence the local water quality. During the Phase I MST Study, the sites Vet-1, Vet-2, Vet-3, Vet-5, and Vet-6 all had sample points that exceeded the FAC Statistical Threshold Value for *E. coli* with counts ranging from 461 to 2,420 MPN/100mL (Fig. 22a). After the Phase I study, a STA was constructed in the Veterans Memorial area. During the long-term monitoring, not one exceedance was recorded (Fig. 22a, b).

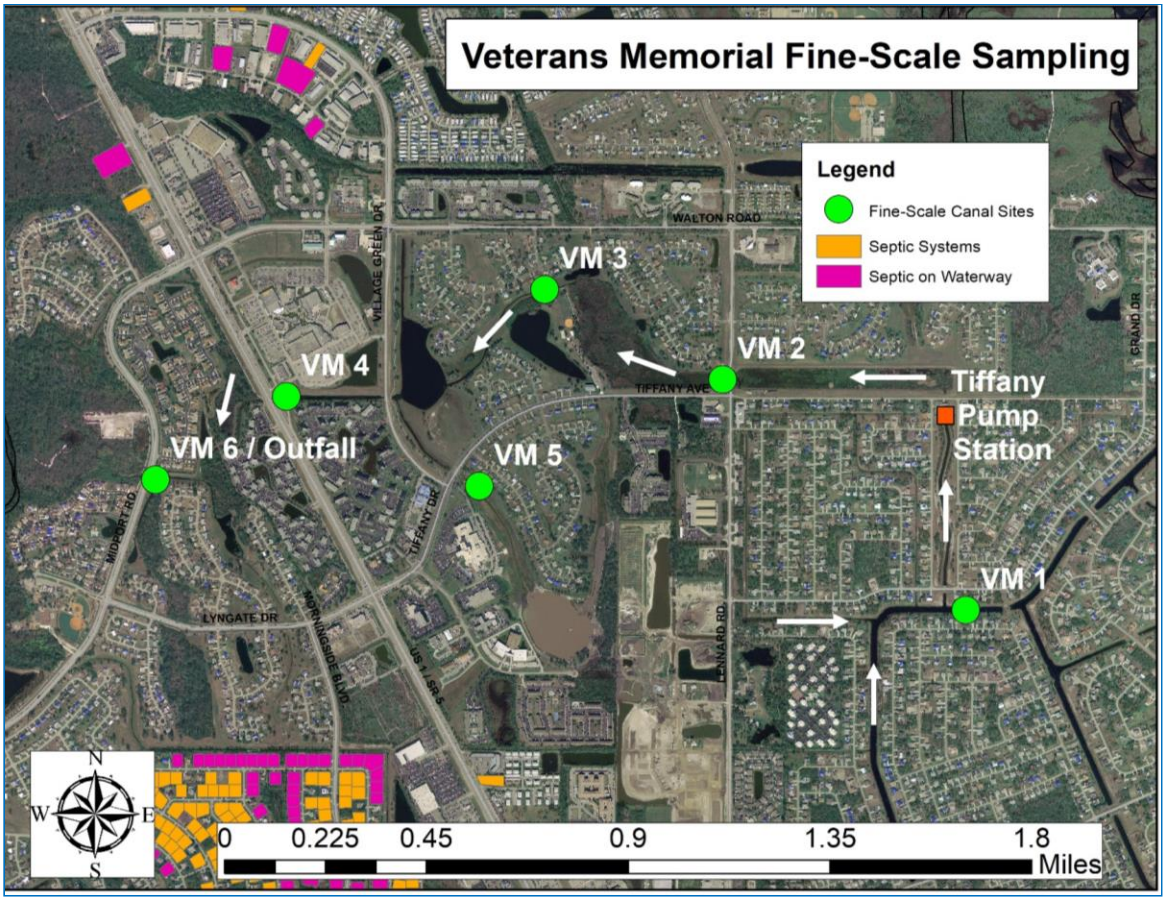

Figure 21. Veterans Memorial (Vet) study site area targeted, fine-scale sampling for fecal indicator bacteria concentrations of surface water showing sampling sites of canals (green circles), septic systems (orange shading), septic systems on waterways (pink shading), and flow direction of water (white arrows) within the system. The stormwater treatment area (STA) is located at VM 6.

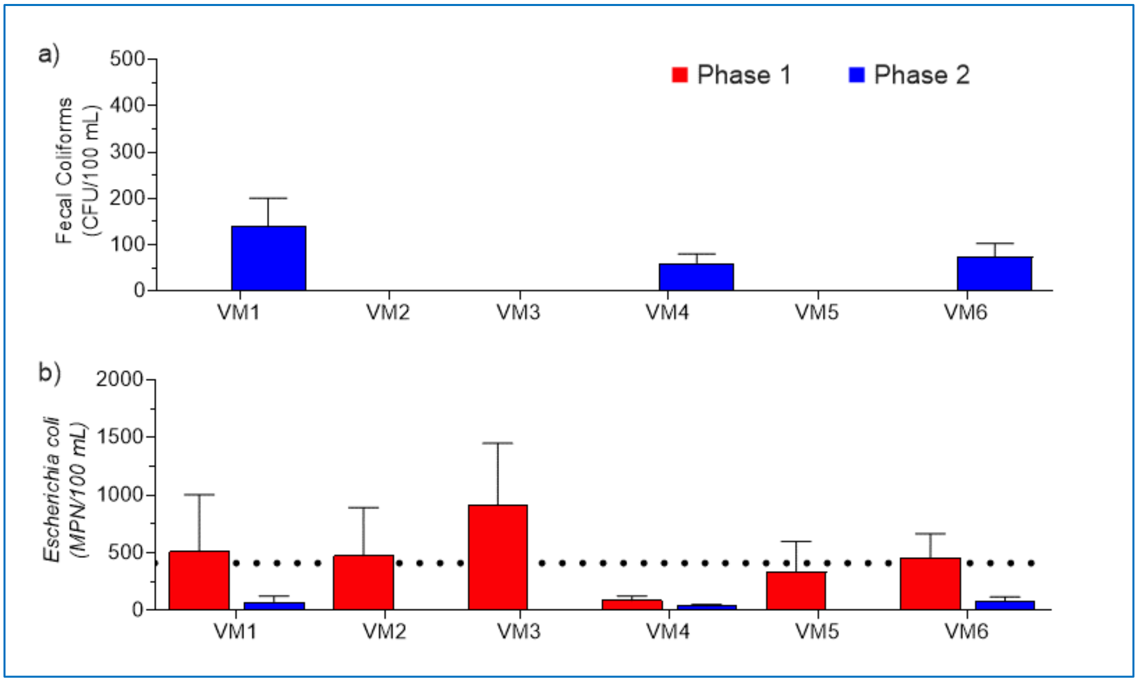

Figure 22. Bacterial counts for Veterans Memorial localized bacteria sampling (average ± standard error), separated by Phase 1 and Phase 2; showing a) fecal coliforms and b) *E. coli* with a dotted line representing the FAC Statistical Threshold Value, which is shown for reference but is applicable to single sample points, not averages.

3.7 By Site Discussion: Long-term Monitoring

C-107

In the Phase I MST Study, the human wastewater tracers, sucralose and acetaminophen, were present at C-107. Further, indicators of stormwater runoff were also present, including diuron, fenuron, and imidacloprid (herbicides and pesticides). In Phase I, fecal indicator bacteria were relatively low at the C-107 site but did increase with rainfall.

During the long-term monitoring, bacteria continued to be relatively low. TDN exceeded the FAC water quality standard at C-107 with generally low concentrations of reactive nitrogen species. TDP was also low, resulting in a relatively high average TDN:TDP ratio (50.2 ± 7.3). $\delta^{15}N$ values for macrophytes at this site indicate a mixed nitrogen source, such as inorganic fertilizers and wastewater. The $\delta^{15}N$ values of phytoplankton were higher in the dry season and lower in the wet season, which indicates inorganic fertilizers or rainfall may be an important nutrient source at this site.

Sagamore

At Sagamore during the Phase I MST sampling, the human molecular marker (HF183) amplified and chemical tracers of wastewater were present, including sucralose, acetaminophen, and carbamazepine. Herbicides and pesticides were also present at this site, indicating the additional influence of stormwater runoff on water quality.

In the long-term monitoring, Sagamore had the highest turbidity of the study (10.7 ± 1.4 NTU) and BOD exceeded the FAC standard. Fecal indicator bacteria counts remained highest at this site for both *E. coli* and fecal coliforms. The long-term monitoring also confirmed elevated nitrogen at Sagamore, inducing ammonium, nitrate + nitrite, and TDN. TDP was also high at Sagamore and both TDN and TDP exceeded FAC surface water quality standards. $\delta^{15}N$ values of macrophytes at Sagamore were enriched in both seasons, indicating a wastewater nitrogen source. Phytoplankton $\delta^{15}N$ values were lower in the dry season and higher in the wet season. This indicates the presence of inorganic fertilizer nitrogen in the dry season and an increase of wastewater nitrogen in the wet season.

Hogpen Slough

The Phase I MST Study detected chemical tracers of human wastewater, stormwater runoff, and macrophyte control at Hogpen. The wastewater tracers, sucralose and acetaminophen, were detected at higher concentrations during the wet season and rain event, indicating that rainfall worsens the wastewater contamination at Hogpen. Nitrate + nitrite was relatively high at this site (average = 0.09 ± 0.02 mg/L)

At Hogpen, during the long-term monitoring the lowest average turbidity of the study was recorded (2.50 ± 0.37 NTU). At this site, average BOD exceeded the FAC standard. Fecal indicator bacteria remained high at this site. $\delta^{15}N$ values indicated variable nitrogen sources at Hogpen. For example, for macrophytes in the dry season the depleted $\delta^{15}N$ value indicated an inorganic fertilizer source, while in the wet season the enriched $\delta^{15}N$ value indicated wastewater. The phytoplankton $\delta^{15}N$ values were enriched during both the wet and dry seasons, further supporting the presence of wastewater nitrogen.

Localized sampling in Hogpen confirmed consistently high fecal indicator bacteria counts. A STA may offer a good solution for improving water quality in Hogpen. This STA would allow water from the north to be cleaned and improve downstream water quality.

Veterans Memorial

Veterans Memorial has historically experienced persistent bacterial issues. During the Phase I MST Study the fecal indicator bacteria counts regularly exceeded the previous FAC standard (400 CFU / 100 mL). The human wastewater tracers sucralose and acetaminophen were also detected at this site and were related to increased rainfall. Further there was chemical evidence to indicate that stormwater runoff also contributed to water quality issues at Veterans Memorial.

Following the Phase I MST report, a STA was constructed in the Veterans Memorial area. After which localized water quality sampling in 2018 and 2019 at Veterans Memorial has not displayed poor water quality. This is an improvement from 2017 when multiple samples exceeded the previous FAC fecal coliform standard. These long-term data illustrate the importance of long-term monitoring to assess the results of remedial management actions.

During the long-term monitoring, BOD exceeded the FAC standard at Veterans Memorial. However fecal indicator bacteria had moderate counts (>100) and *E. coli* counts did not exceed the FAC Statistical Threshold Value. Seasonally, bacteria were slightly lower in the wet season than the dry. Dissolved nutrient concentrations were relatively low, but average TDN did exceed the FAC standard. Interestingly, DIN:SRP and TDN:TDP ratios at Veterans Memorial were the highest of all the sites. Macrophyte $\delta^{15}N$ values at Veterans Memorial were indicative of a mixed source and were more depleted in the dry season than the wet season, indicating increased availability of enriched nitrogen with higher rainfall. As Veterans Memorial is sewered, this could indicate increased stormwater runoff mobilizing wastewater. Phytoplankton $\delta^{15}N$ values at Veterans Memorial in both seasons also indicated a wastewater nitrogen source.

Elkcam

In the Phase I MST Report, sucralose, acetaminophen, and carbamazepine were found at Elkcam, indicating the presence of untreated wastewater. Chemicals tracers of stormwater runoff were also detected during Phase I. Fecal indicator bacteria were high, particularly during the wet season.

In the long-term monitoring, chlorophyll *a* concentrations were moderate (average 6.57 ± 2.0 µg/L) and higher in the wet season. BOD exceeded the FAC standard in both seasons. Both fecal coliforms and *E. coli* were higher during the wet season than the dry season, but the values for both seasons were relatively low. The reactive nutrients, ammonium, nitrate + nitrite, and SRP, were somewhat elevated at Elkcam compared to other study sites. TDN exceeded the FAC standard in both seasons, while TDN only exceeded the standard in the wet season. $\delta^{15}N$ values of macrophytes and phytoplankton were enriched, indicating a wastewater nutrient source.

Monterrey

At Monterrey, sucralose and carbamazepine were detected during the Phase I MST Study, indicating a wastewater influence. Herbicides and pesticides were also detected, indicating that stormwater runoff may also affect water quality at this site. Despite this, fecal indicator bacteria were not high at Monterrey.

At Monterrey, turbidity was slightly elevated compared to other sites during the long-term monitoring (5.12 ± 0.48 NTU). Fecal indicator bacteria counts continued to be low at Monterrey. However, this site had elevated BOD that exceeded the FAC standard, indicating high microbial activity. Dissolved nutrient concentrations were moderately high compared to other sites. TDN and TDP exceeded the FAC standard at Monterrey. In fact, this site had the highest average TDP observed in the long-term monitoring. The average $\delta^{15}N$ values of macrophytes and phytoplankton were all in the range that indicated a wastewater nutrient source.

E-8

During the Phase I MST study, chemical wastewater and stormwater tracers were observed at E-8. This site had low fecal indicator bacteria counts, that were higher in the wet season than the dry season. Dissolved nutrient concentrations at this site also varied seasonally.

In the long-term monitoring, turbidity was low (3.21 ± 0.47 NTU), compared to other sites in the study. Chlorophyll *a* concentrations were relatively high (7.54 ± 1.8 µg/L), BOD exceeded the FAC standard, and bacterial counts were generally low (< 100). Dissolved nutrient concentrations were relatively low at this site and the averages of TDN and TDP were just under the FAC standard. Macrophytes collected at E-8 had enriched $\delta^{15}N$ values, indicating a wastewater nitrogen source. Phytoplankton $\delta^{15}N$ values were more variable and had an enriched wastewater signal in the dry season, but a more depleted value in the wet season, indicative of inorganic fertilizers.

Horseshoe A-18

Chemical tracers of human wastewater and stormwater runoff were both present at A-18 during the Phase I MST Study. Fecal indicator bacteria were low and peaked in the wet season. Dissolved nutrient concentrations at this site were higher in the wet season.

During the long-term monitoring, the highest average chlorophyll *a* concentration of the study (11.2 ± 3.0 µg/L) was at A-18. Fecal indicator bacteria counts were relatively low at A-18 (> 50). Despite this, BOD still exceeded the FAC standard. Dissolved nutrient concentrations were relatively low at A-18, but average TDN still exceeded the FAC standard. The average TDP was just below the FAC standard. Macrophytes and phytoplankton collected at A-18 had enriched $\delta^{15}N$ values indicative of a wastewater nutrient source.

Southbend / Horseshoe A-22

At the A-22 site, chemical markers of wastewater, stormwater, and macrophyte control were detected during the Phase I MST Study. The presence of acetaminophen during the wet season indicated that the presence of untreated wastewater in this system is increased by rainfall. This site had moderate levels of fecal indicator bacteria, which were highest in the wet season. DIN was elevated at A-22 during the wet season.

During the long-term monitoring, turbidity was relatively high (4.75 ± 0.69 NTU) and BOD exceeded the FAC standard. Fecal indicator bacteria counts were moderate (average for fecal coliforms = 171 ± 116 CFU/100 mL and *E. coli* = 120

± 95 MPN/100 mL). These fecal indicator bacteria counts were higher in the wet season, further connecting the water quality at this site with rainfall. Ammonium was higher in the wet season and the highest average concentration of the long-term monitoring was observed at A-22 (0.13 ± <0.01 mg/L). Interestingly, this site also had the highest SRP. DIN, TDN, and TDP were all relatively high at A-22. Macrophytes and phytoplankton collected at A-22 had enriched $\delta^{15}N$ values, indicative of a wastewater nutrient source.

Conclusions

This study provided further insight into factors likely affecting water quality in the North Fork. In the Sagamore drainage basin of CPSL serviced by septic systems, there is evidence that wastewater is present in the groundwater, as well as the adjacent and downstream surface waters, including the North Fork. Because there is no reuse water application in these areas that could be contributing to this wastewater signal, it can be concluded that septic systems are not protective of local water quality in CPSL surface waters.

Objective 1 of this study examined the couplings of groundwater and surface water in the Sagamore basin of CPSL. Multiple lines of evidence indicated that septic tank effluent was impacting localized water quality. For example, the widespread presence of sucralose in both groundwaters and adjacent canals indicated the presence of human wastewater. $\delta^{15}N$ values for groundwater in the non-sewered area were enriched and within the range expected for a wastewater nitrogen source (> 3 ‰), while the sewered well had depleted $\delta^{15}N$ values. Macrophytes and phytoplankton collected at canal sites adjacent to septic systems also had elevated $\delta^{15}N$ values indicating uptake of wastewater nitrogen. Further, the dissolved nutrient concentrations were indicative of a negative groundwater influence on adjacent and downstream surface waters. For example, the elevated DIN, DIN:SRP, TDN, and TDN:TDP in the groundwater near septic systems may be the result of wastewater influence. This is supported by the lower nitrogen concentrations at the sewered site. There was not a clear relationship established between bacterial loading from groundwater to adjacent surface waters, which may be an artifact of the limited locations available for groundwater well installation.

Objective 2 of this study sought to determine nitrogen sources contributing to eutrophication and fecal pollution of the North Fork using $\delta^{15}N$ analyses of macrophytes and phytoplankton at long-term monitoring sites. Nitrogen sources varied by site and season. The $\delta^{15}N$ values for macrophytes and phytoplankton at some sites were highly indicative of a wastewater influence, such as Sagamore, Monterrey, E-8, A-18, Elkcam, and A-22. This is supported by the high dissolved nutrients and fecal indicator bacteria at these sites. These findings also reinforce the conclusions of the Phase I study and Lapointe et al. (2017), which indicated that wastewater was contributing nutrients to the North Fork and St. Lucie Estuary. Other sites had a more variable, mixed signal, such as Hogpen and Veterans Memorial. Similarly, fecal indicator bacteria counts were also variable at Hogpen and Veterans Memorial. While Hogpen and Veterans Memorial were both variable in $\delta^{15}N$ and bacteria, the trends in dissolved nutrient concentrations were different between the sites, with Hogpen having higher DIN and Veterans Memorial having very low SRP. This reinforces the need to assess water quality on a site-by-site basis. The lower $\delta^{15}N$ value of the C-107 indicates more of an influence of inorganic fertilizers and rainfall, particularly in phytoplankton samples. The dissolved nutrient concentrations and bacteria were also relatively low at C-107. In conjunction with the data from Phase I and ongoing monitoring, the addition of $\delta^{15}N$ monitoring allowed for confirmation of the Phase I finding that wastewater is present throughout the North Fork and that for many locations connecting septic systems to centralized sewer will help improve local water quality. Further, the $\delta^{15}N$ values revealed the seasonality of nitrogen sources at the various sites, which will allow the development of site-specific solutions for water quality improvement. For example, the construction of artificial wetlands can help remove nitrogen (Lee et al. 2009; Mallin et al. 2012) and enteric bacteria (Vymazal 2005; Mallin et al. 2012). Finally, the long-term monitoring conducted provides an in depth understanding of localized water quality at these sites, which will enable site specific management actions to be undertaken by CPSL.

Recommendations

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

Recommendations for water quality improvement

- CPSL has been very proactive in implementing an ambitious septic-to-sewer program. We recommend continued focus on connecting septic systems to centralized wastewater treatment at hotspots confirmed in this study (Sagamore, Monterrey, E-8, A-18, Elkcam, and A-22), as well as other septic systems near waterways to mitigate the water quality issues.
- Given the success demonstrated at Veterans Memorial, stormwater improvements, such as the constructions of STAs and wetlands, should be continued and expanded to mitigate stormwater runoff issues. This will increase in importance as severe storm events increase with climate change.
- Ongoing water quality research and monitoring to continuously evaluate the effectiveness of infrastructure improvements is highly recommended. Long-term monitoring will allow for continued re-assessment to ensure that management actions achieve environmental goals.

Recommendations for further research and monitoring

- Continued monitoring of water quality at groundwater wells and adjacent canals following sewering is recommended to document the recovery of water quality in the groundwater and surface waters.
- The continuation of long-term water quality monitoring is highly recommended to provide a baseline to gauge status and trends as a result

of septic-to-sewer programs, stormwater treatment, or other infrastructure improvements made within the system.

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