

Caloosahatchee River – North Fort Myers Nutrient and Bacteria Study: Year 2



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Executive Summary

Nutrient and bacterial pollution of coastal waters is a growing global issue with many ecological and public health implications. In Southwest Florida, increasing population densities combined with aging wastewater infrastructure and extreme weather events have led to deteriorating water quality conditions. As such, bacterial contamination and harmful algal blooms (HABs) have resulted in closures of water bodies for recreational use. The Caloosahatchee River, one of the major sources of freshwater in Southwest Florida, is impaired for nutrients, chlorophyll *a*, dissolved oxygen (DO), and fecal coliforms. A total maximum daily load (TMDL) has been adopted into state rule and approved by the EPA for nutrients, chlorophyll *a*, and DO (602-304.800 F.A.C., effective August 7, 2009). Along the Caloosahatchee River in Lee County, North Fort Myers has experienced persistent bacterial pollution at North Shore Park.

To address this human and environmental health issue, Lee County Division of Natural Resources (LCDNR), Florida Department of Environmental Protection (FDEP), and Harbor Branch Oceanographic Institute-Florida Atlantic University (HBOI-FAU) conducted a multi-year microbial source tracking (MST) study. This MST study combined groundwater and surface water sampling to understand the couplings of septic systems, groundwater, and surface waters in North Fort Myers and identify factors contributing to this persistent bacterial pollution. In the year 1 study, human wastewater from septic systems was identified as a major contributor to poor water quality in North Fort Myers, but many unusual environmental conditions also occurred, such as extreme rainfall and Hurricane Irma. Therefore, the study was continued for a second year with EPA 319 funds granted through FDEP to assess if human wastewater from septic systems continued to be a major source of bacterial and nutrient pollution in North Fort Myers during varied environmental conditions and over multiple years.

The year 1 study began in 2017 with the installation of ten shallow groundwater monitoring wells and the selection of ten surface water sites within the major watersheds of North Fort Myers: Hancock Creek, a central drainage feature between the US-41 and US-41 Business bridges, and Powell Creek. Water samples were collected from these locations twice seasonally (wet / dry) over two project years (2017-2018 and 2019-2020). Groundwater samples were collected from the wells to determine environmental parameters, bacterial abundance, nutrient concentrations and molar ratios, the presence of chemical tracers of wastewater and stormwater, and aqueous stable isotope values of ammonium ($\delta^{15}\text{N-NH}_4^+$) and nitrate ($\delta^{15}\text{N-NO}_3^-$). Surface water samples were analyzed to determine environmental parameters, nutrient concentrations, bacterial abundance, and the presence of molecular markers and chemical tracers. Particulate organic matter (POM) was collected at each surface water site, as a proxy for phytoplankton, and analyzed to determine stable nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotope values, as well as elemental composition (C:N:P), which can be interpreted to indicate the nutrient sources available for primary production (e.g., algal blooms and plant growth). POM samples were also collected opportunistically during HABs that occurred in Lee County during the study period, including blue-green algae (*Microcystis aeruginosa*) and red tide (*Karenia brevis*), and analyzed as above. Additional surface water samples were collected monthly during 2017-2018 for higher resolution monitoring of nutrients and bacterial abundances.

Groundwater monitoring in year 2 of the study confirmed the influence of septic systems from year 1 within the North Fort Myers study area. Water levels in the study area were high throughout the entire study period with an overall mean depth to water table of $2.5 \pm <0.1'$. This suggests septic systems may not always meet the minimum Florida Administrative Code requirements for the separation of septic system drainfields and the seasonal high water table ($\sim 3.5'$). For depth to groundwater measurements recorded during sampling events, 100% were $<3.5'$ in the central drainage basin and Powell Creek, while 58% were $<3.5'$ in Hancock Creek. Fecal indicator bacteria (FIB) abundance in groundwater from all three low elevation watersheds near dense septic systems occasionally exceeded FDEP surface water Ten Percent Threshold Values (TPTV) for both enterococci (≥ 130 MPN/100 mL) and *Escherichia coli* (*E. coli*; ≥ 410 MPN/100 mL). Biochemical oxygen demand (BOD) also sometimes exceeded FDEP surface water quality standards (>2.4 mg/L) in Powell Creek and Hancock Creek. Further, dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite) concentrations were higher in groundwater wells near dense septic systems compared to the reference well. For example, average ammonium (NH_4^+) concentrations in groundwater wells were especially high (> 1 mg/L) in the Powell Creek watershed (5.53 mg/L) and elevated in the central drainage feature (1.36 mg/L), while nitrate was high in the Hancock Creek watershed (1.08 mg/L). The groundwater DIN to soluble reactive phosphorus (SRP, DIN:SRP) and total nitrogen to total phosphorus (TN:TP) molar ratios within the Powell Creek watershed were high, indicating septic tank effluent moving through the soil, where phosphorus is selectively removed by adsorption relative to nitrogen. Sucralose, an artificial sweetener that is often used as tracer of human wastewater, was detected in every well. Concentrations of sucralose were higher at low elevation wells near dense septic systems, usually exceeding “moderate” levels (100 to 1,000 ng/L) and were often “significant” ($> 1,000$ ng/L). The pharmaceuticals carbamazepine, primidone, and acetaminophen were also frequently detected at low elevation wells near dense septic systems, indicating the presence of wastewater. Chemical tracers relating to stormwater runoff (i.e., pesticides, herbicides) were typically not present in groundwater, however there were detections of the herbicides bentazon and fluridone at some wells, indicating rapid percolation of stormwater to the water table. At low elevation wells near dense septic systems, aqueous $\delta^{15}\text{N}\text{-NH}_4^+$ and $\delta^{15}\text{N}\text{-NO}_3^-$ were within the range expected for wastewater nitrogen (means of $+4.3\text{ ‰}$ and $+5.9\text{ ‰}$, respectively).

Surface water sampling in year 2 confirmed findings from year 1 that there are recurring bacterial pollution issues within the North Fort Myers study area, as well as evidence of wastewater, stormwater runoff, and avian influences on water quality. Enterococci and *E. coli* counts often exceeded the FDEP TPTV, though BOD was generally below the FDEP standard. Surface water samples had elevated nutrient levels, including high concentrations of DIN (overall mean = 0.11 ± 0.01 mg/L) and SRP (overall mean = $0.11 \pm <0.01$ mg/L) observed in all watersheds. Furthermore, concentrations of TN (overall mean = 0.80 ± 0.02 mg/L) exceeded the FDEP surface water protective standard for the Lower Caloosahatchee River (≥ 0.5 mg/L). TP (overall mean = 0.16 ± 0.01 mg/L) also exceeded FDEP criteria for the peninsular stream region (≥ 0.12 mg/L), as well as the Lower Caloosahatchee (≥ 0.04 mg/L). Surface water sites had very low DIN:SRP ratios (overall mean = 1.1 ± 0.1) and low TN:TP ratios (overall mean = 14.6 ± 0.6), indicating strong nitrogen-limitation for primary production. This is critical for addressing blooms of the red tide organism (*Karenia*

brevis) and some blue-green algae species (such as *M. aeruginosa*) that thrive where N:P ratios are low. At least one avian marker (GFD or Gull2) was detected at each surface water site, which suggests some of the bacteria loading may come from birds. However, the human bacteria marker (HF183) was also detected at every site, except SW6, suggesting the widespread influence of human wastewater. Additionally, sucralose was also detected at every surface water site during the study period, usually in “moderate” concentrations and sometimes in “significant” concentrations. Pharmaceuticals, including carbamazepine, primidone, and acetaminophen were detected throughout the study area and were highest at the Powell Creek sites. The presence of these chemical tracers and human associated marker suggests that wastewater is ubiquitously present in surface waters throughout North Fort Myers. Additionally, the detection of the herbicides 2,4-D, bentazon, diuron, fluridone, and imazapyr, as well as the pesticide imidacloprid, indicate that stormwater runoff and chemical macrophyte control also affect surface water quality. $\delta^{15}\text{N}$ values of POM at study sites were within the range of wastewater nitrogen (overall mean = $+4.69 \pm 0.18 \text{ ‰}$). Study POM $\delta^{15}\text{N}$ values were also similar to those of *M. aeruginosa* collected at North Shore Park ($+8.79 \pm 0.04 \text{ ‰}$) and Davis Boat Ramp ($+6.93 \pm 0.81 \text{ ‰}$) in Fort Myers, as well as *K. brevis* from coastal areas ($+3.85 \pm 0.23 \text{ ‰}$). These combined data show the linkage of the local watershed to downstream HABs.

The multiple lines of evidence in this study indicated a widespread influence of human wastewater on local water quality in North Fort Myers and the Caloosahatchee River over both years and all seasons of the study. There is no reuse water application or wastewater treatment plant effluent discharge in the study area; therefore, septic systems are likely the most significant wastewater source in North Fort Myers. This is supported by the very shallow water tables that would not provide adequate separation of the drainfield from the groundwater without the addition of mounding. This was most evident in Powell Creek that had the highest water tables and greatest evidence of septic system contamination, including dominance of NH_4^+ . Other minor sources of wastewater may exist in the area but were outside the scope of this project (e.g., private lift stations, homeless populations, aging wastewater infrastructure, etc.). To improve water quality and reduce nutrient loading to local watersheds and the downstream Caloosahatchee River and Estuary, we recommend decreasing reliance on septic systems within North Fort Myers, which could provide Basin Management Action Plan (BMAP) credits. Further, stormwater also contributes to the degradation of this area; therefore, introduction of stormwater management practices to help decrease the influence of surficial runoff would also be important for restoring this watershed. Human influence that increases bird populations in certain areas (e.g., feeding of birds at the beach, open trash containers) could also be discouraged with informational signs. Finally, an assessment of the methods used to manage freshwater macrophytes may be necessary. These changes will help to reduce localized bacterial pollution and mitigate the downstream effects of worsening HABs.

Summary Table. Compilation of all data (years 1 and 2) collected during the Caloosahatchee River – North Fort Myers Nutrient and Bacteria Study showing overall project means summarized for groundwater and surface water; “BDL” 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), green shading indicates a low concentration or detection, yellow shading indicates a value above background levels or approaching the standard, and red shading indicates exceedance of surface quality water standards or a significant presence. There are no standards for reactive nutrients, so classifications were based on an estimated percent contribution of the FDEP surface water standard for the peninsular stream region; total nitrogen (TN) = 1.54 mg/L and total phosphorus (TP=0.12 mg/L); ammonium (NH₄⁺) and nitrate + nitrite (NO_x) were considered elevated at 10% of the TN standard, dissolved inorganic nitrogen (DIN) was considered elevated at 20% of the TN standard, and phosphate (SRP) was considered elevated at 20% of the TP standard; the numerical classifications and units for each parameter are listed in the legend. Stable nitrogen isotope (δ¹⁵N) values were considered “Significant” when > +3 ‰, “Moderate” when > +2 ‰, and “Low” when < +2‰.

Type	Drainage Basin	Well / Site	Bacteria			Human Molecular Marker	Avian Molecular Markers			Ruminant Molecular Marker	Human Wastewater Chemical Tracers				Stormwater Chemical Tracers						Nutrients & Stable N Isotopes							
			Enterococci	E. coli	BOD	HF183-qPCR	GFD-purified-qPCR	GULL2-qPCR	BacR-qPCR	Sucralose	Acetaminophen	Carbamazepine	Primidone	Bentazon	Diuron	Fluridone	Imazapyr	Imidacloprid	2,4-D	NH ₄	NO ₃	DIN	SRP	TN	TP	δ ¹⁵ N		
Ground-water	Reference	GW10	2.31	BDL	0.59	NA	NA	NA	NA	195	BDL	BDL	BDL	1.78	BDL	1.50	BDL	BDL	BDL	0.24	0.01	0.25	0.07	0.62	0.08	0.60		
	Hancock Creek	GW1	0.88	0.81	0.61	NA	NA	NA	NA	30588	BDL	94.4	9.63	BDL	BDL	BDL	BDL	BDL	BDL	0.23	2.78	3.01	1.55	4.19	1.64	8.90		
		GW2	30.7	BDL	0.39	NA	NA	NA	NA	4490	BDL	14.7	5.05	BDL	BDL	BDL	BDL	BDL	BDL	0.19	0.43	0.62	1.11	1.44	3.07	7.05		
		GW3	1.31	0.94	0.30	NA	NA	NA	NA	4014	BDL	1.32	1.13	BDL	BDL	BDL	BDL	BDL	BDL	0.45	0.02	0.48	0.54	1.03	0.56	3.21		
	Central Drainage	GW4	5.81	BDL	0.36	NA	NA	NA	NA	508	BDL	3.71	29.3	1.41	BDL	BDL	BDL	BDL	BDL	1.45	0.01	1.46	0.32	1.90	0.43	3.26		
		GW5	7.25	74.9	0.68	NA	NA	NA	NA	13913	33.4	7.55	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.31	0.01	1.32	0.49	2.20	0.52	6.20		
		GW6	2.88	BDL	0.66	NA	NA	NA	NA	2241	BDL	1.14	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.31	0.01	1.33	0.56	2.46	0.61	5.74		
	Powell Creek	GW7	35.4	BDL	3.34	NA	NA	NA	NA	169	2.88	0.84	BDL	BDL	BDL	BDL	BDL	BDL	BDL	5.35	0.02	5.37	0.21	5.75	0.31	5.32		
		GW8	0.81	BDL	2.31	NA	NA	NA	NA	1235	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	2.59	0.01	2.60	0.14	2.82	0.20	1.03		
		GW9	305	6.06	2.66	NA	NA	NA	NA	335	BDL	BDL	16.51	22.67	BDL	BDL	BDL	BDL	BDL	8.65	0.01	8.66	0.41	9.76	0.43	5.19		
Surface Water	Hancock Creek	SW9	411	221	0.49	80	89	BDL	BDL	396	BDL	0.39	0.79	1.78	BDL	12.0	28.0	BDL	BDL	0.02	0.03	0.06	0.16	0.46	0.18	2.86		
		SW8	190	350	1.18	21	179	BDL	BDL	724	BDL	2.95	6.70	6.90	2.10	95.0	36.0	4.00	5.33	0.03	0.04	0.08	0.14	0.76	0.20	4.42		
		SW7	209	283	1.10	20	1988	BDL	BDL	643	BDL	2.46	3.56	9.52	3.30	48.0	31.0	4.60	13.1	0.02	0.02	0.05	0.11	0.82	0.17	5.98		
		16-3GR	834	645	1.18	35	10085	7763	BDL	534	BDL	1.73	2.49	10.1	4.10	24.0	16.0	4.70	14.3	0.03	0.03	0.17	0.10	0.87	0.16	5.98		
		SW6	343	116	0.96	BDL	1348	14438	BDL	350	BDL	1.00	1.65	7.48	2.90	3.20	20.0	8.60	29.9	0.02	0.09	0.12	0.06	0.96	0.10	7.25		
	Central Drainage	SW5	883	1310	1.49	406	141	BDL	BDL	710	6.50	1.44	2.29	8.70	3.90	3.30	22.0	7.90	17.4	0.12	0.07	0.18	0.09	1.13	0.19	4.18		
		SW4	1253	1284	0.70	256	203	BDL	BDL	854	BDL	15.6	3.51	1.73	BDL	5.70	38.0	3.80	BDL	0.04	0.07	0.11	0.13	0.69	0.17	4.02		
	Powell Creek	SW1	1000	1161	0.85	36	245	BDL	BDL	851	0.54	15.1	3.71	2.00	BDL	4.50	34.0	3.40	BDL	0.03	0.05	0.09	0.12	0.69	0.17	5.39		
		SW2	482	628	1.11	134	370	BDL	BDL	700	7.38	9.78	3.16	4.25	BDL	3.30	34.0	5.40	4.33	0.04	0.05	0.10	0.10	0.79	0.14	3.50		
		SW3	335	500	1.16	283	153	BDL	BDL	591	4.83	7.31	2.51	4.65	BDL	3.00	23.0	6.90	4.15	0.04	0.06	0.10	0.08	0.83	0.11	3.37		
Legend	Low		<35	<126	<1.0	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	<0.02	<0.02	<0.04	<0.005	<0.25	<0.01	<0.2		
	Moderate		35-129	126-409	1.0-2.3	MDL-99,999	MDL-99,999	MDL-99,999	MDL-99,999	MDL-999	MDL-999	MDL-999	MDL-999	MDL-999	MDL-999	MDL-999	MDL-999	MDL-999	MDL-999	0.02-0.153	0.02-0.153	0.04-0.309	0.005-0.023	0.25-1.53	0.01-0.12	2.0-3.0		
	Significant		≥ 130	≥ 410	≥ 2.4	≥ 100,000	≥ 100,000	≥ 100,000	≥ 100,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 1,000	≥ 0.154	≥ 0.154	≥ 0.308	≥ 0.024	≥ 1.54	≥ 0.12	≥ 3.0		
	Parameter Units		MPN/100mL	MPN/100mL	mg/L	GEU/100mL	TSC/100mL	TSC/100mL	TSC/100mL	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	‰		
			Exceeds Standards / Significant			Approaching Standards			Low Value or Below Detection			NA			Not Analyzed													

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Acronyms Used in the Text

ANOVA	analysis of variance
BDL	below detection limit
BOD	biochemical oxygen demand, biological oxygen demand
BMAP	Basin Management Action Plan
BU	Boston University
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
GEU	Genomic Equivalent Units
GF/F	glass fiber filters
HAB	harmful algal bloom
HBOI-FAU	Harbor Branch Oceanographic Institute-Florida Atlantic University
HSD	honest significant difference
LCDNR	Lee County Division of Natural Resources
LCEL	Lee County Environmental Lab
MDL	method detection limit
mg/L	milligrams per liter
MGM	monthly geometric mean
MPN	most probable number
MST	microbial source tracking
ng/L	nanograms per liter
NH ₄ ⁺	ammonium
NNC	Numeric Nutrient Criteria
NO _x	nitrate + nitrite
NO ₃ ⁻	nitrate
PQL	practical quantitation limit
SE	standard error
SM	standard method
SRP	soluble reactive phosphorus, phosphate
TMDL	Total Maximum Daily Load
TN	total nitrogen
TP	total phosphorus
TKN	total Kjeldahl nitrogen
TPTV	ten percent threshold value
TSC	target sequence copies
µg/L	micrograms per liter
UGA-SIEL	University of Georgia, Center for Applied Isotope Studies Stable Isotope Ecology Laboratory
USEPA	United States Environmental Protection Agency

1. Introduction

1.1 Problem Statement and Project Objective

Coastal water quality is an ongoing, evolving issue for urbanized watersheds throughout the United States (NRC 2000). The North Fort Myers area in Lee County, FL has experienced degraded water quality over the last 30 years (W. Dexter Bender and Associates, Inc. 1995). In particular, persistent bacterial pollution has been documented by Lee County Environmental Lab (LCEL) and the Florida Department of Health (FDOH) at North Shore Park in the Caloosahatchee River between North Fort Myers and Fort Myers proper. To address this public health issue, a microbial source tracking (MST) study was conducted to determine the source of this impairment through a collaborative effort with Lee County Division of Natural Resources (LCDNR), LCEL, Florida Department of Environmental Protection (FDEP), and Harbor Branch Oceanographic Institute - Florida Atlantic University (HBOI-FAU). MST studies target source-specific gene fragments and chemicals as indicators to determine the source(s) of microbial pollution. The objective of this study was to determine if human wastewater from septic systems is a primary source of bacterial and nutrient pollution in North Fort Myers. In the year 1 study, human wastewater from septic systems was identified as a major contributor to poor water quality in North Fort Myers, but many unusual environmental conditions also occurred, such as extreme rainfall and Hurricane Irma (Lapointe et al. 2018). Therefore, a second year of the study was conducted with EPA 319 funding granted through FDEP to assess if human wastewater from septic systems was identified as a major source of bacterial and nutrient pollution in North Fort Myers through varied environmental conditions over multiple years.

Water quality in the North Fort Myers watershed relates to conditions downstream in the Caloosahatchee River and Estuary, such as harmful algal blooms (HABs). For example, blooms of the dinoflagellate *Karenia brevis* have been historically reported off the Lee County coast. *K. brevis* blooms are commonly referred to as “red tides” and can contain high concentrations of brevetoxins, a type of neurotoxin that causes fish kills, shellfish contamination, and negatively affects human respiratory systems when aerosolized (Lee et al. 1989). In recent years, these blooms have become increasingly abundant, especially in nearshore environments (Brand and Compton 2007) and have been linked to nutrient enrichment from riverine inputs (Yentsch et al. 2008). Furthermore, red drift macroalgae blooms began developing off the Lee County coast in 2003 and are associated with increasing nutrient contributions from wastewater sources, as well as rainfall and fertilizers (Lapointe and Bedford 2007). In multiple years, including 2005 and 2018, blooms of the blue-green algae *Microcystis aeruginosa* have occurred in the Caloosahatchee River, Estuary, and residential canals of Lee County (Lapointe et al. 2006). Similar blooms of *M. aeruginosa* occurred on Florida’s east coast in the St. Lucie Estuary in 2005, 2013, and 2016 and were attributed to algal “seeding” from Lake Okeechobee discharges, as well as

within basin blooms from local seeding and nutrient loading (Phlips et al. 2012; Lapointe et al. 2017; Kramer et al. 2018). These HABs can negatively impact local economies through reduced real estate values, declines in business, the mortality of commercial seafood, and by inhibiting ecotourism activities (Anderson et al. 2000). Tributaries in North Fort Myers drain into the Caloosahatchee River, and ultimately the Gulf of Mexico, therefore there is great value in minimizing pollutants flowing downriver and into these important coastal ecosystems.

Over the past fifteen years, HBOI-FAU has worked with Lee County to better understand the relationships between water quality in the Caloosahatchee River and Estuary, coastal red tides, and mass accumulations of red drift macroalgae on its beaches. In 2004-2005, Lapointe and Bedford (2007) incorporated the use of stable nitrogen isotope ($\delta^{15}\text{N}$) values of macroalgae in areas upstream and downstream of the project area to discriminate between wastewater, fertilizer, and atmospheric nitrogen inputs to the beaches and nearshore reefs. They found that $\delta^{15}\text{N}$ values of macroalgae collected in the Caloosahatchee River were within the range of sewage nitrogen and that values increased heading downstream from Ortona Lock to Franklin Lock. Interestingly, $\delta^{15}\text{N}$ values in macroalgae observed by Lapointe and Bedford (2007) in 2004 were consistent with those documented in the red tide *K. brevis* blooms off Sanibel Island in 2005 (Lapointe et al. 2006; Yentsch et al. 2008). These findings came with the recommendation to reduce wastewater loading and better manage Lake Okeechobee freshwater discharges within the watershed to minimize the risk of worsening HABs in Lee County waters (**Fig. 1**).



Figure 1. Images reflecting a degraded ecosystem, including a) a warning sign posted in North Fort Myers and blue-green algae blooms b) in a residential canal of Cape Coral, FL during July 2018 and c) at Prosperity Pointe Marina in North Fort Myers in August 2018.

1.2 Study Site

Lee County, FL encompasses 1,212 square miles and is bisected by the Caloosahatchee River and Estuary. With a population of 770,577 as of 2019, according to US Census data, it is the 8th largest county in the state of Florida. Fort Myers, the county seat, was incorporated in 1885, and has been supported by a robust tourist industry since its establishment. The population in Lee County has grown rapidly in recent years, with a 24.5% increase from 2010-2019 according to US Census data (**Fig. 2**). As the population continues to grow, it is important to address local water quality issues in order to maintain the high quality of life that initially brought residents and visitors to the region.

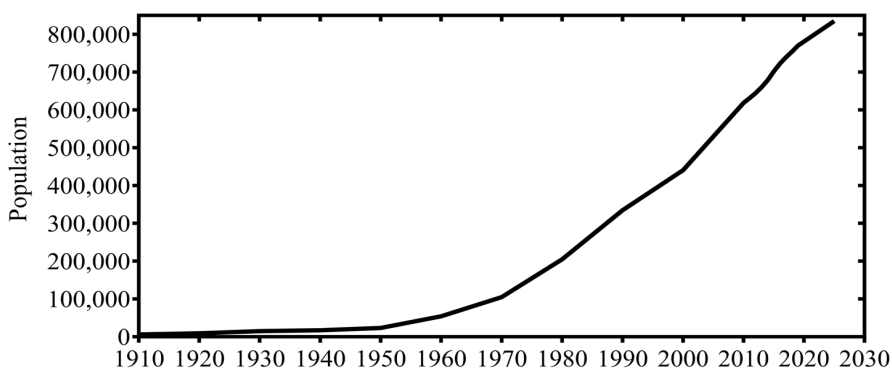


Figure 2. Population growth in Lee County from 1910 projected to 2025, showing rapid growth since 1970.

The Caloosahatchee River and Estuary is a Class III water body with designated uses of fish consumption, recreation, propagation, and maintenance of a healthy population of fish and wildlife. Based on Florida Administrative Code (FAC) Rule 62-302.532, Numeric Nutrient Criteria (NNC) for the Lower Caloosahatchee are 0.040 mg/L total phosphorus (TP) and 5.6 µg/L chlorophyll *a*, which are based on long term averages. Additionally, FAC Rule 62-302.533 designates that daily average percent dissolved oxygen (% DO) saturation must not be below 42% in the Lower Caloosahatchee River. Biochemical oxygen demand (BOD) standards are 2.4 mg/L. In August 2009, FDEP adopted the Caloosahatchee Estuary Basin Total Maximum Daily Load (TMDL) for total nitrogen (TN) based on FAC Rule 62-304.800. The TMDL for the area downstream of the S-79 structure at the Franklin Lock, the estuarine portion of the Caloosahatchee River, was calculated to be 9,086,094 lbs of TN per year. Based on model-simulated flows and concentrations from 2003 to 2005, a 22.8% reduction of TN is required to meet this TMDL and to maintain functionality as Class III designated waters (FAC Rule 62-302). To meet this goal, a long-term average concentration of 0.45 mg/L TN was recommended as “protective” for the Lower Caloosahatchee.

The FDEP first adopted the Caloosahatchee Estuary Basin Management Action Plan (BMAP) in November 2012 to implement the TN TMDL in the Caloosahatchee River and Estuary downstream of the Franklin Lock and Dam. In November 2017, FDEP and local

stakeholders completed the first 5-Year Review to evaluate implementation at the end of the first phase and make recommendations for future phases of the BMAP. The information gathered as part of the 5-Year Review was used to develop an updated BMAP for the Caloosahatchee River and Estuary Watershed. In addition, in January 2019, Executive Order 19-12 (Item C) included a requirement to update and secure all restoration plans, within one year, for waterbodies impacting south Florida communities, including the Caloosahatchee River and Estuary BMAP. The updated Caloosahatchee River and Estuary BMAP was adopted in January 2020. The BMAP requires Lee County and other stakeholders to identify pollutant sources and implement projects and activities to achieve allocated reductions within the watershed.

The urban areas of North Fort Myers were developed along natural canals and creeks that ultimately flow into the Caloosahatchee River and were enhanced with the addition of small “finger” canals to increase the available waterfront property, as well as drainage canals. The western side of North Fort Myers is drained by Hancock Creek, the central area has a drainage feature between US-41 and US-41 Business, and the eastern side is drained by Powell Creek. Throughout this area, there are many waterfront homes with onsite sewage treatment and disposal systems (septic systems; **Fig. 3**). An estimated 2,164 septic systems are located within the study area in North Fort Myers. In Lee County, there are 39,768 “known” and 57,054 “likely” septic systems, however for many land parcels there are no data available on wastewater disposal (FDOT). Septic systems have contributed to the degradation of adjacent surface waters through groundwater discharge and tidal pumping leading to increased nutrient and bacterial concentrations in other Florida counties as well, including Monroe, Palm Beach, Martin, Volusia, Indian River, Brevard, Charlotte, and St. Lucie County (Lapointe and Krupa 1995; Lapointe et al. 1990, 2012, 2015, 2016, 2017, 2018). Further, many septic systems in Florida may not meet FAC requirements due to elevated seasonal high water tables (Bicki and Brown 1990; Lapointe et al. 2018, 2020). A minimum of 6” of cover is required on top of a septic system drainfield, the required drainfield depth is ~1’ (may be less in some soil types), and the required separation from the bottom of the drainfield to the high water table is 2’ (FAC Rule 62E-6). Therefore, at least 3.5’ of separation is needed from the ground surface to the water table to meet the minimum requirements. In some parcels with high water levels, the drainfield has been raised above the natural soil surface to help meet these requirements by adding additional separation between the initial effluent discharge and the groundwater (“mounding”). As such, understanding interactions between septic systems, groundwater, and surface waters in the North Shore Park area of the Caloosahatchee Estuary Basin is essential for determining the best practices to address the recurring bacterial contamination issues and required TN load reductions. Although there are no FDEP nutrient standards for these tidal tributaries in Florida, they can be compared to the NNC for peninsular streams (FAC Rule 62-302.531). Based on these criteria, TP concentrations should not exceed 0.12 mg/L and TN concentrations should not exceed 1.54 mg/L.

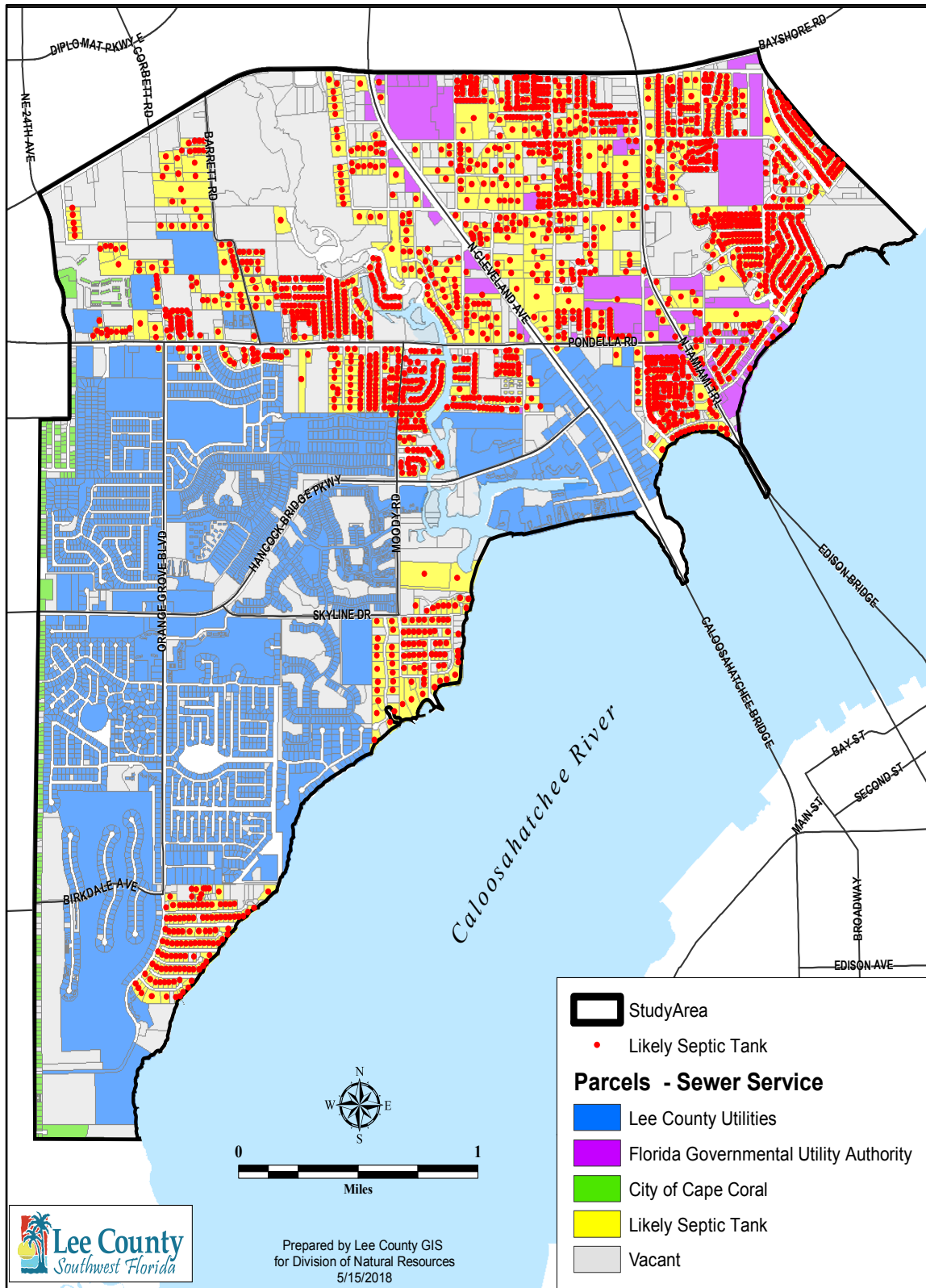


Figure 3. Wastewater service map of the study area within North Fort Myers, FL showing parcels that are “likely” serviced by septic systems (yellow with a red dot to indicate approximate location), vacant parcels (grey), as well as parcels that are connected to Lee County Utilities (blue), Florida Governmental Utility Authority (purple), or the City of Cape Coral Utilities (green).

1.3 Microbial Source Tracking

The presence and density of certain fecal indicator bacteria (FIB) are used as a measure of the safety and suitability of surface water for recreational uses. FIB can originate from point sources (e.g., wastewater treatment plant discharge) and non-point sources (e.g., septic systems, leaking sewer pipes, sewage overflows, urban runoff, pet waste, homeless populations, livestock, agriculture, and wildlife; Byappanahalli et al. 2012). Many factors contribute to urban water pollution, so there is high variability related to weather, rainfall catchment, and drainage infrastructure (Tran et al. 2015; Lapointe et al. 2017).

Fecal coliforms are bacteria found in the lower intestines of warm-blooded animals and are often introduced into the environment through fecal matter. These bacteria can be harmless; however, some strains can cause illness. *Escherichia coli* is the most common fecal coliform and a good indicator of human health risk. Enterococci are a subgroup within the fecal streptococcus group that occur within human digestive systems. While *E. coli* has a low salinity tolerance, enterococci can survive in saltwater. FDEP has designated water quality criteria for enterococci in Class III waters (FAC Rule 62-302.530) based on the Monthly Geometric Mean (MGM; 35 MPN/100 mL) using a minimum of ten samples over a 30-day period or the Ten Percent Threshold Value (TPTV; 130 MPN/100 mL) with no minimum sample size. FDEP water quality criteria for *E. coli* in Class III waters (FAC Rule 62-304.530) MGM and TPTV concentrations are 126 MPN/100 mL and 410 MPN/100 mL, respectively. Some challenges are associated with the use of FIB to assess watershed contamination, including difficulty in discriminating between sources (e.g., fecal or environmental) and short survival times (Scott et al. 2002; Tran et al. 2015). Employing a suite of source tracking tools can address these uncertainties and clarify FIB presence.

Molecular markers can help determine the source of bacterial impairment (Scott et al. 2002). The analytical technique, quantitative polymerase chain reaction (qPCR), allows for the amplification, identification, and quantification of genetic material (i.e., DNA). Specific genetic markers can determine if the source of the bacteria is human (HF183), ruminant (BacR), or avian (GFD, Gull2). HF183 is a molecular marker within the 16S rRNA genes of the *Bacteroides* species which reside in the human colon as part of the normal microbiota and are expelled in excrement. Their detection in environmental waters serves as an indicator of human fecal pollution. In raw sewage, HF183 can be detected in the tens of millions in Genomic Equivalent Units per 100 mL of sample (GEU/100 mL), which can be considered a “high” signal. Septic system effluent exhibits a “moderate” signal, occurring in the hundreds of thousands GEU/100 mL. Treated wastewater is highly variable, ranging from non-detectable to “low” signals of below ten thousand GEU/100 mL to tens of thousands GEU/100 mL (Matthews 2016). Established guidelines do not exist for interpreting HF183 results, but the “high,” “moderate,” and “low” classifications discussed here can be considered as relative guidelines. BacR is a genetic marker within

the 16s rRNA genes of *Bacteroides* species which reside in the digestive tract of most ruminants, such as deer, alpaca, cattle, llama, and goats. Thus, detection of BacR in environmental samples indicates ruminant fecal pollution (Reischer et al. 2006). Similarly, the avian marker GFD is an unclassified *Helicobacter* sp. and indicates the presence of many bird species including gulls, goose, chicken, pigeon, egret, crow, and others (measured in Target Sequence Copies per 100 mL; TSC/100 mL). Gull2 is associated with the bacteria species *Catellibacillus marimammalium*, which is found in the feces of seagulls and other coastal birds. These molecular markers allow for discrimination between FIB sources, however, there are limitations to this method. For example, suspended organic compounds, sediments, or complex biomolecules can inhibit qPCR amplification (Sidstedt et al. 2015). In particular, humic acid is prevalent in some freshwater and can be detrimental to HF183 qPCR analysis (Green and Field 2012). Furthermore, due to holding times, septic tank effluent and sewage may have fewer MST molecular markers than fresh wastewater, and thus reduced amplification efficiency (Boehm et al. 2013).

To supplement molecular marker results, chemical tracers are often useful in MST studies to help identify contributing sources of bacteria. There are some advantages to using chemical tracers over molecular markers, including source specificity, stability, and higher probability of detection (Lim et al. 2017). Chemical tracers, such as the artificial sweetener sucralose, as well as certain human pharmaceuticals, including acetaminophen (over the counter pain reliever), carbamazepine and primidone (prescription anticonvulsants), and meta-Chlorophenylpiperazine (mCPP; psychoactive stimulant), are all useful indicators of human wastewater and can be useful in MST studies. Sucralose is considered a reliable tracer of domestic wastewater because it is not completely broken down by human digestion and is transported conservatively through wastewater treatment plants (WWTPs) and septic systems (Oppenheimer et al. 2011). Furthermore, in Florida sucralose has been widely detected in canals (35%), streams (52%), and rivers (72%), while pharmaceuticals were also present in 8% of canals, 26% of streams, and 27% of rivers (Silvanima et al. 2018). Sucralose has been used with success in MST studies throughout Florida, including in the Loxahatchee River (Stoner et al. 2016), Indian River County canals and the St. Sebastian River (Tarnowski 2014), Charlotte County (Lapointe et al. 2016), Martin County (Lapointe et al. 2017), and Port St. Lucie (Lapointe et al. 2020) to pinpoint areas where septic systems are contaminating the surface water. Treated wastewater and reclaimed water have sucralose concentrations ranging from 10,000 to 40,000 ng/L (FDEP, 2014), while septic tank effluent concentrations are more variable and can range from 2,000 to 67,000 ng/L (Buerge et al. 2009; Lapointe et al. 2016; Yang et al. 2016; Snider et al. 2017). Further, wastewater may be partially treated, and some pharmaceuticals removed in septic systems with advanced treatment technologies (Wilcox et al. 2009). Additionally, many pharmaceuticals and chemical compounds degrade during transport out of the septic holding tank through the drainfield soil (Yang et al. 2016). Despite the limitations,

chemical tracers of human wastewater can be valuable in determining sources contributing to the impairment to an environment.

Herbicide and pesticide chemical tracers can also illustrate other sources of contamination to a waterbody. The herbicides linuron, diuron, and 2,4-Dichlorophenoxyacetic acid (2,4-D) are used primarily for agricultural purposes, so these chemicals suggest the influence of terrestrial runoff from agriculture. Fenuron, triclopyr, and imazapyr are herbicides used for weed control on non-crop land, indicating a residential influence. Fluridone is an herbicide used to control aquatic weeds, such as water lettuce (*Pistia stratiotes*), so its presence suggests that chemical macrophyte control has recently been applied in the water body. Additionally, both 2,4-D and imazapyr can also be used as aquatic herbicides. This is relevant to water quality as decaying macrophytes can be another source of enterococci in a water body (Byappanahalli et al. 2012). Bentazon, an herbicide, and imidacloprid, a pesticide, have multiple uses, including crop treatment and residential applications, which makes them good general tracers for terrestrial runoff. It is interesting to note that imidacloprid has been found in many of Florida's groundwaters and surface waters, most notably in rivers (Silvanima et al. 2018), suggesting that this chemical is resilient in the environment. Pyraclostrobin is a fungicide used in agricultural applications to control the growth of mildews and molds. The presence or absence of these various chemicals can inform the sources of aquatic bacteria and other pollution to the watershed.

Stable carbon ($\delta^{13}\text{C}$) and nitrogen isotope ($\delta^{15}\text{N}$) values of water and plant tissue represent another method of source tracking because they provide insight to the source of nutrients. For example, aqueous (dissolved) $\delta^{15}\text{N}$ from a water sample in the form of ammonium ($\delta^{15}\text{N-NH}_4^+$) and nitrate ($\delta^{15}\text{N-NO}_3^-$) can be used to distinguish between sources of dissolved inorganic nitrogen (DIN; i.e., atmospheric, fertilizer, or wastewater). Ammonium (NH_4^+) is the predominant species of nitrogen in septic system effluent (Bicki et al. 1984; Lapointe et al. 1990; Valiela et al. 1997) with $\delta^{15}\text{N}$ values ranging from +4 ‰ to +5 ‰ (Lapointe and Krupa 1995; Hinkle et al. 2008; Katz et al. 2010). Further, because primary producers integrate nutrients over days to weeks, the chemical signature is representative of longer-term nutrient availability (Lapointe 1985). For example, $\delta^{13}\text{C}$ values of plants can help identify the carbon source because terrestrial carbon is often more depleted (having lower values) than marine carbon, which is more enriched (having higher values; Peterson and Fry 1987). Primary producers $\delta^{15}\text{N}$ values are often used to discriminate between natural and anthropogenic N sources (Risk et al. 2009). The reported $\delta^{15}\text{N}$ values for atmospheric N typically ranges from -3 ‰ to +1 ‰ (Paerl and Fogel, 1994), while synthetic fertilizers range from -2 ‰ to +2 ‰ (Bateman and Kelly 2007). These N sources are both depleted relative to enriched values of +3 ‰ to +19 ‰ for human sewage (Heaton 1986; Costanzo et al. 2001).

This MST effort in North Fort Myers considered bacterial concentrations, chemical indicators, stable isotope values, and environmental parameters with seasonal water tables and land use in the study area to help better understand the drivers contributing to degraded water quality in North Fort Myers.

2. Methods

2.1 Site Descriptions

Sites were located north of the Caloosahatchee River within the Hancock and Powell Creek watersheds, as well as near a central drainage feature (**Fig. 4**). The primary land use for each of these watersheds is residential with houses located directly on the water in some areas. There are also commercial areas within the watershed, specifically along major roads such as US-41, US-41 Business, Hancock Bridge Pkwy., Pondella Rd., and Pine Island Rd. Fort Myers Central Advanced WWTP is closest to the study area and discharges into the south side of the Caloosahatchee River. The study area is primarily serviced by septic systems (**Fig. 4**). There is no application of reuse water within the study area, so this was not a confounding issue for the study. The sites are mostly freshwater with estuarine influence at sites nearest to the Caloosahatchee River.

Ten groundwater sites were selected where wells were installed: a reference site within a less developed, higher elevation area, three sites in Hancock Creek watershed, three sites in the central drainage watershed, and three sites in Powell Creek watershed (**Fig. 4**). Ten surface water sites were also selected: five in Hancock Creek, one at the central drainage feature, and four in Powell Creek. The study area does not encompass the entire watershed but aims to assess water quality immediately upstream of North Shore Park and the Caloosahatchee River. For the purposes of the report, “watershed” refers to the described study area only. Detailed information about the specific location of surface and groundwater sites is available in **Appendix 1**.

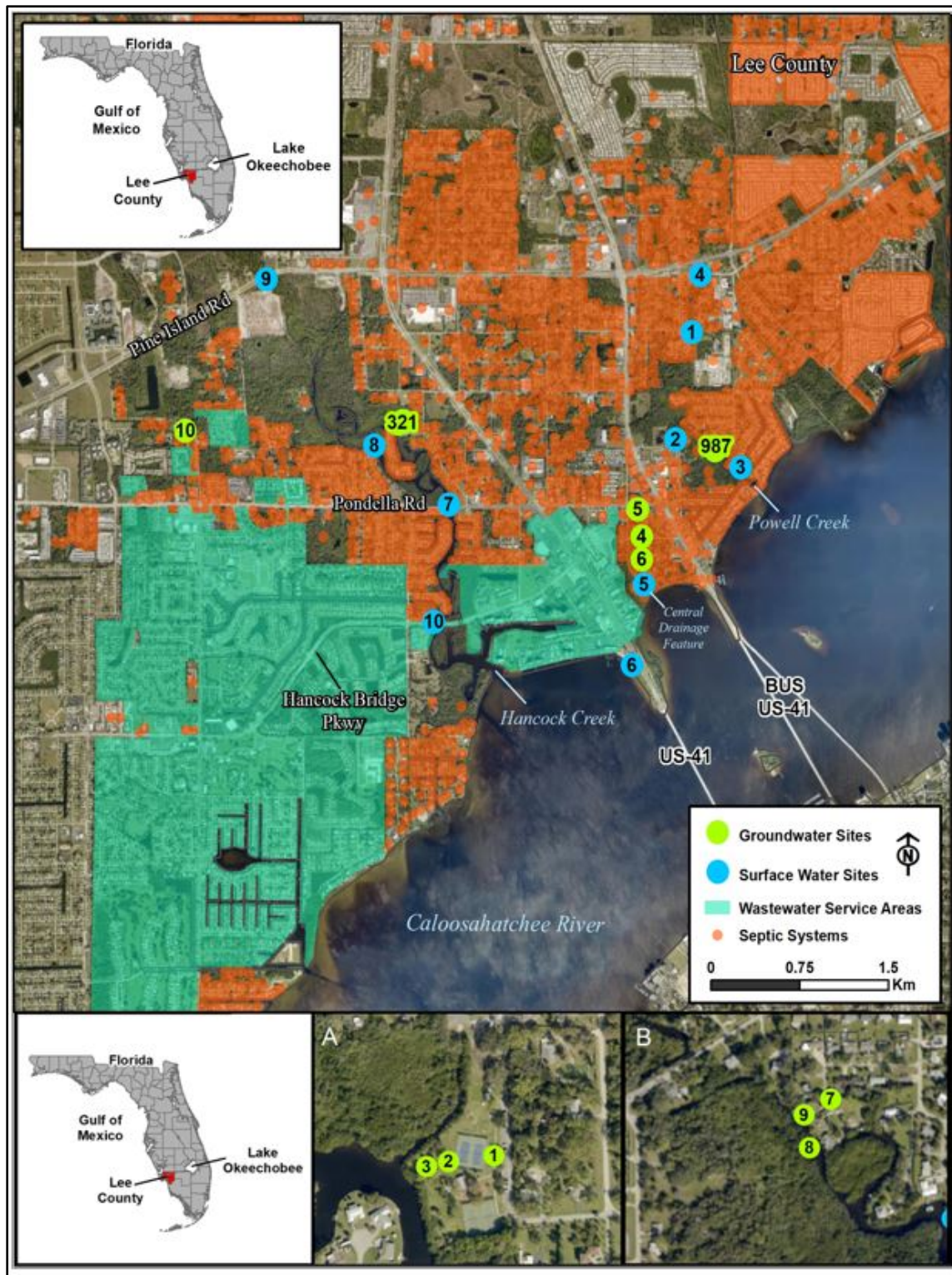


Figure 4. Study area in North Fort Myers, FL showing locations of groundwater wells (GW 1-10, green circles) and surface water sites (SW 1-10, blue circles), as well as areas connected to sewer for wastewater disposal (teal shading) and parcels with septic systems (orange shading). Inset A shows a close up of groundwater sites 1-3 and inset B shows groundwater sites 7-9.

2.1.1 Groundwater Sites

Shallow groundwater wells (~7.3' depth) constructed with 2-inch diameter PVC well casing and locked plastic caps were installed by LCDNR in September 2017 for the purpose of sampling the surficial aquifer within the study area. Three groundwater wells were located in the Hancock Creek watershed, all of which were located within Judd Community Park in a primarily residential area. The first well, NOFOPZ-01 (GW1), was adjacent to the north side of the tennis court near the parking lot and closest to a septic system drainfield (**Fig. 5**). The second well, NOFOPZ-02 (GW2), was adjacent to the north side of the tennis court and west of GW1 (**Fig. 5**). The last well, NOFOPZ-03 (GW3), was located west of GW2, nearest to a canal feeding into Hancock Creek (**Fig. 5**).



Figure 5. Groundwater monitoring wells (GW1-GW3) in Judd Community Park located within the Hancock Creek watershed on the western side of North Fort Myers, FL; the approximate location of the septic tank drainfield is indicated by the green square.

The central drainage watershed contained three groundwater wells, all of which were located in dense residential areas. The wells in this watershed were not as closely aggregated as those in the Hancock and Powell Creek watersheds. The northern most well was NOFOPZ-05 (GW5), which was located at 57 Cypress St. near a septic system drainfield (**Fig. 6**). Just south of GW5 was another well, NOFOPZ-04 (GW4), at 73/75 Cabana Ave. (**Fig. 6**). GW4 was located close to two septic system drainfields (**Fig. 6**). NOFOPZ-06 (GW6) was the most southern well, located at 1104 Seventh Way, and was close to a septic system drainfield (**Fig. 6**). It is noteworthy that not all septic system drainfields in this area are represented on **Fig. 6**, only locations that had visible mounding.



Figure 6. Groundwater monitoring wells (GW4-GW6) located near the “central drainage feature” in North Fort Myers, FL; the approximate location of some septic tank drainfields determined by the presence of mounding are indicated by the green squares. Not all drainfields in this area are represented in this map due to a lack of information.

The Powell Creek watershed included three groundwater wells from within a densely populated residential area. Two wells were located at 127 Dow Ln.; NOFOPZ-07 (GW7) was in the front yard closest to the septic system drainfield and NOFOPZ-08 (GW8) was in the back yard of the property near Powell Creek (**Fig. 7**). The third well, NOFOPZ-09 (GW9), was in the back yard of 131 Dow Ln. and also near Powell Creek (**Fig. 7**).



Figure 7. Groundwater monitoring wells (GW7-GW9) located in Powell Creek watershed on the eastern side of North Fort Myers, FL; the approximate location of the septic tank drainfields are indicated by the green squares.

The reference well, NOFOPZ-10 (GW10), was in a relatively undeveloped area with higher elevation in the western part of North Fort Myers off Pine Island Rd. at 1397 Orchid Dr. (**Fig. 8**). Several sewered businesses and homes were located near this well as well as some septic systems, but land use was less densely occupied relative to the other watersheds.



Figure 8. Reference groundwater monitoring well (GW10) located in a less developed, higher elevation area of western North Fort Myers, FL.

2.1.2 Surface Water Sites

Hancock Creek begins in northwest North Fort Myers near E. Diplomat Pkwy. and flows southeast for about five miles. Hancock Creek is joined by Yellow Fever Creek in a small natural area north of Judd Community Park and then flows through a heavily developed residential area until terminating in the Caloosahatchee River. The most upstream surface water site in the Hancock Creek watershed was HBOI-SW09 (SW9), where samples were collected from the downstream side of the bridge at Yellow Fever Creek and Pine Island Rd., due to inaccessibility of the upstream side of the bridge (**Fig. 9**). This was the most natural site in this watershed and was meant to represent minimal human influence, however it was adjacent to a highway and downstream of a bridge. The next site, HBOI-SW08 (SW8), was located on Hancock Creek adjacent to Craig St. and Thompson St. in a residential area and was collected from a private seawall with permission from the homeowner (**Fig. 9**). Downstream was HBOI-SW07 (SW7), which was collected from Hancock Creek upstream of the Pondella Rd. Bridge (**Fig. 9**). An established LCDNR sampling site, 16-3GR, was the next downstream site in the Hancock Creek watershed. This site was in a heavily developed residential area of Hancock Creek and samples were collected on the upstream side of Hancock Bridge Pkwy. (**Fig. 9**). The final site in the Hancock Creek watershed was HBOI-SW06 (SW6), located at North Shore Park on the shore of the Caloosahatchee River near the terminus of Hancock Creek (**Fig. 9**). At SW6, samples were collected by wading out approximately ten yards from shore with a sampling pole. During sampling at SW6 effort was made to not disturb sediments near or upstream from where water was collected.



Figure 9. Upstream (left) and downstream (right) images of surface water sites within the Hancock Creek watershed from the most upstream site in Yellow Fever Creek (SW9, top) to the terminus at North Shore Park (SW6, bottom).

The central drainage feature is located at the end of River Rd. between US-41 and US-41 Business. This area is residential, and the feature supports a small neighborhood with several waterfront homes. The surface water site HBOI-SW05 (SW5) was located adjacent to River Rd. just before it joins the Caloosahatchee River, where samples were collected from the bank of the drainage feature (**Fig. 10**).

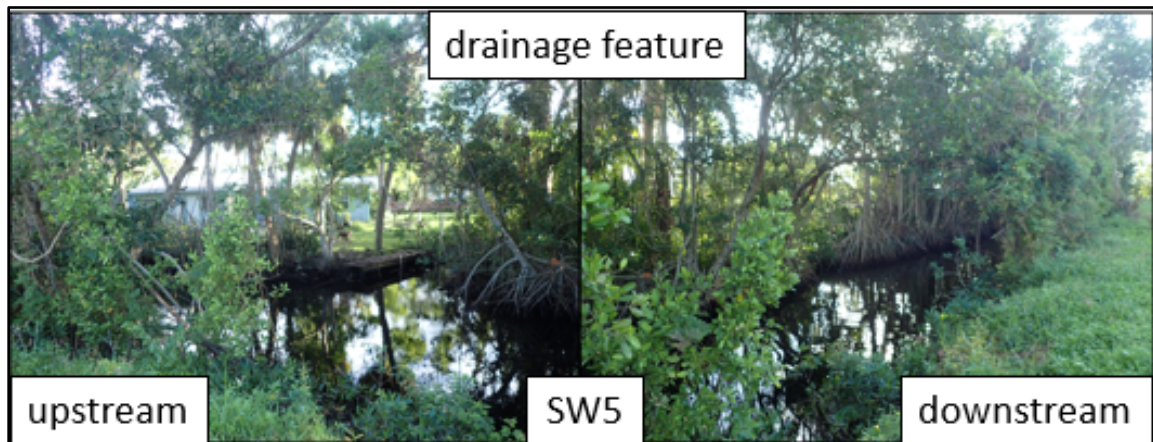


Figure 10. Upstream (left) and downstream (right) images of the central drainage feature (SW5) between US-41 and US-41 Business.

Powell Creek is a four-mile-long creek that begins at Forest Park Dr. and flows southwest, terminating into the Caloosahatchee River north of Business 41. The most upstream site in the Powell Creek watershed, HBOI-SW04 (SW4), receives flow from an upstream residential area, but was undeveloped at the sampling location. SW4 was sampled from the downstream side of the bridge at Bayline Dr., due to a lack of access upstream of the bridge (**Fig. 11**). The next site, HBOI-SW01 (SW1), is in a residential area and was sampled downstream of the bridge at East Mariana Ave. and Whidden Rd., due to unsafe sampling conditions on the upstream side of the bridge (**Fig. 11**). The next site, HBOI-SW02 (SW2), was sampled downstream of a bridge in a residential area at the intersection of Brooks Rd. and Lavin Ln., due to unsafe sampling conditions on the upstream side of the bridge (**Fig. 11**). The most downstream site in the Powell Creek watershed was HBOI-SW04 (SW4) at the terminus of Sunset Dr. This site was residential and was sampled from a kayak launch in a cul-de-sac (**Fig. 11**).



Figure 11. Upstream (left) and downstream (right) images of surface water sites within the Powell Creek watershed from the most upstream site (SW4, top) to where the creek meets the Caloosahatchee River (SW3, bottom).

2.2 Rainfall

Rainfall data over the study period (January 2017 to May 2020) was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (<https://www.ncdc.noaa.gov/data-access>). Fort Myers station US1FLLE0037 was selected as the primary data source due to its close proximity to the study area and high temporal coverage (92.6%). This data was supplemented with data from other nearby stations in Fort Myers (US1FLLE0055, 3.1%; USW00012835, 2.0%; US1FLLE0056, 0.1%; US1FLLE0039, 0.1%) and Cape Coral (US1FLLE0053, 2.2%) for days in which data at the primary station is missing.

2.3 Sample Collection

Surface and groundwater sampling were conducted by HBOI-FAU and LCEL following established FDEP standard operating procedures (SOPs; FAC Rule 62-160). Surface water samples were collected with a Van Dorn, Niskin bottle, or sampling pole depending on the site and sample date. Sites were sampled upstream of structures when possible, however many sites in the study area did not permit this. Any sites that were not accessible upstream of structures are noted in section 2.1. Samples were collected four times during the wet season (October 17-18, 2017, November 14-15, 2017, August 27-28, 2019, and September 25-26, 2019) and four times during the dry season (February 13-14, 2018, March 13-14, 2018, February 10-11, 2020, and March 10-11, 2020). In addition to the above sampling events, surface water samples were also collected monthly from October 2017 to May 2018 for bacterial analyses, nutrient, BOD, and color.

2.3.1 Groundwater Sampling

Depth to water in the groundwater wells was measured with a 100 m Geotech Water Meter Reader during sample collection and weekly, as possible, during the project period. Other environmental parameters of groundwater in the wells were also measured by LCDNR or LCEL staff using calibrated multiparameter probes, including pH, salinity (ppt), conductivity (μS), temperature ($^{\circ}\text{C}$), and DO ($\%$, mg/L ; **Fig. 12**). Per FDEP protocols, the well volume was calculated and triple the well volume was purged before sampling.

Singular water samples were collected into clean high-density polyethylene (HDPE) bottles for determination of enterococci and *E. coli* counts, BOD concentrations, and color (**Fig. 12**). Triplicate water samples were collected into HDPE clean bottles to determine concentrations of ammonium (NH_4^+), nitrate + nitrite (NO_x), SRP, total Kjeldahl nitrogen (TKN), total nitrogen (TN), and total phosphorus (TP). These samples were immediately submerged in ice in a dark cooler and delivered to LCEL for analyses within specified holding times. At LCEL samples were analyzed by standard NELAC approved methods.



Figure 12. Images from the study including, a) North Shore Park at sunrise, b) hand-filtering a surface water sample, c) pumping groundwater from a monitoring well, d) collecting water samples, e) removing a groundwater filter from a vacuum pump, and f) the temporary laboratory set up for HBOI sample processing.

Water samples for analysis of chemical tracers were collected in 1 L amber glass bottles, stored on ice, and shipped overnight to the FDEP Central Laboratory, Tallahassee, FL where they were analyzed following standard methods, available on the FDEP website (<https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops>). During all events, concentrations of the human wastewater tracers acetaminophen, carbamazepine, primidone, and sucralose were determined. The herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), bentazon, and triclopyr, as well as the psychoactive stimulant meta-Chlorophenylpiperazine (mCPP) were tested for in both seasons of year 1 (2017-2018). In the dry season of year 1 (2018) water samples were also analyzed to determine concentrations of an insecticide (imidacloprid), agricultural fungicide (pyraclostrobin), and other herbicides, including diuron, fenuron, fluridone, imazapyr, and linuron. Additional human wastewater tracers were also included in the suite of analytes during both seasons in year 2 (2019-2020), including ibuprofen, hydrocodone, and naproxen.

Groundwater was also collected for analysis of aqueous stable nitrogen isotope values of ammonium ($\delta^{15}\text{N-NH}_4^+$) and nitrate ($\delta^{15}\text{N-NO}_3^-$). These samples were collected into 1 L HDPE bottles and immediately stored on ice in the field. In year 1, the samples were vacuum filtered through 47 mm GF/F and the filtrate was frozen until shipment to University of Georgia, Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL) for analysis. In year 2, because UGA-SIEL ceased to perform these analyses, samples were acidified and stored at room temperature until shipment to Boston University (BU). At UGA-SIEL and BU, the water samples were run through ammonia (NH_3) diffusion. This involved increasing the pH of the dissolved sample to convert NH_4^+ to gaseous NH_3 , 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^+ , then proceeding with the standard diffusion and NH_3 capture on an acidified filter. The filter was then analyzed as a typical solid sample on a Carlo Erba Isotope Ratio Mass Spectrometer for $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$.

2.3.2 Surface Water Sampling

As done for groundwater, environmental parameters were measured at all surface water sites by HBOI-FAU or LC staff with calibrated multiparameter probes, including a YSI ProODO, YSI Pro1030, and/or a YSI ProPlus. Water clarity was also assessed visually using a secchi disk when possible.

Surface water samples were collected and handled similarly to groundwater for determination of enterococci and *E. coli* counts, BOD, color, NH_4^+ , NO_x , SRP, TKN, TN, TP, and chemical tracers. Additionally, surface water samples for analysis of molecular markers were collected at each site in clean 500 mL HDPE bottles. These samples were

immediately stored on ice in a dark cooler and shipped overnight to the FDEP Central Laboratory where they were analyzed for the human marker HF183, the ruminant marker BacR, and the bird markers GFD and Gull2.

At surface water sites, particulate organic matter (POM) was collected as a proxy for phytoplankton to document ambient nutrient signatures. For POM collections, surface water was collected with a clean secondary vessel (Van Dorn, Niskin bottle, or 1 L bottle). Samples were then coarse filtered into a 1 L HDPE bottle at the site through a 200 μm nylon netting to remove macrodetritus and microzooplankton, per Savoye et al. (2003). The filtered samples were immediately submerged in ice and stored in a dark cooler. Upon return to the lab (within 6 hours), POM samples were filtered through pre-combusted 47 mm glass fiber filters (GF/F) using a vacuum pump (**Fig. 12**) and the volume filtered was recorded. In year 1 the filters were frozen at 20°C until analysis. Due to a change in the recommended procedure by the analytical lab (UGA), in year 2 the filters were dried at 60°C, folded, and wrapped in aluminum foil until analysis. The POM filters were analyzed for stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes, as well as elemental composition (%C, %N, and %P) at UGA-SIEL on a Thermo Delta V Environmental Analysis - Isotope Ratio Mass Spectrometer coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo ConFlo III Interface (see the following for methods: <http://sisbl.uga.edu/ratio.html#top>). In year 1, %P was analyzed following the methodology of Aspila et al. (1976) on a Technicon Autoanalyzer II with an IBM-compatible, Labtronics, Inc. DP500 software data collection system (D'Elia et al. 1997). In year 2, %P samples were analyzed at the University of Missouri Soil and Plant Testing Laboratory by Inductively Coupled Plasma Atomic Emission Spectroscopy (Viso and Zachariadis 2018) because UGA ceased to perform these services. C:N:P data were compared to a modified Redfield ratio of 360:30:1 (Redfield 1958) to characterize temporal and spatial variation in algal nutrient status. C:N ratios > 6.6 indicate increasing N-limitation, while C:P ratios > 106 and N:P ratios > 16 both indicate increasing P-limitation (Atkinson and Smith 1983; Lapointe 1987; Lapointe et al. 2015). Opportunistic phytoplankton samples were also collected during HAB events of blue-green algae (*M. aeruginosa*; October 2017 and July 2018) and red tide (*K. brevis*; March 2018) and analyzed similarly for stable isotope values and elemental composition.

2.4 Data Analysis

For nutrient concentrations and bacterial counts, half of the methods detection limit (MDL) was substituted for any results flagged as below detection limits (“U”) or less than the criterion of detection (“T”). Molar ratios of nutrient concentrations (DIN:SRP and TN:TP) were calculated using molar concentrations (μM). For chemical tracers, any results that were not above the practical quantitation limit (PQL) and were flagged with “U” or a “T” were considered as non-detections and zeros were substituted prior to data analyses.

Molecular markers were handled similarly to chemical tracers for consistency. For all data, results flagged as between the laboratory detection limit and the PQL (“I”) and estimated values (“J”), such as exceeded detection limits for FIB counts, were included in analyses.

When possible, data were compared to a standard or point of reference to provide context to the values observed. For example, bacterial counts were compared to the FDEP Ten Percent Threshold Value (TPTV) for enterococci (130 MPN/100 mL) and *E. coli* (410 MPN/100 mL). Bacterial standards for enterococcus apply to marine waters and those for *E. coli* are applicable to freshwaters. Bacterial standards do not apply to groundwater. Thus, these water quality standards are not applicable at all sites, however they are mentioned to serve as points of reference. Biochemical oxygen demand (BOD) was compared to the FDEP surface water standard (2.4 mg/L). Further, due to the proximity of the study sites to the Lower Caloosahatchee and the lack of applicable standards for tidal tributaries, nutrient results were compared to NNC for both the Lower Caloosahatchee and peninsular streams. For total nitrogen (TN) the FDEP surface water protective standard for the Lower Caloosahatchee is 0.5 mg/L and 1.54 mg/L for peninsular streams. For total phosphorus (TP) the FDEP surface water standard for the Lower Caloosahatchee is 0.04 mg/L and 0.12 mg/L for peninsular streams. There are no criteria for chemical tracers, but sucralose concentrations were classified as “moderate” from 100-1,000 ng/L (0.1-1.0 µg/L) and “significant” at > 1,000 ng/L (1.0 µg/L; FDEP 2014).

Descriptive statistics, including mean (average), median, standard deviation, standard error, minimum, and maximum, were calculated for all parameters and normality of the data was assessed. In conjunction with land use, these data were considered together to infer possible sources of bacterial contamination and water quality issues. To determine what factors were influential on water quality in the study area, parameters were then statistically compared between watersheds, wells/sites, project year (year 1, year 2), and season (dry, wet) with analysis of variance (ANOVA) if assumptions were met or the non-parametric Mann-Whitney U test (comparisons of two) or Kruskal-Wallis test (comparisons of three or more) if ANOVA assumptions were not met. Log transformation, with the addition of a constant for negative ranging variables, was attempted for all parameters before non-parametric statistics were employed. Differences were considered significant at $p < 0.05$. Significant main tests for ANOVA were followed by Tukey Honest Significant Difference test (HSD) and Kruskal-Wallis was followed by Dunn’s multiple comparisons with a Bonferroni correction. Analyses were conducted in MiniTab 19 and SPSS 27; figures were created in GraphPad Prism 8. Results were interpreted with an eye towards potential solutions to this chronic public health issue and to make recommendations for future studies.

3. Results

3.1 Rainfall

All sampling events were conducted on days with little to no precipitation (**Fig 13**). Mean annual rainfall in the study area from 2017-2020 ranged from a low of 48.64" in 2018 to a high of 81.85" in 2017. The most significant rainfall event occurred on September 10-11, 2017, when Hurricane Irma passed through, contributing 10.14" of precipitation over a two-day period. Each year there were rainy seasons from ~May through October and dry seasons from ~November through April in the study area, with some variation between years. On average, 50.78" of rain fell during the rainy season each year, accounting for about 80% of the annual total. The cumulative rainfall fourteen days prior to the October 2017, November 2017, February 2018, and March 2018 sampling events were 2.13", 0.33", 0.90", and 0.36", respectively. The cumulative rainfall fourteen days prior to the August 2019, September 2019, February 2020, and March 2020 sampling events were 4.80", 0.62", 2.75", and 0.62", respectively.

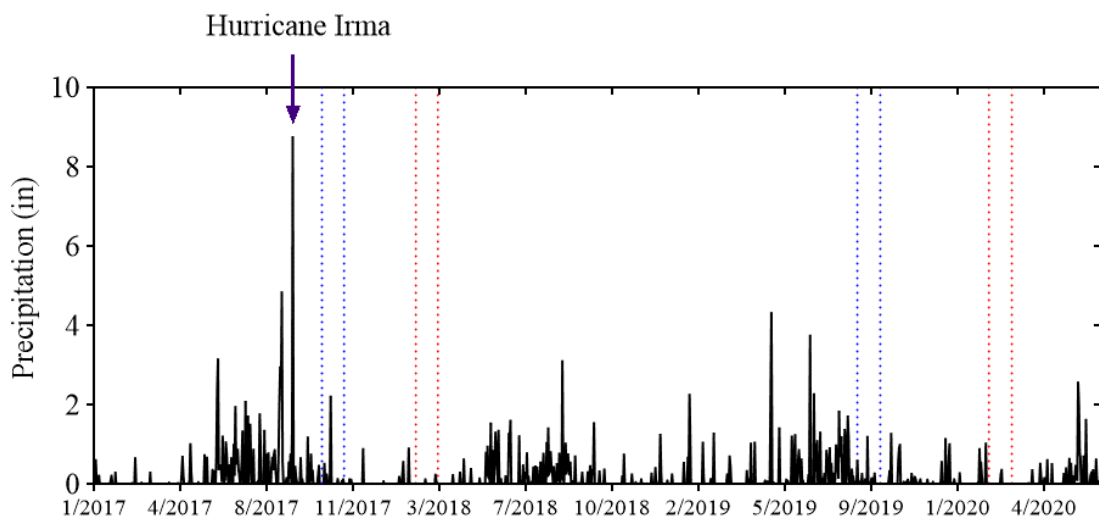


Figure 13. Daily rainfall (in) measured in North Fort Myers, FL from January 2017 through May 2020, showing Hurricane Irma (purple arrow), the wet season sampling events (blue lines), and the dry season sampling events (red lines).

3.2 Groundwater

3.2.1 Environmental Parameters

Based on the long-term weekly monitoring, depth to water in the study area was generally low with a range of <0.1 to 5.3' and an overall mean of $2.5 \pm <0.1'$ (median of 2.4'). Most of the measurements (~87%) did not meet the minimum depth to water needed for a septic system to function properly without the addition of mounding. The reference site (GW10) had the deepest depth to water recorded with a mean depth of $4.3 \pm <0.1'$. The other

watersheds were more similar, with mean depths of $2.3 \pm 0.1'$ in Hancock Creek watershed, $2.3 \pm <0.1'$ in the central drainage basin, and $2.2 \pm <0.1'$ in Powell Creek watershed. Seasonal differences were observed in depth to water table, with higher water tables in the wet seasons and lower in the dry seasons, but depth to water table remained shallow ($<3.5'$) in most wells throughout the study (**Fig. 14**).

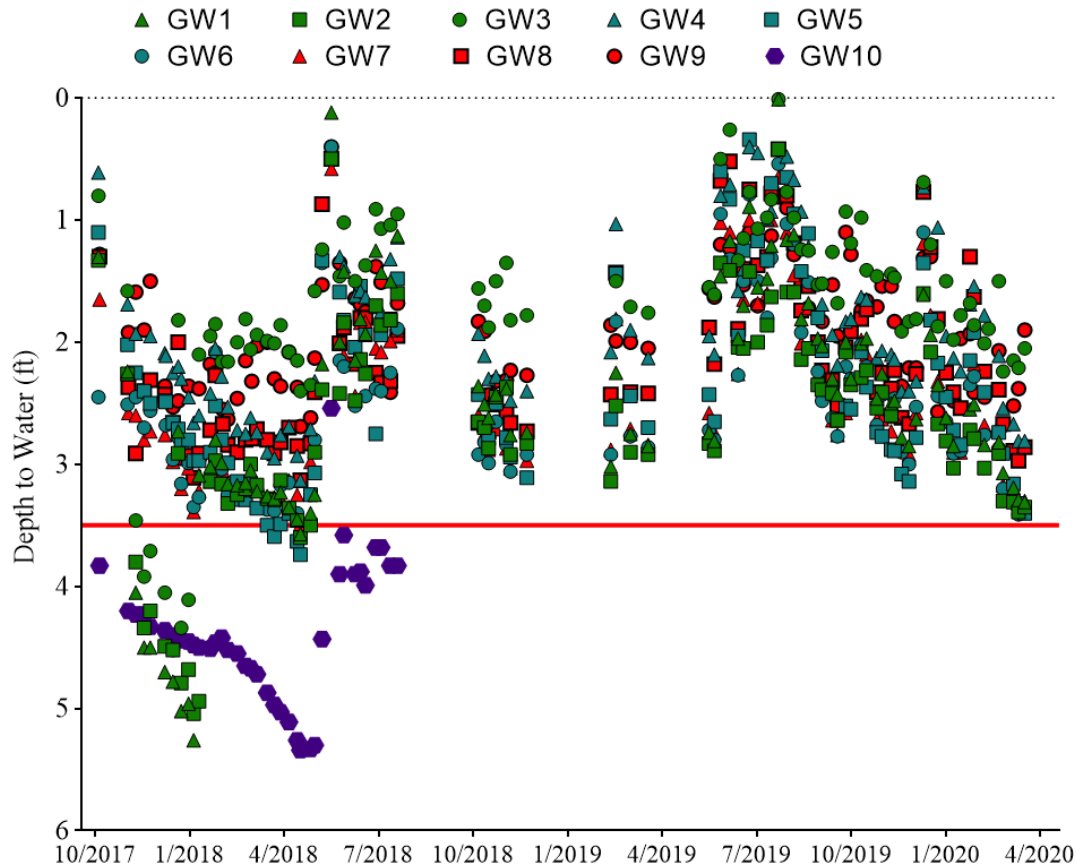


Figure 14. Depth to water table measured in North Fort Myers, FL over the study period by site and watershed (reference area = purple, Hancock Creek = green, central drainage = teal, and Powell Creek = red); the red line indicates the approximate minimum separation required from the ground surface (at zero) to the water table required by FAC Rule 64E-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' drainfield (can be less in some sediments), and 2' from the bottom of the drainfield to the high water table; for a total of 3.5' minimum 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 between the drainfield and the groundwater.

In North Fort Myers, the depth to water table observed during the sampling events ranged from 0.6 to 6.8' with an overall mean of $2.8 \pm 0.1'$. During sampling events, depth to water was variable seasonally (ANOVA, $p = 0.01$) and by watershed (ANOVA, $p < 0.01$), but not by project year (ANOVA, $p = 0.80$; **Fig. 15**). No interactions were significant (all $p > 0.05$). By season, the depth to water table was lower in the wet season (mean = $2.5 \pm 0.2'$)

than the dry season (mean = $3.2 \pm 0.2'$). By watershed, water tables were significantly deeper at the reference well (GW10; mean = $4.2 \pm 0.1'$) and in Hancock Creek watershed (mean = $3.5 \pm 0.3'$) than in the central drainage watershed (mean = $2.3 \pm 0.2'$) or Powell Creek (mean = $2.2 \pm 0.1'$). Median water level values for each watershed followed this same trend (**Table 1**). In the central drainage basin and Powell Creek, 100% of the depth to water measurements recorded during sampling events were too shallow to support adequate treatment of wastewater from a septic system without the addition of mounding ($<3.5'$). In Hancock Creek, 58% of depth to water measurements were too shallow and varied seasonally with 50% of dry season measurements $<3.5'$ and 67% of wet season measurements $<3.5'$. In the reference well, 100% of depth to water measurements recorded during sampling events were $>3.5'$.

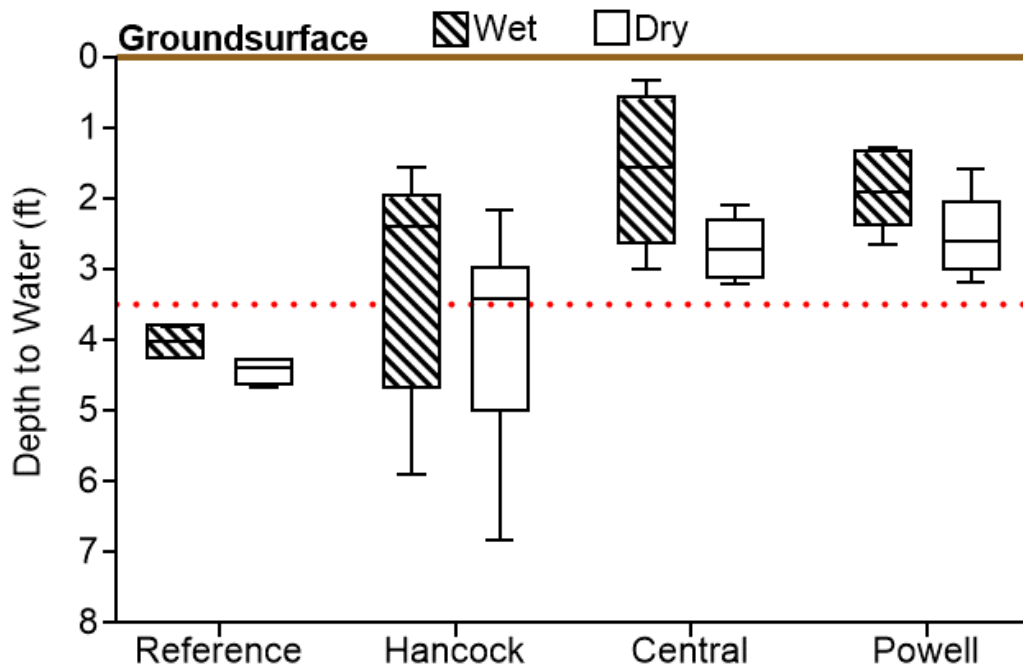


Figure 15. Depth to water table (ft; bars indicate minimum and maximum values) measured at the ten wells during sampling events by watershed and season (wet/dry); the red line indicates the approximate minimum separation required from the ground surface (at zero) to the water table required by FAC Rule 64E-6, values above this line indicate septic systems in this area may not be compliant with current requirements for new septic systems.

Table 1. Environmental parameters of groundwater by watershed and well (medians); Cond. = conductivity, Temp. = temperature, DO = dissolved oxygen,

Watershed	Well	Count	Depth to Water (ft)	pH	Salinity (ppt)	Cond. (µS)	Color (CU)	Temp. (°C)	DO (%)	DO (mg/L)
Reference	Watershed Median / GW10	8	4.3	7.0	0.31	640	58	23.9	4.3	0.37
Hancock Creek	Watershed Median	24	3.1	6.5	0.49	996	104	24.5	13.8	1.19
	GW1	8	3.3	6.6	0.56	1127	118	24.4	17.7	1.52
	GW2	8	5.1	6.6	0.53	1067	101	25.1	32.6	2.59
	GW3	8	2.3	6.5	0.37	756	104	24.7	4.8	0.24
Central Drainage	Watershed Median	24	2.5	6.7	0.50	1006	72	25.5	3.4	0.28
	GW4	8	2.3	6.8	0.68	1360	68	25.7	3.3	0.27
	GW5	8	2.4	6.3	0.37	767	154	25.7	4.2	0.33
	GW6	8	2.8	6.7	0.50	1006	76	25.4	3.3	0.26
Powell Creek	Watershed Median	24	2.2	6.7	0.70	1411	73	23.8	11.9	1.03
	GW7	8	2.6	6.7	0.35	728	24	23.7	13.3	1.04
	GW8	8	2.3	6.8	0.70	1411	85	24.5	19.0	1.59
	GW9	8	1.8	6.7	1.19	2310	124	23.4	6.0	0.50

Groundwater pH ranged from 6.18 to 7.12. Low elevation wells near dense septic systems had lower pH than the reference well (GW10) and season or project year did not change this pattern. For example, pH was significantly variable by watershed (ANOVA, $p < 0.001$), but not season (ANOVA, $p = 0.12$) or project year (ANOVA, $p = 0.451$). There were significant interactions between watershed and season (ANOVA, $p = 0.001$), as well as watershed and project year (ANOVA, $p < 0.001$) that were driven by variability in pH at the reference site (GW10). Despite these interactions, the patterns observed in groundwater pH were consistent with the lowest pH in the Hancock watershed (mean = 6.5), followed by the central drainage and Powell Creek watersheds (mean for both = 6.6), and the reference well having the highest (mean = 7.0). Median pH values followed the same pattern (**Table 1**).

Salinity in North Fort Myers groundwater ranged from 0.16 to 2.3 ppt. Groundwater salinity was variable by watershed (Kruskal-Wallis test, $p = 0.001$), but not season (Mann-Whitney U test, $p = 0.285$) or project year (Mann-Whitney U test, $p = 0.086$). Near the Caloosahatchee River, groundwater salinities were higher. For example, the highest median watershed salinity was observed in Powell Creek (0.70 ppt), followed by the central drainage (0.50 ppt), and Powell Creek (0.49) watersheds (**Table 1**). The reference well was located most upland, and the groundwater was significantly less saline (median = 0.31 ppt) than the other watersheds, which were all similar (**Table 1**).

Conductivity in North Fort Myers groundwater ranged from 76 to 4,319 µS and had patterns similar to salinity. For example, groundwater conductivity was variable by watershed (Kruskal-Wallis test, $p = 0.001$), but not season (Mann-Whitney U test, $p = 0.22$) or project year (Mann-Whitney U test, $p = 0.16$). The watershed with the highest median conductivity was Powell Creek (1,411 µS), followed by the central drainage (1006 µS),

and Hancock Creek watersheds (996 μS), while the reference well had the lowest median groundwater conductivity (640 μS ; **Table 1**).

Color of groundwater in North Fort Myers ranged from 17.6 to 696 CU. Groundwater color was variable by project year (Mann-Whitney U test, $p = 0.016$) and season (Mann-Whitney U test, $p = 0.007$), but not watershed (Kruskal-Wallis test, $p = 0.506$). In year 1, color was higher (median = 111 CU) than in year 2 (median = 60 CU). By season, groundwater color was higher in the wet season (median = 119 CU) than the dry season (60 CU). Though not significantly lower, the reference well (GW10) had the lowest median color (58 CU), followed by the central drainage (72 CU), Powell Creek (73 CU), and Hancock Creek (104 CU) watersheds (**Table 1**).

Temperature of groundwater in North Fort Myers ranged from 21.7 to 30.4°C. Groundwater temperature was variable by watershed (Kruskal-Wallis test, $p = 0.028$) and season (Mann-Whitney U test, $p < 0.001$), but not project year (Mann-Whitney U test, $p = 0.086$). The central drainage watershed, which had the highest median groundwater temperature (25.5°C), was significantly different than Powell Creek, which had the lowest median groundwater temperature (23.8°C; **Table 1**). The other watersheds were similar in temperature. Median temperatures were higher in the wet season (27.8°C) than the dry season (23.0°C).

Dissolved oxygen (DO) of groundwater in North Fort Myers ranged from 0.04 to 5.03 mg/L. Due to low groundwater recharge rates that inflated oxygen at some wells (particularly in the Hancock Creek and Powell Creek watersheds), DO (both % and mg/L) were not compared for this project. Median DO values as measured are shown in **Table 1**.

3.2.3 Bacterial Abundance

Groundwater enterococci counts ranged from <0.5 MPN/100mL (the lower limit of detection) to $>2,420$ MPN/100mL (the upper limit of detection). Enterococci abundance in groundwater was not significantly different by watershed (Kruskal-Wallis test, $p = 0.566$), season (Mann-Whitney U test, $p = 0.870$), or project year (Mann-Whitney U test, $p = 0.624$). Though not significantly different, enterococci counts were highest in the Powell Creek watershed (mean = 114 ± 100 MPN/100mL), followed by the Hancock Creek (mean = 11.0 ± 8.1 MPN/100mL), central drainage (mean = 5.3 ± 2.3 MPN/100mL), and reference (mean = 2.3 ± 0.69 MPN/100mL) watersheds (**Fig. 16a**). When median is examined, differences by watershed were minimal. The well with the highest median enterococci counts was GW7 (27.5; **Table 2**).

Table 2. Fecal indicator bacteria abundance and biochemical oxygen demand (BOD) concentrations of groundwater (medians) by watershed (reference well, Hancock Creek, central drainage, and Powell Creek) and site; gray shading indicates watershed medians.

Watershed	Well	Count	Enterococci (MPN/100mL)	<i>E. coli</i> (MPN/100mL)	BOD (mg/L)
Reference	Watershed Median / GW10	8	2.0	0.5	0.45
Hancock Creek	Watershed Median	24	0.5	0.5	0.15
	GW1	8	0.8	0.5	0.15
	GW2	8	0.5	0.5	0.15
	GW3	8	0.5	0.5	0.15
Central Drainage	Watershed Median	24	1.5	0.5	0.28
	GW4	8	2.0	0.5	0.15
	GW5	8	0.8	0.5	0.50
	GW6	8	1.5	0.5	0.30
Powell Creek	Watershed Median	24	1.0	0.5	2.95
	GW7	8	27.5	0.5	3.65
	GW8	8	0.5	0.5	2.25
	GW9	8	1.0	0.5	3.20

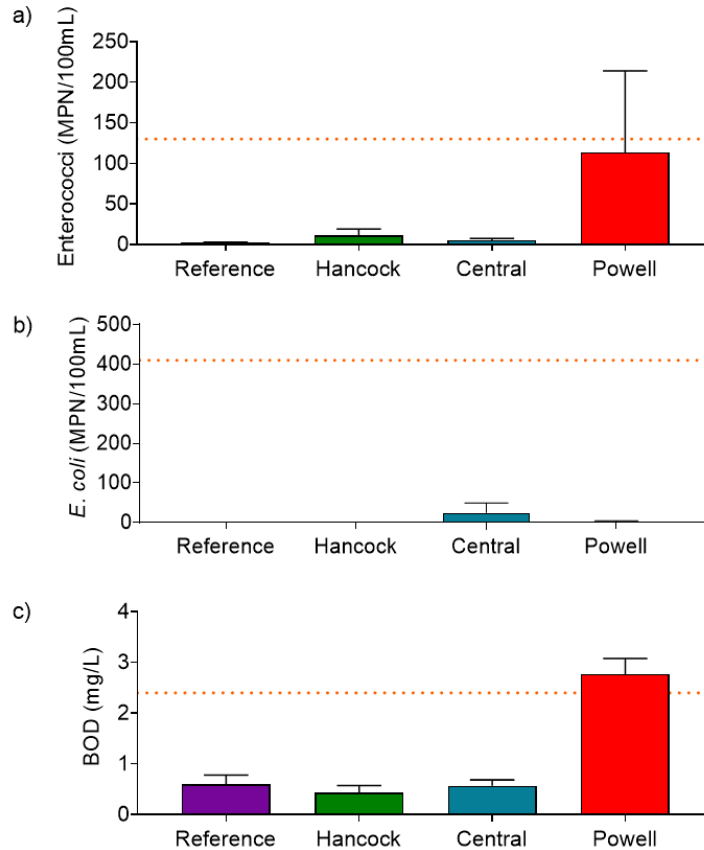


Figure 16. Fecal indicator bacteria abundance for a) enterococci and b) *Escherichia coli* (*E. coli*), as well as biochemical oxygen demand (BOD) concentrations of groundwater (mean ± standard error) by watershed (reference well, Hancock Creek, central drainage, and Powell Creek); dotted lines indicate FDEP Ten Percent Threshold Value (TPTV) surface water criteria for enterococci (marine water; ≥ 130 MPN/100 mL), FDEP surface water standards for BOD (2.4 mg/L), and FDEP TPTV surface water criteria for *E. coli* (fresh water; ≥ 410 MPN/100 mL). Surface water standards do not apply to groundwater or the study area and are shown as a point of reference only.

Groundwater *E. coli* counts ranged from 0.5 to 579 MPN/100 mL. The abundance of groundwater *E. coli* was not variable by watershed (Kruskal-Wallis test, $p = 0.560$), season (Mann-Whitney U test, $p = 0.583$), or project year (Mann-Whitney U test, $p = 0.786$). Though not significantly different, the highest groundwater *E. coli* counts were observed in the central drainage watershed (mean = 25 MPN/100mL), followed by the Powell Creek (2 MPN/100mL) and Hancock Creek (0.75 MPN/100mL) watersheds, as well the reference well (0.5 MPN/100mL; **Fig. 16b**). There was no difference in median *E. coli* counts between watersheds or wells (**Table 2**).

Groundwater BOD ranged from 0.15 to 5.2 mg/L. BOD concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not by season (Mann-Whitney U test, $p = 0.583$) or project year (Mann-Whitney U test, $p = 0.204$). Powell Creek watershed had the highest groundwater BOD concentration (median = 2.95 mg/L), followed by the reference well (0.45 mg/L), central drainage watershed (0.28 mg/L), and Hancock Creek watershed (0.15 mg/L; **Table 2**). Though the FAC standard is not applicable to groundwater, Powell Creek exceeded the value recommended as protective for the Lower Caloosahatchee River (2.4 mg/L; **Fig. 16c**).

3.2.4 Nutrient Concentrations and Molar Ratios

Groundwater NH_4^+ was variable by watershed (Kruskal-Wallis test, $p < 0.001$) and project year (Mann-Whitney U test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.184$; **Fig. 17a**). Powell Creek had a significantly higher median NH_4^+ concentration (4.06 mg/L) than all the other watersheds. The median groundwater NH_4^+ concentration of the central drainage watershed (1.38 mg/L) was higher than Hancock Creek watershed (0.32 mg/L) and the reference well (0.24 mg/L), which were similar to each other (**Table 3**). Median NH_4^+ concentrations were higher in year 1 (1.42 mg/L) than in year 2 (0.70 mg/L).

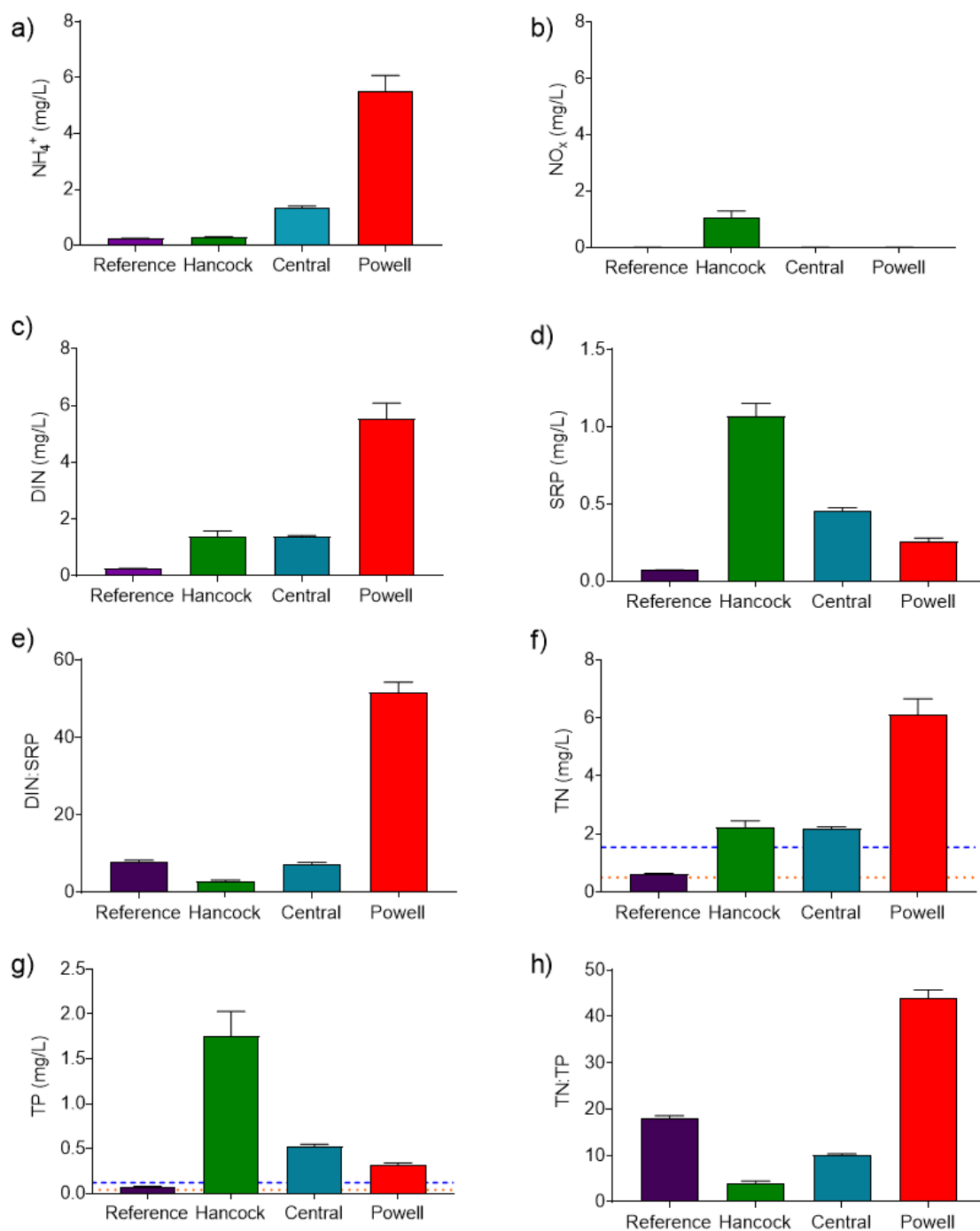


Figure 17. Nutrient concentrations and molar ratios of groundwater (mean $\pm$ standard error) in North Fort Myers, FL by watershed (reference, Hancock Creek, central drainage, and Powell Creek), including a) ammonium (NH_4^+), b) nitrate + nitrite (NO_x), c) dissolved inorganic nitrogen (DIN), d) soluble reactive phosphorus (SRP), e) the molar ratio of DIN:SRP, f) total nitrogen (TN) with an orange dotted line indicating the FDEP surface water protective standard for the Lower Caloosahatchee (≥ 0.5 mg/L) and blue dashed line indicating the FDEP surface water standard for peninsular streams (≥ 1.54 mg/L), g) total phosphorus (TP) with an orange dotted line indicating the FDEP surface water standard for the Lower Caloosahatchee (≥ 0.04 mg/L) and blue dashed line indicating the FDEP surface water standard for peninsular streams (≥ 0.12 mg/L), and h) the molar ratio of TN:TP.

Table 3. Nutrient concentrations and molar ratios of groundwater (median) by watershed (reference well, Hancock Creek, central drainage, and Powell Creek) and site; gray shading indicates watershed medians.

Watershed	Well	Count	NH ₄ (mg/L)	NO _x (mg/L)	DIN (mg/L)	SRP (mg/L)	DIN:SRP	TKN (mg/L)	TN (mg/L)	TP (mg/L)	TN:TP
Reference	<i>Watershed Median / GW10</i>	8	0.24	0.005	0.25	0.07	7.91	0.6	0.6	0.07	17.6
Hancock Creek	<i>Watershed Median</i>	72	0.32	0.037	0.54	0.78	2.19	1.1	1.3	0.99	3.6
	GW1	24	0.28	2.997	3.09	1.66	3.94	1.4	4.1	1.80	5.2
	GW2	24	0.17	0.106	0.38	1.04	1.25	1.0	1.4	1.80	1.5
	GW3	24	0.52	0.005	0.54	0.56	2.17	1.0	1.1	0.58	4.4
Central Drainage	<i>Watershed Median</i>	72	1.38	0.005	1.39	0.43	7.29	2.1	2.1	0.48	10.2
	GW4	24	1.39	0.005	1.40	0.30	10.40	1.9	2.0	0.34	11.9
	GW5	24	1.27	0.005	1.27	0.45	5.32	2.2	2.2	0.50	9.5
	GW6	24	1.17	0.005	1.19	0.60	5.00	2.1	2.1	0.66	8.8
Powell Creek	<i>Watershed Median</i>	70	4.06	0.005	4.12	0.18	46.86	4.8	4.8	0.25	45.5
	GW7	24	3.70	0.008	3.81	0.11	70.57	4.7	4.7	0.25	47.2
	GW8	24	1.31	0.005	1.32	0.09	43.26	1.7	1.7	0.15	31.7
	GW9	24	8.61	0.005	8.62	0.38	45.63	9.5	9.5	0.39	48.5

Groundwater NO_x concentrations were generally low across the North Fort Myers study area (**Fig. 17b**). NO_x concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.329$) or project year (Mann-Whitney U test, $p = 0.612$). Groundwater in the Hancock Creek watershed had significantly higher median concentrations of NO_x (0.037 mg/L) than the other watersheds, which were all similar (medians = 0.005 mg/L; **Table 3**).

Groundwater DIN concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$; **Fig. 17c**), but not season (Mann-Whitney U test, $p = 0.649$) or project year (Mann-Whitney U test, $p = 0.136$). The median groundwater DIN concentrations of all watersheds were significantly different from each other. The highest median DIN was observed in the Powell Creek watershed (4.12 mg/L), followed by the central drainage watershed (1.39 mg/L), Hancock Creek watershed (0.54 mg/L), and the reference well (0.25 mg/L; **Table 3**).

Groundwater SRP concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.725$) or project year (Mann-Whitney U test, $p = 0.210$; **Fig. 17d**). The median SRP concentrations of watersheds were all significantly different from each other. The highest median SRP concentration was in Hancock Creek watershed (0.78 mg/L), followed by the central drainage watershed (0.43 mg/L), Powell Creek watershed (0.18 mg/L), and the reference well (0.07 mg/L; **Table 3**).

The ratios of DIN:SRP in groundwater were variable by watershed (Kruskal-Wallis test, $p < 0.001$) and project year (Mann-Whitney U test, $p = 0.17$; **Fig. 17e**), but not season (Mann-Whitney U test, $p = 0.729$). Median DIN:SRP ratios were significantly different in all watersheds, except the central drainage and the reference well, which were similar. The highest median DIN:SRP ratio of groundwater was in the Powell Creek watershed (46.86), followed by the reference well (7.91), central drainage watershed (7.29), and then Hancock Creek watershed (2.19; **Table 3**). Year 1 had significantly higher median DIN:SRP ratios (8.51) than year 2 (6.09).

Groundwater TN concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.673$) or project year (Mann-Whitney U test, $p = 0.300$; **Fig. 17f**). Median groundwater TN concentrations of all watersheds were significantly different from each other. The highest median TN concentration was in the Powell Creek watershed (4.8 mg/L), followed by the central drainage watershed (2.1 mg/L), Hancock Creek watershed (1.3 mg/L), and the reference well (0.6 mg/L; **Table 3**).

Groundwater TP concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.466$) or project year (Mann-Whitney U test, $p = 0.297$; **Fig. 17g**). Median groundwater TP concentrations of all watersheds were significantly different from each other. The highest median groundwater TP concentration was in the Hancock Creek watershed (0.99 mg/L), followed by the central drainage watershed (0.48 mg/L), Powell Creek watershed (0.25 mg/L), and the reference well (0.07 mg/L; **Table 3**).

The ratios of TN:TP in groundwater were variable by watershed (Kruskal-Wallis test, $p < 0.001$) and project year (Mann-Whitney U test, $p < 0.034$; **Fig. 17h**), but not season (Mann-Whitney U test, $p = 0.730$). Median groundwater TN:TP ratios were all significantly different from each other, except Powell Creek watershed and the reference well, which were similar. The highest TN:TP was observed in the Powell Creek watershed (45.5), followed by the reference site (17.6), central drainage watershed (10.2), and Hancock Creek watershed (3.6; **Table 3**). Year 1 median groundwater TN:TP ratios were significantly higher (12.3) than year 2 (9.2).

3.2.5 Microbial Source Tracking: Chemical Tracers

Sucralose was ubiquitous in groundwater of the North Fort Myers study area, ranged from 0 to 58,000 ng/L, and was higher at low elevation wells near dense septic systems (**Fig. 18a**). Concentrations of sucralose in groundwater were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not by season (Mann-Whitney U test, $p = 0.113$) or project year (Mann-Whitney U test, $p = 0.419$). The highest median sucralose concentrations were observed in Hancock Creek (6,400 ng/L) and the central drainage watershed (1,850 ng/L), which both had levels that would be considered “significant” ($> 1,000$ ng/L; **Table 4**). Powell Creek had the lowest concentrations of sucralose in the groundwater (median = 86 ng/L), followed by the reference watershed, which was also relatively low (median = 200 ng/L; **Table 4**).

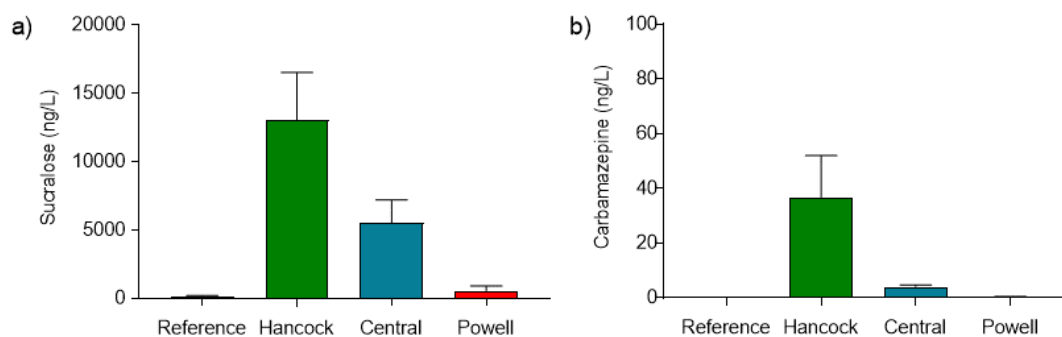


Figure 18. Human wastewater chemical tracer concentrations of groundwater (mean $\pm$ standard error) observed in North Fort Myers, FL by watershed (reference area, Hancock Creek, central drainage, and Powell Creek), including a) the artificial sweetener sucralose and b) the anticonvulsant pharmaceutical, carbamazepine.

Table 4. Chemical tracer concentrations of groundwater (median) observed in North Fort Myers, FL by site and watershed (reference area, Hancock Creek, central drainage, and Powell Creek); gray shading indicates watershed medians. Acetaminophen, diuron, fenuron, hydrocodone, ibuprofen, imazapyr, imidicloprid, linuron, MCP, naproxen, pyraclostrobin, triclopyr, and 2,4-D are not shown because all site medians < 0.001.

Watershed	Well	Count	Human Wastewater Tracers			Stormwater Tracers	
			Sucralose (ng/L)	Carbamazepine (ng/L)	Primidone (ng/L)	Bentazon (ng/L)	Fluridone (ng/L)
Reference	<i>Watershed Median / GW10</i>	8	200	0	0	2.1	1.5
Hancock Creek	<i>Watershed Median</i>	24	6400	9.2	0	0	0
	GW1	8	24500	46.5	8	0	0
	GW2	8	1400	13.5	2.6	0	0
	GW3	8	3150	1.1	0	0	0
Central Drainage	<i>Watershed Median</i>	24	1850	3.2	0	0	0
	GW4	8	350	3.2	30.5	1.5	0
	GW5	8	13500	7.1	0	0	0
	GW6	8	1200	1.1	0	0	0
Powell Creek	<i>Watershed Median</i>	24	86.5	0	0	0	0
	GW7	8	68.5	0	0	0	0
	GW8	8	63	0	0	0	0
	GW9	8	275	0	16.5	21.5	0

Carbamazepine was detected in groundwater near densely aggregated septic systems in the Hancock Creek and the central drainage watersheds at very low concentrations (**Table 4**) and ranged from 0 to 310 ng/L. Concentrations of carbamazepine in groundwater were variable by watershed (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.519$) or project year (Mann-Whitney U test, $p = 0.892$; **Fig. 18b**). The highest carbamazepine concentrations were found in groundwater of the Hancock Creek watershed (median = 9.2 ng/L) and the central drainage watershed (3.2 ng/L; **Table 4**). In the Powell Creek watershed and reference well, the median concentration of carbamazepine in the groundwater was 0 ng/L (**Table 4**).

Primidone was not detected in the reference well but was detected in all other watersheds (**Table 4**) with a range of 0 to 47 ng/L. Concentrations of primidone were not variable by project year (Mann-Whitney U test, $p = 0.235$), watershed (Kruskal-Wallis test, $p = 0.361$) or season (Mann-Whitney U test, $p = 0.866$). The highest median primidone concentrations were at GW4 in the central drainage watershed (30.5 ng/L) and GW9 in the Powell Creek watershed (16.5 ng/L; **Table 4**).

Bentazon was detected in groundwater at three of the four watersheds in very low concentrations (**Table 4**) with a range of 0 to 44 ng/L. Concentrations of bentazon were variable by watershed (Kruskal-Wallis test, $p = 0.030$), but not season (Mann-Whitney U test, $p = 0.569$) or project year (Mann-Whitney U test, $p = 0.569$). By watershed, the highest median bentazon concentrations were observed in the reference well (2 ng/L) with the other watersheds having medians of 0 ng/L (**Table 4**). When means are examined, Powell Creek has the highest bentazon concentrations (7.56 ± 4.08 ng/L), followed by the reference well (1.78 ± 0.64 ng/L), central drainage basin (0.47 ± 0.27 ng/L), and Hancock Creek (no detections). By site, GW9 in Powell Creek had the highest median bentazon concentration (21.5 ng/L).

Some chemical tracers were not widely in detected groundwaters of the North Fort Myers study area. Acetaminophen was only detected three times; in GW7 on 11/15/2017 (23 ng/L), in GW5 on 08/27/2019 (27 ng/L), and again in GW5 on 09/16/2019 (240 ng/L). The chemical tracers mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any groundwater wells during the study. Fluridone was only detected at the reference well (**Table 4**).

3.2.6 Aqueous Stable Isotopes

$\delta^{15}\text{N-NH}_4^+$ values of groundwater in North Fort Myers were generally enriched above +3 ‰ (**Fig. 19a**) with a range of -5.72 to +12.05 ‰. Groundwater $\delta^{15}\text{N-NH}_4^+$ values were variable by project year (ANOVA, $p < 0.001$) and watershed (ANOVA, $p < 0.001$), but not season (ANOVA, $p = 0.052$). There was also a significant interaction between project year and watershed (ANOVA, $p = 0.010$), but no other interactions were significant (ANOVA, all $p > 0.050$). Even though the main test was significant, multiple comparisons between project years did not reveal significant differences between year 1 and year 2. Despite this, mean year 1 groundwater $\delta^{15}\text{N-NH}_4^+$ values ($+5.57 \pm 0.5$ ‰) were higher than mean values from year 2 ($+2.62 \pm 0.5$ ‰). Similarly, multiple comparisons by watershed did not reveal significant differences in groundwater $\delta^{15}\text{N-NH}_4^+$ values. The most enriched mean groundwater $\delta^{15}\text{N-NH}_4^+$ values were in central drainage watershed ($+5.54 \pm 0.6$ ‰), followed by Hancock Creek watershed ($+4.51 \pm 0.9$ ‰), Powell Creek watershed ($+2.77 \pm 0.6$ ‰), and the reference well ($+2.20 \pm 1.1$ ‰; **Fig. 19a**). While season did not have significant effects on groundwater $\delta^{15}\text{N-NH}_4^+$ values, the wet season mean ($+4.72 \pm 0.5$ ‰) was higher than the dry season mean ($+3.4 \pm 0.6$ ‰). Multiple comparisons of the

interaction between project year and watershed revealed that in year 2, Powell Creek watershed had significantly higher $\delta^{15}\text{N-NH}_4^+$ values (mean = $+2.51 \pm 1.1$ ‰) than the reference well (mean = $+0.98 \pm 1.1$ ‰). Interestingly, in year 2 the central drainage watershed actually had the highest mean groundwater $\delta^{15}\text{N-NH}_4^+$ values ($+3.92 \pm 0.6$ ‰), but due to the high variability observed (range from -0.75 to 5.96 ‰), the difference was not significant. Similarly, in year 2 Hancock Creek had a large groundwater $\delta^{15}\text{N-NH}_4^+$ range (from -5.72 to +6.74‰), which lowered the watershed mean in that year ($+1.99 \pm 1.0$ ‰). The reference well had the smallest range in year 2 (from -1.08 to + 3.06 ‰). For detailed $\delta^{15}\text{N-NH}_4^+$ data, see **Appendix 2**.

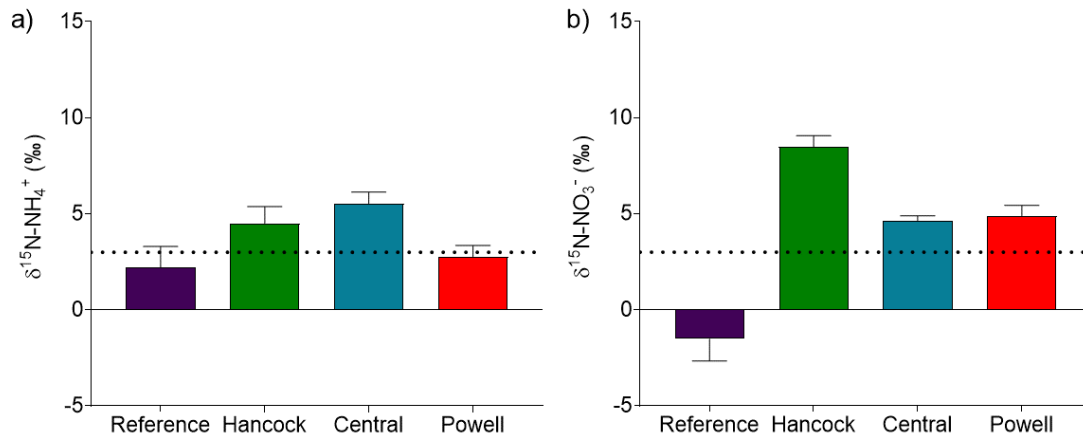


Figure 19. Aqueous stable nitrogen isotope values of groundwater (mean $\pm$ standard error) observed in North Fort Myers, FL by watershed (reference well, Hancock Creek, central drainage, and Powell Creek), including a) ammonium ($\delta^{15}\text{N-NH}_4^+$) and b) nitrate ($\delta^{15}\text{N-NO}_3^-$), with a dotted line to represent the approximate value for wastewater (+ 3 ‰), such as septic tank effluent.

$\delta^{15}\text{N-NO}_3^-$ values of groundwater in North Fort Myers were widely variable with a range of -10.5 to +44.1 ‰ (**Fig. 19b**). Prior to statistical analyses, $\delta^{15}\text{N-NO}_3^-$ values were transformed ($\log_{10}[x+10]$). $\delta^{15}\text{N-NO}_3^-$ values of groundwater in North Fort Myers were variable by season (ANOVA, $p = 0.008$), but not by project year (ANOVA, $p = 0.794$) or watershed (ANOVA, $p = 0.139$). The interaction of project year and season was significant (ANOVA, $p < 0.001$) and the interaction of project year and watershed was marginally significant (ANOVA, $p = 0.046$). No other interactions were significant. Wet season $\delta^{15}\text{N-NO}_3^-$ values of groundwater (mean = $+7.89 \pm 1.9$ ‰) were significantly higher than in the dry season (mean = $+2.71 \pm 1.5$ ‰). Though not statistically significant, the mean year 1 groundwater $\delta^{15}\text{N-NO}_3^-$ values ($+3.25 \pm 1.2$ ‰) were lower than the mean values for year 2 ($+7.17 \pm 2.0$ ‰). While watershed was also not a significant factor, $\delta^{15}\text{N-NO}_3^-$ values of groundwater were highest in Hancock Creek watershed ($+8.49 \pm 2.7$ ‰), followed by Powell Creek watershed ($+4.90 \pm 2.6$ ‰), the central drainage watershed ($+4.65 \pm 1.2$ ‰), and the reference well (-1.52 ± 2.8 ‰).

3.2 Surface Water

3.2.1 Environmental Parameters

Surface water pH ranged from 5.5 to 8.0 during the study (see **Table 5** for medians). Surface water pH was variable by watershed (Kruskal-Wallis test, $p = 0.022$), season (Mann-Whitney U test, $p = 0.001$), and project year (Mann-Whitney U test, $p = 0.033$), but not by site (Kruskal-Wallis test, $p = 0.408$). Multiple comparisons showed that Hancock Creek had a higher median pH (7.5) than the central drainage feature (7.0), while Powell Creek was similar to both (median = 7.4). In the wet season, median pH was significantly higher (7.5) than the dry season (7.2). Annually, median pH was significantly lower in year 1 (7.3) than year 2 (7.4).

Table 5. Environmental parameters of surface water (median) observed by watershed (Hancock Creek, a central drainage feature, and Powell Creek) and site; Temp. = temperature, DO = dissolved oxygen, Cond. = conductivity; gray shading indicates watershed medians.

Watershed	Site	Count	pH	Salinity (ppt)	Cond. (µS)	Color (CU)	Temp. (°C)	DO (%)	DO (mg/L)
Hancock Creek	Watershed Median	40	7.5	1.97	3702	65	23.9	58.0	5.12
	SW9	8	7.5	0.34	685	43	22.9	33.7	2.91
	SW8	8	7.5	1.96	3702	70	24.6	54.2	4.79
	SW7	8	7.5	5.08	9015	72	24.5	60.3	5.30
	16-3GR	8	7.5	5.75	10227	69	24.5	69.0	6.07
	SW6	8	7.5	7.39	12857	69	24.2	90.4	7.87
Central Drainage	Watershed Median / SW5	8	7.0	5.83	9077	62	23.5	57.0	4.79
Powell Creek	Watershed Median	32	7.4	0.32	655	52	23.6	55.2	4.63
	SW4	8	7.3	0.25	563	46	23.6	60.5	4.85
	SW1	8	7.3	0.25	567	42	23.7	56.5	4.87
	SW2	8	7.4	1.30	2531	53	23.5	51.1	4.32
	SW3	8	7.4	2.68	4857	53	23.6	61.2	5.14

Surface water salinity ranged from 0 to 13 ppt during the study (see **Table 5** for medians). Generally, salinity was lowest at upstream sites and increased going downstream towards the estuarine Caloosahatchee River. Salinity was variable by watershed (Kruskal-Wallis test, $p = 0.015$), site (Kruskal-Wallis test, $p = 0.031$), and season (Mann-Whitney U test, $p < 0.001$), but not project year (Mann-Whitney U test, $p = 0.795$). Multiple comparisons showed that median surface water salinities in Powell Creek (0.32 ppt) were significantly lower than in Hancock Creek (1.97 ppt). The central drainage feature had the highest median salinity (5.83 ppt), but the variability was so great that it was statistically similar to the other watersheds. Even though the main test was significant, multiple comparisons of surface water salinity by site did not reveal significant differences. Median surface water salinity was higher in the dry season (6.1 ppt) than the wet season (0.3 ppt).

Surface water conductivity ranged from 6 to 21,853 μS during the study. Conductivity was lowest at upstream sites and increased going downstream towards the estuarine Caloosahatchee River (**Table 5**). Surface water conductivity was variable by watershed (Kruskal-Wallis test, $p = 0.016$), site (Kruskal-Wallis test, $p = 0.027$), and season (Mann-Whitney U test, $p < 0.001$), but not project year (Mann-Whitney U test, $p < 0.855$). Multiple comparisons of surface water conductivity by watershed showed that Powell Creek had significantly lower conductivity (median = 655 μS) than Hancock Creek (median = 3,702 μS ; **Table 5**). Like salinity, while the central drainage feature had the highest median conductivity (9,077 μS), the variability observed in the watershed was so great that it was statistically similar to both other watersheds. Even though the main test was significant, multiple comparisons of surface water conductivity by site did not reveal significant differences. The dry season had significantly higher median conductivity (9,547 μS) than the wet season (553 μS).

Surface water color ranged from 12 to 212 CU during the study. Color was log transformed prior to statistical analyses. Surface water color was variable by season (ANOVA, $p < 0.001$) and project year (ANOVA, $p = 0.015$), but not watershed (ANOVA, $p = 0.204$) or site within watershed (nested ANOVA, $p = 0.168$). There was a significant 3-way interaction effect between watershed, year, and season (ANOVA, $p = 0.044$). Seasonally, surface water color was higher in the wet season (median = 86.1 CU), than the dry season (median = 49.3 CU). Annually, surface water color was higher in year 1 (63.7 CU) than year 2 (50.0 CU). Though no significant differences were found by watershed, the highest median color was observed in Hancock Creek (65 CU), followed by the central drainage feature (62 CU), and Powell Creek (52 CU; **Table 5**). For monthly color data from the additional sampling events, see **Appendices 3 and 4**.

Surface water temperatures ranged from 17.9 to 29.6°C during the study. Water temperature was variable by season (Mann-Whitney U test, $p < 0.001$), but not by watershed (Kruskal-Wallis test, $p = 0.986$), site (Kruskal-Wallis test, $p = 0.999$), or project year (Mann-Whitney U test, $p = 0.564$). Seasonally, median surface water temperature was higher in the wet season (26.5°C) than the dry season (20.9°C). By watershed, median water temperatures varied very little (less than 2°C; **Table 5**).

Surface water DO ranged from 0.37 to 8.2 mg/L during the study (see **Table 5** for median values). DO (mg/L) was variable by site within watershed (nested ANOVA, $p < 0.001$) and season (ANOVA, $p < 0.001$), but not by watershed (ANOVA, $p = 0.160$) or project year (ANOVA, $p = 0.194$) with no significant interactions between factors. Multiple comparisons showed that within Hancock Creek, the mean DO concentration was significantly lower at the most upstream site, SW9 (3.0 ± 0.3 mg/L), than all other sites. DO concentrations at the most downstream site in Hancock Creek, SW6 (7.4 ± 0.5 mg/L), were significantly higher than all other sites, except 16-3GR (5.7 ± 0.5 mg/L), which was

similar. In Powell Creek, mean DO concentrations at SW2 (3.7 ± 0.5 mg/L) were significantly lower than other sites. The dry season had higher mean DO (5.4 ± 0.2 mg/L) than the wet season (4.2 ± 0.3 mg/L).

3.3.2 Bacterial Abundance

Surface water enterococci counts ranged from <5 MPN/100mL (the lower limit of detection) to $>2,420$ MPN/100mL (the upper limit of detection). Enterococci abundance in the North Fort Myers study area was variable by watershed (Kruskal-Wallis test, $p = 0.023$), site (Kruskal-Wallis test, $p = 0.037$), and season (Mann-Whitney U test, $p = 0.007$), but not by project year (Mann-Whitney U test, $p = 0.051$). Although watershed was significantly different in the main test, pairwise comparisons revealed no significant differences in surface water enterococci counts between watersheds, likely due to very high variability between sampling events. Similarly, while the main statistical test for site was significant, no significant differences in surface water enterococci counts were observed between sites in pairwise comparisons. Despite the lack of statistical differences, there was variability in surface water enterococci counts by watershed and site (**Table 6, Fig. 20a**). The highest median enterococci counts were observed in the central drainage feature (888 MPN/100mL), followed by Powell Creek (353 MPN/100mL), and Hancock Creek (123 MPN/100mL; **Table 6**). In Hancock Creek, 16-3GR had the highest enterococci counts, often exceeding the TPTV criteria for the downstream waters of the Lower Caloosahatchee River (**Table 6, Fig. 20a**), followed by SW7. The sites upstream and downstream of dense residential areas, such as SW9 and SW6, tended to have lower enterococci counts (**Table 6, Fig. 20a**). Surface water in the central drainage feature had elevated enterococci counts and also exceeded the Lower Caloosahatchee TPTV criteria (**Fig. 20a**), which is particularly relevant at this site because sampling was conducted just upstream from the river (**Fig. 4**). In Powell Creek, surface water enterococci counts were elevated, and all sites exceeded the Lower Caloosahatchee TPTV criteria (**Fig. 20a**). In Powell Creek, sites displayed a trend of higher enterococci counts upstream that decreased downstream. This may be an effect of dilution with Caloosahatchee River in the lower sites that are more tidally influenced and experience greater water exchange with the river. Seasonal effects were observed in enterococci counts with higher median counts in the dry season (518 MPN/100mL), than the wet season (134 MPN/100mL). Though not significantly variable by project year, median enterococci counts were higher in year 1 (423 MPN/100mL) than year 2 (154 MPN/100mL). For monthly enterococci count data from the additional sampling events see **Appendices 3 and 4**.

Table 6. Fecal indicator bacteria abundance and biochemical oxygen demand (BOD) concentrations of surface waters (median) observed by watershed (Hancock Creek, a central drainage feature, and Powell Creek), and site; gray shading indicates watershed medians.

Watershed	Site	Count	Enterococci (MPN/100mL)	<i>E. coli</i> (MPN/100mL)	BOD (mg/L)
Hancock Creek	Watershed Median	40	123	227	0.90
	SW9	8	52	194	0.45
	SW8	8	79	349	0.80
	SW7	8	164	199	1.00
	16-3GR	8	677	564	1.05
	SW6	8	21	93	0.90
Central Drainage	Watershed Median / SW5	8	888	659	1.05
Powell Creek	Watershed Median	32	353	548	0.85
	SW4	8	1259	1224	0.65
	SW1	8	184	692	0.80
	SW2	8	455	644	1.05
	SW3	8	378	441	1.00

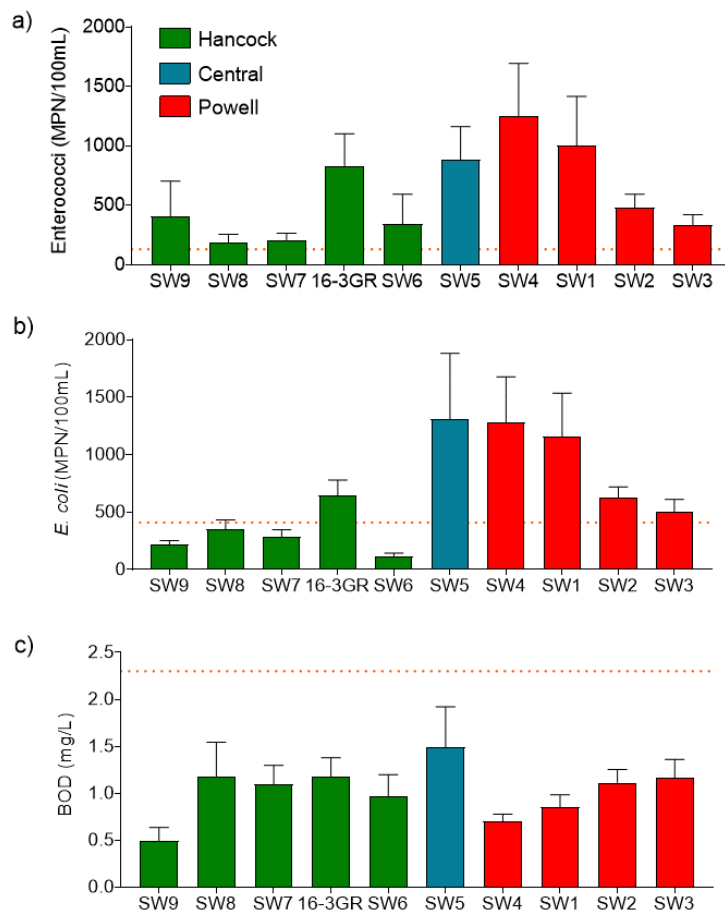


Figure 20. Fecal indicator bacteria abundance, including a) enterococci and b) *Escherichia coli* (*E. coli*), and c) biochemical oxygen demand (BOD) concentrations of surface waters (mean $\pm$ standard error) in North Fort Myers, FL by site and watershed (Hancock Creek, a central drainage feature, and Powell Creek); orange dotted lines indicate FDEP Ten Percent Threshold Value (TPTV) surface water criteria for enterococci (marine water; ≥ 130 MPN/100mL), FDEP surface water standards for BOD (2.4 mg/L), and FDEP TPTV surface water criteria for *E. coli* (fresh water; ≥ 410 MPN/100mL). Marine standards do not apply to the study area and are shown for a point of reference only.

Surface water *E. coli* counts ranged from 38 to >4,840 MPN/100mL. *E. coli* abundance in the North Fort Myers study area was variable by watershed (Kruskal-Wallis test, $p < 0.001$), site (Kruskal-Wallis test, $p < 0.001$), and season (Mann-Whitney U test, $p = 0.002$), but not by project year (Mann-Whitney U test, $p = 0.355$). Pairwise comparisons by watershed showed that Powell Creek had significantly higher median surface water *E. coli* counts (548 MPN/100mL) than Hancock Creek (227 MPN/100mL; **Table 6**). The central drainage feature had the highest median *E. coli* counts (659 MPN/100mL), but the variability in that watershed was also largest (standard error of mean = 1,613 MPN/100mL) and the number of sites lowest ($n = 1$); therefore, differences between the central drainage watershed and the other watersheds were not statistically significant. Pairwise comparisons by site showed that SW6 had significantly lower surface water *E. coli* counts than 16-3GR, which is the next site upstream in Hancock Creek (**Fig. 20 b**). Higher median surface water *E. coli* counts were observed in the dry season (596 MPN/100mL) than the wet season (267 MPN/100mL). Although project year was not significantly variable, median *E. coli* counts were higher in year 1 (461 MPN/100mL) than year 2 (292 MPN/100mL). For monthly *E. coli* count data from the additional sampling events see **Appendices 3 and 4**.

Surface water BOD concentrations in the North Fort Myers study area ranged from 0.15 to 4.4 mg/L. BOD concentrations were variable by season (Mann-Whitney U test, $p < 0.001$), but not by watershed (Kruskal-Wallis test, $p = 0.314$), site (Kruskal-Wallis test, $p = 0.061$), or project year (Mann-Whitney U test, $p = 0.065$). By watershed, the highest BOD was at the central drainage feature (1.05 mg/L), followed by Hancock Creek (0.90 mg/L), and Powell Creek (0.85 mg/L), representing a total range in median watershed BOD concentrations of 0.20 mg/L. Pairwise comparisons did not reveal significant differences in surface water BOD concentrations between sites, but SW9, the most upstream site in Hancock Creek, had the lowest median BOD (0.45 mg/L), followed by SW4, the most upstream site in Powell Creek (0.65 mg/L; **Fig. 20c**). The other sites all had median BOD concentrations between 0.80 mg/L and 1.05 mg/L (**Table 6**). Surface water median BOD concentrations were significantly higher in the dry season (1.30 mg/L) than the wet season (0.74 mg/L). While not significantly different, median BOD concentrations were higher in year 1 (1.17 mg/L) of the project, than year 2 (0.87 mg/L). For monthly BOD concentration data from the additional sampling events see **Appendices 3 and 4**.

3.3.3 Nutrient Concentrations and Molar Ratios

Surface water NH_4^+ concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), site (Kruskal-Wallis test, $p < 0.001$), and season (Mann-Whitney U test, $p < 0.001$), but not project year (Mann-Whitney U test, $p = 0.052$; **Fig 21a**). Multiple comparisons showed the central drainage feature had significantly higher median NH_4^+ concentrations (0.06 mg/L) than Hancock Creek (0.02 mg/L) and Powell Creek (0.04 mg/L; **Table 7**). Within each watershed, multiple comparisons did not reveal any differences in median NH_4^+ concentrations by site. Surface water NH_4^+ concentrations in the wet season had a

wider range (0.29 mg/L) and higher median (0.03 mg/L) than the dry season (range = 0.06 mg/L, median = 0.02). Years 1 and 2 had similar median surface water NH_4^+ concentrations (0.03 mg/L). For monthly NH_4^+ data from the additional sampling events, see **Appendices 5 and 6**.

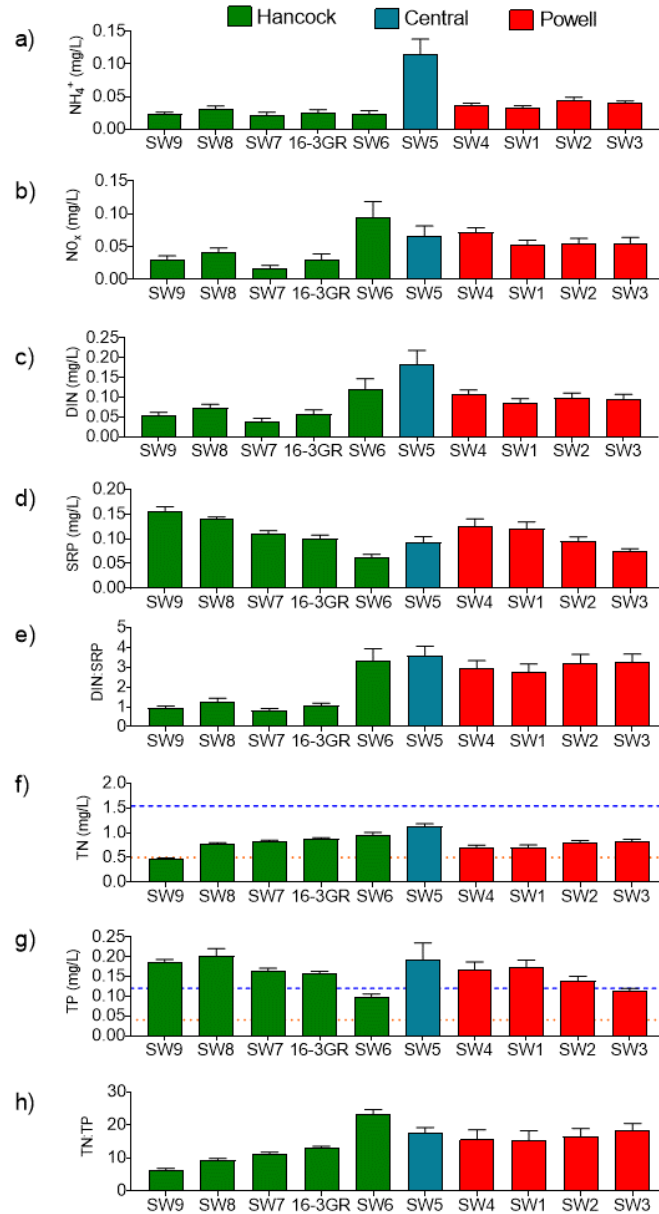


Figure 21. Nutrient concentrations and molar ratios (mean $\pm$ standard error) of surface water in North Fort Myers, FL by site and watershed (Hancock Creek, a central drainage feature, and Powell Creek), including a) ammonium (NH_4^+), b) nitrate + nitrite (NO_x), c) dissolved inorganic nitrogen (DIN), d) soluble reactive phosphorus (SRP), e) the molar ratio of DIN:SRP, f) total nitrogen (TN) with an orange dotted line indicating the FDEP surface water protective standard for the Lower Caloosahatchee (≥ 0.5 mg/L) and blue dashed line indicating the FDEP surface water standard for peninsular streams (≥ 1.54 mg/L), g) total phosphorus (TP) with an orange dotted line indicating the FDEP surface water standard for the Lower Caloosahatchee (≥ 0.04 mg/L) and blue dashed line indicating the FDEP surface water standard for peninsular streams (≥ 0.12 mg/L), and e) the molar ratio of TN:TP.

Table 7. Surface water nutrient concentrations and molar ratios (median) observed by watershed (Hancock Creek, a central drainage feature, and Powell Creek) and site; gray shading indicates watershed medians.

Watershed	Site	Count	NH4 (mg/L)	NOx (mg/L)	DIN (mg/L)	SRP (mg/L)	DIN:SRP	TKN (mg/L)	TN (mg/L)	TP (mg/L)	TN:TP
Hancock Creek	<i>Watershed Median</i>	120	0.02	0.01	0.03	0.115	1.09	0.76	0.79	0.16	11.6
	SW9	8	0.02	0.03	0.06	0.151	0.74	0.41	0.45	0.18	5.3
	SW8	8	0.03	0.03	0.08	0.135	1.23	0.73	0.76	0.17	9.4
	SW7	8	0.01	0.01	0.01	0.093	0.64	0.84	0.84	0.16	11.5
	16-3GR	8	0.02	0.01	0.04	0.084	0.99	0.87	0.88	0.15	13.2
	SW6	8	0.02	0.01	0.03	0.055	2.27	0.86	0.90	0.11	21.1
Central Drainage	<i>Watershed Median / SW5</i>	8	0.06	0.03	0.09	0.065	3.11	1.05	1.10	0.17	17.4
Powell Creek	<i>Watershed Median</i>	96	0.04	0.05	0.09	0.083	3.06	0.72	0.80	0.13	14.4
	SW4	8	0.03	0.08	0.12	0.122	2.41	0.58	0.67	0.17	8.7
	SW1	8	0.03	0.05	0.08	0.117	2.37	0.54	0.61	0.16	9.1
	SW2	8	0.04	0.05	0.09	0.086	2.97	0.72	0.79	0.14	11.8
	SW3	8	0.04	0.04	0.08	0.074	3.10	0.79	0.86	0.12	15.0

Surface water NO_x concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), site (Kruskal-Wallis test, $p < 0.001$), and season (Mann-Whitney U test, $p < 0.001$), but not project year (Mann-Whitney U test, $p = 0.050$; **Fig. 21b**). Multiple comparisons showed that Powell Creek had significantly higher median surface water NO_x concentrations (0.05 mg/L) than Hancock Creek (0.01 mg/L), while the central drainage feature (0.03 mg/L) was similar to both Hancock Creek and Powell Creek (**Table 7**). Within each watershed, multiple comparisons did not reveal any differences in surface water NO_x concentrations by site. Wet season NO_x concentrations had a wider range (0.27 mg/L) and a higher median (0.07 mg/L) than the dry season (range = 0.11 mg/L, median = 0.01 mg/L). For monthly NO_x data from the additional sampling events, see **Appendices 5 and 6**.

Surface water DIN concentrations were variable by watershed (Kruskal-Wallis test, $p < 0.001$), site (Kruskal-Wallis test, $p < 0.001$), season (Mann-Whitney U test, $p < 0.001$), and project year (Mann-Whitney U test, $p = 0.033$; **Fig. 21c**). Multiple comparisons showed that median surface water DIN concentrations were significantly higher in Powell Creek and the central drainage feature (both 0.09 mg/L) than in Hancock Creek (0.03 mg/L; **Table 7**). Within each watershed, multiple comparisons did not reveal any differences in surface water DIN concentrations by site. Wet season DIN concentrations had a wider range (2.70 mg/L) and a higher median (0.10 mg/L) than the dry season (range = 0.04 mg/L, median = 0.03 mg/L). Median surface waters DIN concentrations were significantly higher in year 1 (0.09 mg/L) than year 2 (0.07 mg/L). For monthly DIN data from the additional sampling events, see **Appendices 5 and 6**.

Surface water SRP concentrations were variable by watershed (Kruskal-Wallis test, $p = 0.033$), site (Kruskal-Wallis test, $p < 0.001$), season (Mann-Whitney U test, $p < 0.001$), and project year (Mann-Whitney U test, $p < 0.001$; **Fig. 21d**). Multiple comparisons did not find significant differences in SRP concentrations by watershed. The highest median SRP was in Hancock Creek (0.115 mg/L), followed by Powell Creek (0.083 mg/L), and the

central drainage feature (0.065 mg/L; **Table 7**). Within Hancock Creek, multiple comparisons showed that SW6 had significantly lower surface water SRP concentrations than any other site. Within Powell Creek, all sites were similar. Dry season surface water SRP concentrations had a wider range (0.22 mg/L) and a higher median (0.11 mg/L) than the wet season (range = 0.18 mg/L, median = 0.08 mg/L). Median SRP concentrations were significantly higher in year 1 (0.10 mg/L) than year 2 (0.09 mg/L). For monthly SRP data from the additional sampling events, see **Appendices 5 and 6**.

Surface water DIN:SRP ratios were generally low (<5) for the North Fort Myers area. The ratio of DIN:SRP was variable by watershed (Kruskal-Wallis test, $p < 0.001$), site (Kruskal-Wallis test, $p < 0.001$), and season (Mann-Whitney U test, $p < 0.001$, but not project year (Mann-Whitney U test, $p = 0.399$; **Fig. 21e**). Multiple comparisons showed that surface water DIN:SRP ratios were significantly lower in Hancock Creek (median = 1.09), than in the central drainage basin (3.11) or Powell Creek (3.06; **Table 7**). Multiple comparisons showed that within Hancock Creek, the most downstream site, SW6, was significantly higher than all of the other sites (SW7, SW8, and SW9), except 16-3GR. Wet season DIN:SRP ratios of surface water had a wider range (1.93) and a higher median (3.46) than the dry season (range = 0.56, median = 1.10). For monthly DIN:SRP data from the additional sampling events, see **Appendices 5 and 6**.

Surface water TN was elevated throughout the study area. TN was variable by watershed (Kruskal-Wallis test, $p < 0.001$), site (Kruskal-Wallis test, $p < 0.001$), season (Mann-Whitney U test, $p < 0.001$), and project year (Mann-Whitney U test, $p < 0.001$; **Fig. 21f**). Multiple comparisons showed that TN concentrations were significantly higher in the central drainage feature (1.10 mg/L) than in Hancock Creek (0.76 mg/L) or Powell Creek (0.72 mg/L; **Table 7**). Within Hancock Creek, SW9, the most upstream site, had significantly lower TN than all the downstream sites. Within Powell Creek, all sites were similar. Wet season TN concentrations had a wider range (1.21 mg/L) and a higher median (0.89 mg/L) than the dry season (range = 0.95 mg/L, median = 0.75). Median TN concentrations were significantly higher in year 2 (0.89 mg/L) than year 1 (0.75 mg/L). For monthly TN data from the additional sampling events, see **Appendices 5 and 6**.

Surface water TP was elevated throughout the study area. TP was variable by site (Kruskal-Wallis test, $p < 0.001$), season (Mann-Whitney U test, $p < 0.001$), and project year (Mann-Whitney U test, $p < 0.001$), but not by watershed (Kruskal-Wallis test, $p = 0.071$; **Fig. 21g**). Median surface water TP concentrations were highest in the central drainage feature (0.17 mg/L), followed by Hancock Creek (0.16 mg/L), and Powell Creek (0.13 mg/L; **Table 7**). Within Hancock Creek, multiple comparisons showed that SW6 had significantly lower surface water TP concentrations than any other site. Within Powell Creek, all sites were similar. Dry season surface water TP concentrations had a wider range (1.1 mg/L) and a higher median (0.16 mg/L) than the wet season (range = 0.20 mg/L, median = 0.13 mg/L).

Median TP concentrations were significantly higher in year 1 (0.17 mg/L) than year 1 (0.14 mg/L). For monthly TP data from the additional sampling events, see **Appendix Appendices 5 and 6**.

TN:TP ratios of surface water were generally low throughout the study area. Surface water TN:TP ratios were variable by watershed (Kruskal-Wallis test, $p = 0.010$), site (Kruskal-Wallis test, $p < 0.001$), season (Mann-Whitney U test, $p < 0.001$), and project year (Mann-Whitney U test, $p < 0.001$; **Fig. 21h**). Multiple comparisons showed that TN:TP ratios of surface water in Hancock Creek (11.6) were significantly lower than either Powell Creek (14.4) or the central drainage feature (17.4; **Table 7**). Within Hancock Creek, multiple comparisons showed that TN:TP ratios were significantly lower upstream at SW9 and SW8, than downstream at 16-3GR and SW6; while the middle site, SW7, was significantly different from the most downstream site, SW6, but was similar to the next site downstream, 16-3GR. Wet season TN:TP ratios of surface water had a wider range (45.3) and a higher median (15.0) than the dry season (range = 34.3, median = 9.6). Median TN:TP ratios of surface water were significantly higher in year 2 (15.0) than year 1 (10.2). For monthly TN:TP data from the additional sampling events, see **Appendices 5 and 6**.

3.3.4 Microbial Source Tracking: Molecular Markers

Concentrations of the human marker HF183 spanned from below detection limit (BDL) to 1,820 GEU/100mL at SW3 in the October sampling event in year 1 (**Fig. 22a**). HF183 concentrations in surface water were variable by watershed (Kruskal-Wallis test, $p = 0.001$), but not by site (Kruskal-Wallis test, $p = 0.055$), season (Mann-Whitney U test, $p = 0.247$), or project year (Mann-Whitney U test, $p = 0.490$). Multiple comparisons of HF183 concentrations revealed that the central drainage feature (median = 230 GEU/100mL) was significantly higher than in Hancock Creek (median = 0 GEU/100mL), but not Powell Creek (median = 0 GEU/100mL; **Table 8**).

Table 8. Molecular marker concentrations in surface waters (median) by watershed (Hancock Creek, a central drainage feature, and Powell Creek) and site; gray shading indicates watershed medians.

Watershed	Site	Count	Human Marker	Avian Markers		Ruminant Marker
			HF183-qPCR (GEU/100 mL)	GFD-qPCR (TSC/100 mL)	GULL2-qPCR (TSC/100 mL)	BacR-qPCR (TSC/100mL)
Hancock Creek	<i>Watershed Median</i>	40	0	255	0	0
	SW9	8	0	0	0	0
	SW8	8	0	80	0	0
	SW7	8	0	615	0	0
	16-3GR	8	0	6,950	4,550	0
	SW6	8	0	95	0	0
Central Drainage	<i>Watershed Median</i>	8	230	0	0	0
	SW5					
Powell Creek	<i>Watershed Median</i>	32	0	0	0	0
	SW4	8	0	0	0	0
	SW1	8	0	0	0	0
	SW2	8	95	0	0	0
	SW3	8	0	0	0	0

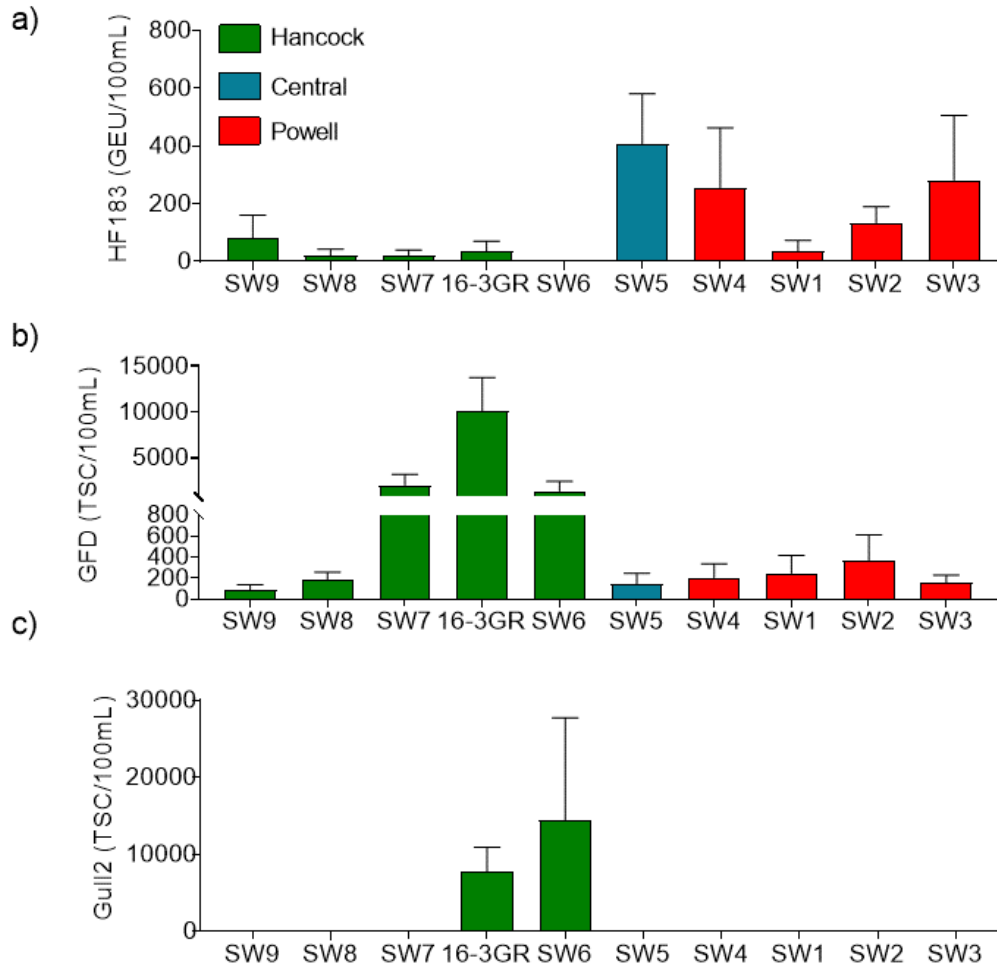


Figure 22. Molecular markers concentrations in surface waters (mean $\pm$ standard error) of North Fort Myers, FL by site and watershed (Hancock Creek, a central drainage feature, and Powell Creek), including a) the human marker, HF183, and the avian markers b) GFD and c) Gull2.

Concentrations of the avian marker GFD fluctuated from BDL to 32,600 TSC/100mL at 16-3GR, which was the highest observed concentration. GFD concentrations were variable by watershed (Kruskal-Wallis test, $p = 0.014$), site (Kruskal-Wallis test, $p = 0.001$), and season (Mann-Whitney U test, $p = 0.007$), but not by project year (Mann-Whitney U test, $p = 0.385$). Multiple comparisons by watershed did not find any significant differences in GFD concentrations (**Table 8**). Multiple comparisons by site showed that within Hancock Creek, SW9, the most upstream site, had a significantly lower median (0 TSC/100mL) than 16-3GR (6,950 TSC/100mL; **Fig. 22b**). The other sites were all similar. Significantly higher median GFD concentrations were observed in the wet season (275 TSC/100mL), than the dry season (0 TSC/100mL). Mean GFD concentrations in the wet season ($2,472 \pm 961$ TSC/100mL) were also higher than in the dry season (488 ± 223 TSC/100mL).

The avian marker Gull2 was only detected in Hancock Creek and concentrations ranged from BDL to 107,000 TSC/100mL (**Fig. 22c**). Gull2 concentrations were variable by site (Kruskal-Wallis test, $p = 0.001$), but not by season (Mann-Whitney U test, $p = 0.992$) or project year (Mann-Whitney U test, $p = 0.094$; **Table 8**). Multiple comparisons by site did not find any significant differences in Gull2 concentrations.

The ruminant marker BacR was not detected during the study resulting in median values of zero for all sites (**Table 8**).

3.3.5 Microbial Source Tracking: Chemical Tracers

Sucralose was ubiquitous in surface waters of the study area in concentrations ranging from 0 to 1,600 ng/L. Sucralose varied by watershed (Kruskal-Wallis test, $p = 0.033$) and site (Kruskal-Wallis test, $p = 0.037$), but not season (Mann-Whitney U test, $p = 0.214$) or project year (Mann-Whitney U test, $p = 0.607$). Multiple comparisons by watershed did not find any significant differences in surface water sucralose concentrations. Though no significant differences were found between watersheds, the highest surface water sucralose concentrations were observed in Powell Creek (mean = 749 ± 66 ng/L), followed by the central drainage feature (mean = 710 ± 74 ng/L) and Hancock Creek (mean = 529 ± 46 ng/L; for medians see **Table 9**). Though the main statistical test was significant for site, multiple comparisons did not find any significant differences (**Fig. 23 a**).

Table 9. Surface water chemical tracer concentrations (median) observed in North Fort Myers, FL by site and watershed (Hancock Creek, a central drainage feature, and Powell Creek); gray shading indicates watershed medians. Acetaminophen, fenuron, hydrocodone, ibuprofen, linuron, MCPP, naproxen, pyraclostrobin, and triclopyr are not shown because all site medians < 0.001.

Watershed	Site	Count	Human Wastewater Tracers			Stormwater Tracers					
			Sucralose (ng/L)	Carbamazepine (ng/L)	Primidone (ng/L)	2-4-D (ng/L)	Bentazon (ng/L)	Diuron (ng/L)	Fluridone (ng/L)	Imazapyr (ng/L)	Imidicloprid (ng/L)
Hancock Creek	Watershed Median	40	490	1.70	2.00	7.1	6.35	2.9	24.0	28	4.6
	SW9	8	330	0.50	0	0	1.85	0	12.0	28	0
	SW8	8	720	2.90	6.70	3.7	5.80	2.1	95.0	36	4.0
	SW7	8	750	2.70	5.00	9.7	9.40	3.3	48.0	31	4.6
	16-3GR	8	595	1.70	2.40	11.9	11.50	4.1	24.0	16	4.7
	SW6	8	285	1.10	0	27.2	7.45	2.9	3.2	20	8.6
Central Drainage	Watershed Median / SW5	8	645	1.65	0	6.7	7.00	3.9	3.3	22	7.9
Powell Creek	Watershed Median	32	715	11.50	2.10	0	2.85	0	3.9	34	4.6
	SW4	8	850	14.50	2.20	0	1.45	0	5.7	38	3.8
	SW1	8	920	13.50	2.15	0	1.90	0	4.5	34	3.4
	SW2	8	665	9.55	2.45	4.0	4.35	0	3.3	34	5.4
	SW3	8	570	5.85	2.10	3.3	4.40	0	3.0	23	6.9

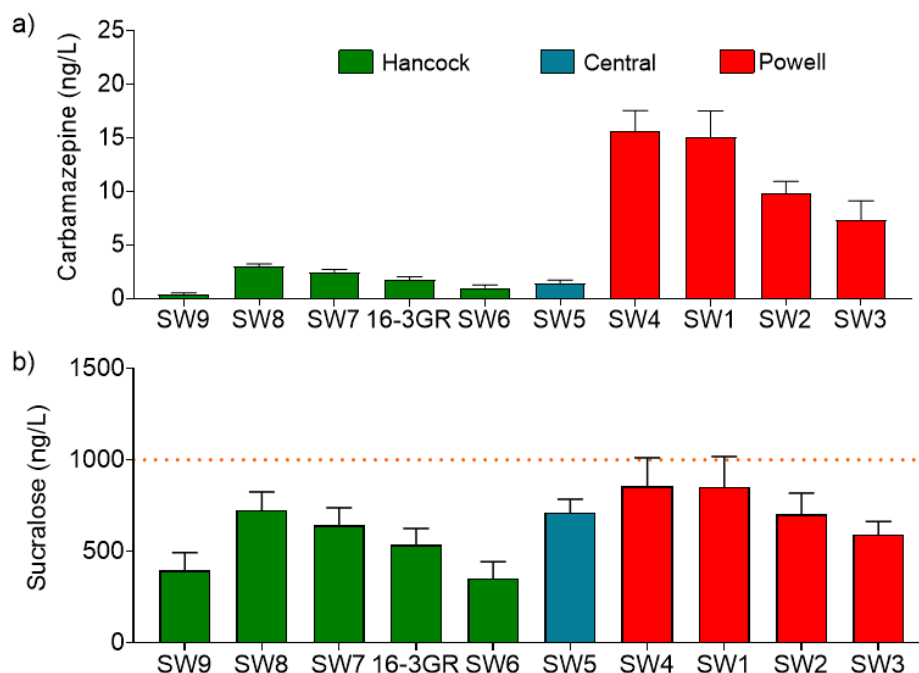


Figure 23. Surface water human chemical tracers concentrations (mean $\pm$ standard error) observed in North Fort Myers, FL by site, watershed (Hancock Creek, a central drainage feature, and Powell Creek), including a) the artificial sweetener sucralose, with an orange dotted line indicating “significant” concentrations, as well as b) the anticonvulsant pharmaceutical carbamazepine.

Carbamazepine ranged from 0 to 28 ng/L and was ubiquitous in surface waters throughout the study area in low levels (**Fig. 23b**). Surface water carbamazepine concentrations varied by watershed (Kruskal-Wallis test, $p < 0.001$) and site (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.355$) or project year (Mann-Whitney U test, $p = 0.331$). Multiple comparisons showed that Powell Creek had significantly higher carbamazepine concentrations in surface water (mean = 12 ± 1.1 ng/L) than Hancock Creek (mean = 1.7 ± 0.2 ng/L) or the central drainage feature (mean = 1.4 ± 0.2 ng/L; see **Table 9** for medians). There were no significant differences by site within the same watershed (**Fig. 23b**).

Surface water acetaminophen concentrations ranged from 0 to 38 ng/L. Acetaminophen concentrations varied by watershed (Kruskal-Wallis test, $p = 0.002$), but not site (Kruskal-Wallis test, $p = 0.065$), season (Mann-Whitney U test, $p = 0.465$) or project year (Mann-Whitney U test, $p = 0.477$). Multiple comparisons showed that acetaminophen concentrations were significantly higher in the central drainage feature (mean = 6.5 ± 3.6 ng/L) and Powell Creek (mean = 3.2 ± 0.16 ng/L), than in Hancock Creek (no detection).

Primidone was ubiquitous throughout the study area in low concentrations with a range of 0 to 14 ng/L. Surface water primidone concentrations varied by season (Mann-Whitney U test, $p < 0.001$), but not by watershed (Kruskal-Wallis test, $p = 0.478$), site (Kruskal-Wallis

test, $p = 0.093$), or project year (Mann-Whitney U test, $p = 0.567$). While the medians for both seasons were 0 ng/L, mean surface water primidone concentrations were higher in the dry season (6.6 ± 2.1 ng/L) than the wet season (4.17 ± 2.1 ng/L). Though no significant differences were found between watersheds, the highest surface water primidone concentrations were observed in Powell Creek (mean = 3.2 ± 0.7 ng/L), followed by Hancock Creek (mean = 3.0 ± 0.5 ng/L) and the central drainage feature (mean = 2.3 ± 1.8 ng/L; for medians see **Table 9**).

2-4-D was only tested for in year 1 and concentrations ranged from 0 to 56 ng/L. Surface water 2-4-D concentrations varied by watershed (Kruskal-Wallis test, $p = 0.014$) and site (Kruskal-Wallis test, $p = 0.005$), but not season (Mann-Whitney U test, $p = 0.910$). Multiple comparisons showed that surface water 2-4-D concentrations were significantly higher in Hancock Creek (mean = 12.5 ± 3.4 ng/L) than Powell Creek (mean = 2.2 ± 0.01 ng/L), while the central drainage feature was similar to both (mean = 17.4 ± 13 ng/L) due to its high variability (see **Table 9** for medians). Though the main statistical test was significant for site, multiple comparisons did not find any significant differences.

Bentazon was only tested for in year 1 and concentrations ranged from 0 to 17 ng/L. Surface water bentazon concentrations varied by watershed (Kruskal-Wallis test, $p = 0.009$) and site (Kruskal-Wallis test, $p = 0.002$), but not season (Mann-Whitney U test, $p = 0.027$). Multiple comparisons showed that surface water bentazon concentrations were significantly higher in the central drainage feature (mean = 8.7 ± 3.1 ng/L) and Hancock Creek (mean = 7.2 ± 1.1 ng/L) than in Powell Creek (mean = 3.2 ± 0.04 ng/L; see medians in **Table 9**). Though the main statistical test was significant for site, multiple comparisons did not find any significant differences.

Diuron was only tested for during the dry season of year 1 and concentrations ranged from 0 to 4.1 ng/L. Surface water diuron concentrations did not vary by watershed (Kruskal-Wallis test, $p = 0.059$) or site (Kruskal-Wallis test, $p = 0.437$). Though no significant differences were found between watersheds, the highest surface water diuron concentrations were observed in the central drainage feature (one detection = 3.9 ng/L, followed by Hancock Creek (mean = 2.5 ± 0.1 ng/L), and Powell Creek (no detection; **Table 9**). Within Hancock Creek, SW9, the most upstream site, was the only location where no diuron was detected in the surface water.

Fluoridone was only tested for during the dry season of year 1 and was ubiquitous throughout the study area (**Table 9**) with concentrations ranging from 3 to 95 ng/L. Surface water fluoridone concentrations did not vary by watershed (Kruskal-Wallis test, $p = 0.203$) or site (Kruskal-Wallis test, $p = 0.437$). Though no significant differences were found between watersheds, the highest surface water fluoridone concentrations were observed in Hancock Creek (mean = 36.4 ± 17 ng/L), followed by Powell Creek (mean = $4.1 \pm <0.01$

ng/L) and the central drainage feature (3.3 ng/L; for medians see **Table 9**). As such, the sites with the highest fluoridone concentrations, SW8 (95 ng/L), followed by SW7 (48 ng/L), 16-3-GR (24 ng/L), and SW9 (12 ng/L), were all in Hancock Creek.

Imazapyr was only tested for during the dry season of year 1 and was ubiquitous throughout the study area (**Table 9**) with concentrations ranging from 16 to 38 ng/L. Surface water imazapyr concentrations did not vary by watershed (Kruskal-Wallis test, $p = 0.290$) or site (Kruskal-Wallis test, $p = 0.437$). Though no significant differences were found between watersheds, the highest surface water imazapyr concentrations were observed in Powell Creek (mean = 32.3 ± 3.2 ng/L), followed by Hancock Creek (mean = 26.2 ± 3.6 ng/L), and the central drainage feature (22 ng/L).

Imidacloprid was only tested for during the dry season of year 1 and was ubiquitous throughout the study area, except for at SW9 (**Table 9**) with concentrations ranging from 0 to 8.6 ng/L. Surface water imidacloprid concentrations did not vary by watershed (Kruskal-Wallis test, $p = 0.474$) or site (Kruskal-Wallis test, $p = 0.437$). Though no significant differences were found between watersheds, the highest surface water imidacloprid concentrations were observed in the central drainage feature (one detection = 7.9 ng/L), followed by Powell Creek (mean = 4.9 ± 0.01 ng/L) and Hancock Creek (mean = 4.4 ± 0.1 ng/L).

The chemical tracers hydrocodone, ibuprofen, naproxen, mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any surface water sites during the study. Fenuron, linuron, and pyraclostrobin were only tested for in the dry season of year 1. mCPP and triclopyr were tested for in both seasons in year 1, but not detected. Hydrocodone, ibuprofen, and naproxen were tested for in both seasons in year 2, but not detected.

3.3.6 Particulate Organic Matter Stable Isotopes and Elemental Composition

Surface water POM $\delta^{15}\text{N}$ values were generally enriched $> +3$ ‰ throughout the study area (overall mean = $+4.69 \pm 0.2$ ‰; **Fig. 24a**) with values ranging from -8.45 to +11.9 ‰. POM $\delta^{15}\text{N}$ values were variable by season (Mann-Whitney U test, $p < 0.001$), watershed (Kruskal-Wallis test, $p = 0.006$), and site (Kruskal-Wallis test, $p < 0.001$), but not project year (Mann-Whitney U test, $p = 0.756$). The dry season had significantly higher median surface water POM $\delta^{15}\text{N}$ values ($+5.56$ ‰) than the wet season ($+3.96$ ‰). POM $\delta^{15}\text{N}$ values were significantly higher in Hancock Creek, than Powell Creek. As such, median surface water POM $\delta^{15}\text{N}$ values were highest in Hancock Creek ($+5.53$ ‰), followed by the central drainage feature ($+5.20$ ‰), and Powell Creek ($+4.01$ ‰). Within Hancock Creek, POM $\delta^{15}\text{N}$ values at the most upstream site (SW9, median = 2.83) were significantly lower than all the downstream sites, except for the nearest (SW8). There were no significant differences by site within Powell Creek. For site and watershed POM $\delta^{15}\text{N}$ means, see **Appendix 7**.

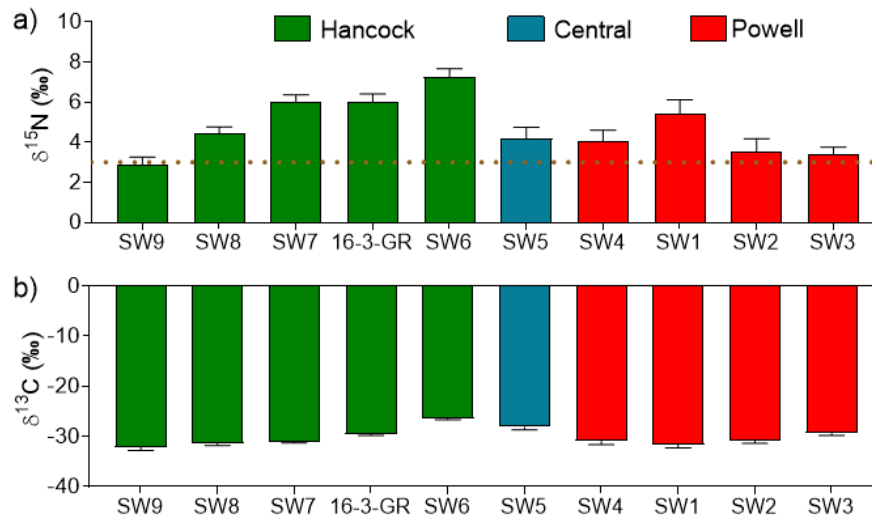


Figure 24. Stable isotope values (mean $\pm$ standard error) of particulate organic matter, a proxy for phytoplankton, collected from surface water sites in North Fort Myers by watershed (Hancock Creek, a central drainage feature, and Powell Creek), showing values for a) nitrogen ($\delta^{15}\text{N}$) and b) carbon ($\delta^{13}\text{C}$) with a dotted line to represent the approximate value for unprocessed wastewater (+ 3‰), such as septic tank effluent.

Surface water POM $\delta^{13}\text{C}$ values were generally depleted throughout the study area suggesting a terrestrial carbon source (**Fig. 24**) with values ranging from -38.3 to -24.8 ‰. POM $\delta^{13}\text{C}$ values were variable by project year (Mann-Whitney U test, $p < 0.001$), watershed (Kruskal-Wallis test, $p < 0.001$), and site (Kruskal-Wallis test, $p < 0.001$), but not season (Mann-Whitney U test, $p = 0.983$). Median surface water POM $\delta^{13}\text{C}$ values were significantly higher in year 2 (-29.0 ‰) than in year 1 (-31.4 ‰). By watershed, the central drainage feature has significantly more enriched POM $\delta^{13}\text{C}$ values (median = -26.9 ‰), than Hancock Creek (median = -29.9 ‰) or Powell Creek (median = -30.8 ‰). Within Hancock Creek, the most upstream site (SW9) had significantly more depleted POM $\delta^{13}\text{C}$ values (median = -31.3 ‰) than all other sites. There were no significant differences by site within Powell Creek. For site and watershed POM $\delta^{13}\text{C}$ means, see **Appendix 7**.

Surface water POM C:N ratios ranged from 0.96 to 13.7 (**Fig. 25a**). POM C:N ratios were variable by project year (Mann-Whitney U test, $p < 0.001$), season (Mann-Whitney U test, $p < 0.001$), watershed (Kruskal-Wallis test, $p = 0.016$), and site (Kruskal-Wallis test, $p = 0.028$). Median POM C:N ratios were higher in year 1 (8.70) than in year 2 (8.08). Median wet season POM C:N ratios (8.76) were significantly higher than in the dry season (8.29). Powell Creek surface water POM C:N ratios were significantly higher than Hancock Creek. Accordingly, Powell Creek had the highest median POM C:N ratio (8.76), followed by the central drainage feature (8.32), and Hancock Creek (8.29). Pairwise comparisons did not reveal significant differences by site in POM C:N ratios. For site and watershed POM C:N ratio means, see **Appendix 7**.

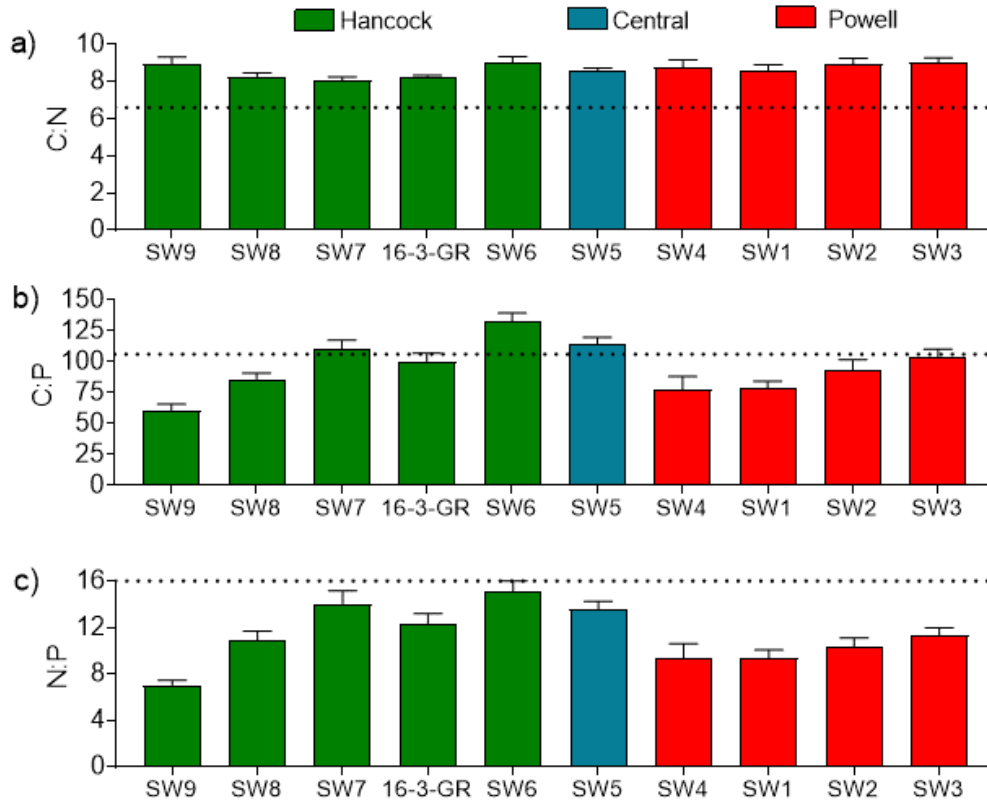


Figure 25. Elemental composition (mean $\pm$ standard error) of particulate organic matter, a proxy for phytoplankton, by watershed, including Hancock Creek, a central drainage feature, and Powell Creek, showing ratios of a) carbon to nitrogen (C:N), b) carbon to phosphorous (C:P), and c) nitrogen to phosphorus (N:P); dotted lines indicate shifts in nutrient limitation where C:N ratios > 6.6 indicate increasing N-limitation, C:P ratios > 106 indicate increasing P-limitation, and N:P ratios > 16 indicate increasing P-limitation (Atkinson and Smith 1983, Lapointe 1987, Lapointe et al. 2015).

Surface water POM C:P ratios ranged from 21.9 to 221.3 (**Fig. 25b**). The POM C:P ratios were variable by project year (Mann-Whitney U test, $p < 0.001$), watershed (Kruskal-Wallis test, $p = 0.001$), and site ($P < 0.001$), but not season (Mann-Whitney U test, $p = 0.362$). Median surface water POM C:P ratios were higher in year 2 (105.8) than year 1 (81.3). While not significant, median wet season POM C:P ratios were higher (93.8) than in the dry season (90.2). The central drainage feature had significantly higher POM C:P ratios (median = 109) than Hancock Creek (median = 93.0) and Powell Creek (median = 83.6). In Hancock Creek, the most upstream site (SW9) had significantly lower POM C:P ratios (median = 54.4) than all other sites in that watershed, except the site immediately downstream (SW8, median = 85.5). In Powell Creek watershed, there were no significant differences in POM C:P ratios by site. For site and watershed POM C:P ratio means, see **Appendix 7**.

Surface water POM N:P ratios ranged from 2.05 to 26.6 (**Fig. 25c**). POM N:P ratios were variable by project year (Mann-Whitney U test, $p < 0.001$), watershed (Kruskal-Wallis test,

$p = 0.001$), and site ($P < 0.001$), but not season (Mann-Whitney U test, $p = 0.687$). Median N:P ratios were higher in year 2 (12.6) than year 1 (9.35). The wet season median POM N:P ratio (11.1) was similar to the dry season ratio (11.4). All watersheds had significantly different POM N:P ratios. The central drainage feature had the highest median POM N:P ratio (12.4), followed by Hancock Creek (11.1), and Powell Creek (10.8). In Hancock Creek, the most upstream site (SW9) had significantly lower POM N:P ratios (median = 6.78) than all other sites in that watershed. In Powell Creek watershed, there were no significant differences in POM N:P ratios by site. For site and watershed POM N:P ratio means, see **Appendix 7**.

HAB POM samples were collected during blue-green algae blooms in October 2017 and July 2018, as well as during red tide events in March 2018. Blue-green algae POM samples from Davis Boat Ramp in the Caloosahatchee River from October 2017 had similar $\delta^{13}\text{C}$ values to those from the study area ($-29.9 \pm 0.19 \text{ ‰}$) and enriched $\delta^{15}\text{N}$ values within the range of wastewater ($+6.93 \pm 0.81 \text{ ‰}$). Further, blue-green algae POM samples collected during a bloom at North Shore Park (SW6, see cover photo) also had enriched $\delta^{13}\text{C}$ values like the study area ($-28.8 \pm 0.02 \text{ ‰}$) and enriched $\delta^{15}\text{N}$ values ($+8.79 \pm 0.04 \text{ ‰}$). Red tide samples collected in coastal areas of Lee County had heavier $\delta^{34}\text{C}$ values than the samples collected in the study area ($-11.3 \pm 1.7 \text{ ‰}$). This is reflective of the more enriched marine dissolved inorganic carbon found in coastal areas compared to the freshwater canals. The $\delta^{15}\text{N}$ values of red tide samples averaged $+3.85 \pm 0.23 \text{ ‰}$ in the coastal area, showing the linkage with upstream wastewater sources. See **Appendix 7** for mean stable isotope values and elemental composition of blue-green algae and red tide samples.

4. Discussion by Watershed

4.1 Reference Well

While not pristine, the results from the groundwater reference well (GW10) were much different than those from the wells near densely aggregated septic systems. The reference well had the deepest water table with a median depth to water of 4.3' and all of the depth to water measurements recorded during sampling events were >3.5'. Deeper water tables were measured in the dry season (median = 4.4') than the wet season (median = 4.0'). FIB abundance and BOD concentrations were low to below detection in the reference well with maximum detections of 6 MPN/100mL for enterococci, 0.5 MPN/100mL for *E. coli*, and 1.6 mg/L for BOD. Generally, in the reference well mean groundwater concentrations of NH_4^+ (0.24 ± 0.01 mg/L), NO_x ($0.01 \pm <0.01$ mg/L), DIN (0.25 ± 0.01 mg/L), and TN (0.62 ± 0.02 mg/L) were lower than the wells near more densely aggregated septic systems. Mean SRP (0.07 ± 0.002 mg/L) and TP (0.08 ± 0.003 mg/L) concentrations were also lowest in the reference well. DIN:SRP (mean = 7.86 ± 0.32 mg/L) and TN:TP (mean = 18.0 ± 0.56 mg/L) ratios both indicated N-limitation (<20). Sucralose concentrations were relatively low (mean = 195.3 ± 26 ng/L) compared to other groundwater wells, while carbamazepine, primidone, and acetaminophen were all BDL. The herbicides bentazon (mean = 1.78 ± 0.64 ng/L) and fluridone (1.50 ng/L) were the only other chemical tracers present at the reference site and their detection suggests rapid percolation of stormwater to the water table. Groundwater $\delta^{15}\text{N-NH}_4^+$ values were slightly enriched (mean = $+2.20 \pm 1.1$ ‰), while $\delta^{15}\text{N-NO}_3^-$ values were more depleted (mean = -1.52 ± 2.8 ‰) as result of fractionation associated with nitrification. GW10 was the most upland, undeveloped groundwater well monitored in the study, compared to the dense residential land uses in the other lower elevation watersheds. While there were some indications of anthropogenic impact (sucralose, bentazon, fluridone) to the groundwater, the magnitude was lower than in the other watersheds with septic systems.

4.2 Hancock Creek Watershed

Hancock Creek groundwater showed signs of contamination from septic systems. The median depth to water table in the Hancock Creek watershed was shallow (3.1') with higher water tables measured during the wet season (2.4') relative to the dry season (3.4'). 58% of depth to water measurements recorded during sampling events were <3.5'. Therefore, septic systems in Hancock Creek watershed area may not have adequate separation between the drainfield and the high water table without additional mounding, particularly in the wet season. FIB abundance was low in groundwater of the Hancock Creek watershed compared to the other watersheds containing septic systems with maximum detections of 195 MPN/100mL for enterococci and 4 MPN/100mL for *E. coli*. Despite this, BOD concentrations were comparatively high (3.2 mg/L). Nutrients were generally high in Hancock Creek groundwater. For example, NO_x (mean = 1.08 ± 0.21 mg/L), SRP (mean = 1.07 ± 0.08 mg/L), and TP (mean = 1.76 ± 0.27 mg/L) concentrations in Hancock Creek

were the highest in the study, while DIN (mean = 1.37 ± 0.20 mg/L) was also well above background levels observed in the reference well (mean = 0.25 ± 0.01 mg/L). Interestingly, NH_4^+ was relatively low in Hancock Creek (mean = 0.29 ± 0.02 mg/L) compared to the central drainage watershed and Powell Creek, indicating microbial nitrification to NO_x . DIN:SRP (mean = 1.09 ± 0.12) and TN:TP (mean = 4.11 ± 0.38) were the lowest of the study, indicating this system is highly P-enriched. Indicators of wastewater were present in Hancock Creek groundwater. In particular, sucralose concentrations were especially high (mean = $13,030 \pm 3,507$ ng/L), and at GW1 (maximum value = 58,000 ng/L) these concentrations approached levels previously measured in raw wastewater (40,000 to 67,000 ng/L; Lapointe et al. 2016; Silvanima et al. 2018). Further, the pharmaceuticals carbamazepine (mean = 36.8 ± 15 ng/L) and primidone (mean = 5.27 ± 1.7 ng/L) were observed at each well in the Hancock Creek watershed. However, no stormwater chemical tracers were observed in groundwater, suggesting rapid percolation of stormwater does not affect groundwater quality in the Hancock Creek watershed. Finally, groundwater $\delta^{15}\text{N}$ - NH_4^+ values observed in Hancock Creek were within the range of wastewater (mean = $+4.51 \pm 0.87$ ‰), while $\delta^{15}\text{N}$ - NO_3^- values were even more enriched (mean = $+8.49 \pm 2.7$ ‰) because of microbial fractionation. These data combined indicate a strong link between wastewater and groundwater quality in the Hancock Creek watershed.

Surface water in Hancock Creek also suggested wastewater contamination, as well as the influence of bird populations and stormwater runoff. Bacterial abundance was high in surface waters of Hancock Creek. For example, both enterococci (mean = 397 ± 97 MPN/100mL) and *E. coli* (mean = 323 ± 44 MPN/100mL) counts exceeded FDEP standards, whereas BOD (mean = 0.98 ± 11 mg/L) was below the FDEP surface water standard. The highest bacterial abundances within Hancock Creek were observed at 16-3GR in a dense residential area, which had mean values of 834 ± 267 MPN/100mL for enterococci, 645 ± 135 MPN/100mL for *E. coli*, and 1.78 mg/L for BOD. Nutrients were also high in Hancock Creek surface waters. For example, while Hancock Creek had the lowest mean surface water concentrations of NH_4^+ (0.03 ± 0.002 mg/L) and NO_x (0.04 ± 0.002 mg/L) of the three watersheds, these concentrations are still elevated above background levels and indicative of nutrient pollution. Further, the highest mean NO_x concentrations of the study (0.09 ± 0.02 mg/L) were observed at the most downstream site, SW6. In Hancock Creek watershed, SRP (0.11 ± 0.004 mg/L) showed a trend of higher concentrations upstream, decreasing downstream. TN concentrations (mean = 0.78 ± 0.02 mg/L) exceeded the FDEP surface water standard for the Lower Caloosahatchee (>0.50 mg/L), while TP concentrations (mean = 0.16 ± 0.01 mg/L) exceeded the FDEP standard for the Lower Caloosahatchee (>0.04 mg/L) and peninsular streams (>0.12 mg/L). Hancock Creek surface waters had the lowest DIN:SRP (mean = 0.65 ± 0.05) and TN:TP (mean = 12.6 ± 0.6) ratios, supporting that this system is highly P-enriched. Human wastewater influence was detectable as the human bacterial marker HF183 was often detected in relatively low levels throughout Hancock Creek (mean = 31.3 ± 18

GEU/100mL). Additionally, the avian bacteria markers GFD (mean = $2,738 \pm 969$ TSC/100mL) and Gull2 (mean = $4,440 \pm 2,748$ TSC/100mL) were highest in Hancock Creek and were most elevated at downstream sites (16-3GR and SW6 for both, and SW7 for GFD), suggesting a significant influence of bird populations on water quality. This was corroborated by the strong guano smell frequently observed at sites SW7 and 16-3GR. There was a colony of bats under the bridge at SW7, but samples were collected upstream of this influence at this site. The ubiquitous presence of “moderate” levels of sucralose (mean = 529 ± 46 ng/L), as well as carbamazepine (mean = 1.71 ± 0.18 ng/L) and primidone (mean = 3.04 ± 0.54 ng/L) demonstrate the influence of domestic wastewater on the surface water in Hancock Creek. Several chemical tracers of stormwater runoff were also consistently detected in Hancock Creek surface water samples, including the herbicides bentazon (mean = 7.15 ± 1.1 ng/L), diuron (mean = 2.48 ± 0.7 ng/L), fluridone (mean = 36.4 ± 17 ng/L), imazapyr (mean = 26.2 ± 3.6 ng/L), and 2,4-D (mean = 12.5 ± 3.4 ng/L) and the pesticide imidacloprid (mean = 4.38 ± 1.4 ng/L). Hancock Creek POM $\delta^{15}\text{N}$ values were consistently within the range of wastewater (mean = $+5.30 \pm 0.22$ ‰), except for the most upstream site at Yellow Fever Creek (SW9; mean = $+2.86 \pm 0.40$ ‰), indicating that phytoplankton are assimilating nitrogen from wastewater sources. In POM samples, C:N ratios generally exceeded 6.6 (mean = 8.49 ± 0.12), suggesting N-limitation. The mean POM N:P ratio was lower than 16 (11.8 ± 0.47), further supporting that this watershed was N-limited. The mean C:P ratio was below 106 (97.7 ± 3.6), suggesting slight P-enrichment. These data combined reveal a widespread presence of wastewater throughout Hancock Creek, as well as the influence of bird populations and stormwater runoff.

Water quality concerns at North Shore Park (SW6) were one of the motivating factors for this study. Surface water FIB counts were generally higher upstream in Hancock Creek than at North Shore Park, implicating the creek as a source of this bacterial contamination. The human wastewater tracers sucralose and carbamazepine were detected at North Shore Park, at concentrations generally lower than at upstream sites, which may reflect the greater dilution of Hancock Creek septic tank effluent as it terminates into the Caloosahatchee River. During the present study, the mean POM $\delta^{15}\text{N}$ values were highest ($+7.25 \pm 0.42$ ‰) at North Shore Park, supporting the conclusion that this site is strongly influenced by wastewater. Sampling during a *Microcystis* bloom at North Shore Park on July 19, 2018 (see report cover photo) revealed high NH_4^+ (mean = 0.02 mg/L) and SRP (mean = 0.49 mg/L) concentrations and a $\delta^{15}\text{N}$ value of $+8.79$ ‰ in the algal tissue (unpublished data), indicating wastewater as the nitrogen source supporting this HAB, which is consistent with previous research (Lapointe and Bedford 2007; Yentsch et al. 2008). Finally, the Gull2 marker was highest at North Shore Park and birds were almost always present in the water at this site, indicating that there is also an avian influence on water quality. Thus, surface water quality issues at North Shore Park can be tied to upstream and local sources.

4.3 Central Drainage Watershed

The central drainage watershed showed evidence of contamination from septic systems. Water tables were shallow in the central drainage watershed with a median depth to water of 2.5' and lower depth to water measured during the wet season (median = 1.8') than the dry season (median = 2.7'). 100% of the depth to water measurements recorded during sampling events were <3.5'. Therefore, septic systems in the central drainage watershed may not have adequate separation between the drainfield and the seasonal high water table without additional mounding, particularly in the wet season. In the central drainage watershed groundwater, enterococci counts were low (mean = 5.31 ± 2.3 MPN/100mL), however *E. coli* counts were the highest observed in the study (mean = 25.4 ± 24 MPN/100mL). Despite this, groundwater BOD was lowest in the central drainage watershed (mean = 0.57 mg/L). Nutrients were elevated in the central drainage watershed. For example, NH_4^+ concentrations were high (1.36 ± 0.05 mg/L), resulting in high DIN concentrations (1.37 ± 0.05 mg/L). Further, SRP (0.46 ± 0.02 mg/L), TN (2.18 ± 0.06 mg/L), and TP (0.52 ± 0.03 mg/L) concentrations were also all high. Interestingly, in the central drainage basin NO_x concentrations were relatively low ($0.01 \pm <0.001$ mg/L), indicating poor soil conditions for nitrification and denitrification. In groundwaters of the central drainage watershed, low DIN:SRP (7.32 ± 0.36) and TN:TP (10.0 ± 0.30) ratios were observed, which are similar to municipal wastewater (5:1) and indicate that this watershed is N-limited. Wastewater contamination was evident in the central drainage watershed as "significant" sucralose concentrations were observed in groundwater of the central drainage watershed ($5,554 \pm 1,665$ ng/L), indicating the linkage between septic systems and groundwater. Carbamazepine (mean = 36.1 ± 15 ng/L) and primidone (mean = 5.27 ± 1.7 ng/L) were also observed at each well in the central drainage watershed further revealing the influence of septic systems on groundwater. The herbicide bentazon was also detected in groundwater of the central drainage watershed (mean = 7.56 ± 4.1 ng/L), which suggests that chemicals applied to the ground surface can be transported rapidly to the very shallow groundwater via percolation. Both groundwater $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ values in this watershed were within the range of wastewater ($+5.54 \pm 0.59$ ‰ and $+4.65 \pm 1.2$ ‰, respectively). The combined data support the influence of wastewater, as well as stormwater infiltration, on groundwater in the central drainage watershed.

Surface water in the central drainage feature also showed evidence of contamination by septic systems, as well as the influence of stormwater runoff. The central drainage feature had high surface water enterococci (883 ± 278 MPN/100mL) and *E. coli* ($1,310 \pm 570$ MPN/100mL) counts that greatly exceeded FDEP standards, while BOD (1.5 ± 0.43 mg/L) did not. Nutrient concentrations were high in the central drainage feature. For example, the highest mean NH_4^+ concentrations were observed at this site (0.12 ± 0.02 mg/L) and SRP concentrations were elevated as well (0.09 ± 0.01 mg/L), leading to the highest average surface water DIN concentrations (0.18 ± 0.04 mg/L) and DIN:SRP ratio (2.36 ± 0.25) of the study, which is similar to the average ratio of municipal wastewater (~5). There were

also high concentrations of TN in the central drainage feature (mean = 1.13 ± 0.05 mg/L) that exceeded the FDEP surface water standard for the Lower Caloosahatchee (>0.5 mg/L), while TP concentrations (mean = 0.19 ± 0.04) exceeded the FDEP standard for the Lower Caloosahatchee (>0.04 mg/L) and peninsular streams (>0.12 mg/L). Human influence was supported by the highest HF183 concentration of the study (mean = 406 ± 175 GEU/100mL). Low concentrations of GFD were also observed (mean = 141 ± 103 TSC/100mL), indicating a subtle avian influence. To further support the influence of human wastewater, sucralose was present in “moderate” concentrations in surface water (mean = 710 ± 74 ng/L). There were also detections of the pharmaceuticals carbamazepine (mean = 1.44 ± 0.26 ng/L), primidone (mean = 2.29 ± 1.8 ng/L), and acetaminophen (mean = 6.50 ± 3.6 ng/L), further supporting that there is wastewater contamination in this watershed. Chemical tracers of surficial runoff were also detected in the central drainage feature surface water, including the herbicides bentazon (mean = 8.70 ± 3.1 ng/L), diuron (3.90 ng/L), fluridone (3.30 ng/L), imazapyr (22.0 ng/L), and 2,4-D (mean = 17.4 ± 13 ng/L), and the pesticide imidacloprid (7.90 ng/L). Surface water herbicide concentrations were typically greater during the wet season when stormwater runoff is highest. Surface water POM $\delta^{15}\text{N}$ values from the central drainage feature were within the range for wastewater (mean = $+4.18 \pm 0.57$ ‰) demonstrating the uptake of wastewater nitrogen into the ecosystem. C:N ratios in POM samples exceeded 6.6 (mean = $+8.54 \pm 0.19$ ‰) and N:P ratios were lower than 16 (mean = 13.6 ± 0.65), indicating N-limitation. POM C:P ratios slightly exceeded 106 (mean = 114.7 ± 4.99), indicating P-limitation. The combined data reveal the influence of wastewater, as well as stormwater runoff on surface water quality in the central drainage watershed of North Fort Myers.

4.4 Powell Creek Watershed

There was evidence of the influence of wastewater and stormwater infiltration on groundwater quality within the Powell Creek watershed. Water tables in the Powell Creek watershed were the shallowest in the study with a median depth of 2.2'. The highest water tables were observed during the wet season (median = 1.9') compared to the dry season (median = 2.6'). 100% of the depth to water measurements recorded during sampling events were $<3.5'$. Without sufficient mounding, septic systems along Powell Creek are likely not compliant with current FAC code. This is an issue that warrants further investigation and site-by-site inspections. Bacterial abundance was elevated in Powell Creek groundwater with the highest enterococci counts observed during the study (mean = 114 ± 100 MPN/100mL), as well as detections of *E. coli* (mean = 2.40 ± 1.9 MPN/100mL). Groundwater BOD (mean = 2.78 ± 0.31 mg/L) also exceeded FDEP surface water standards (>2.4 mg/L). Nutrient concentrations in groundwater were high in Powell Creek, especially nitrogen. For example, concentrations of NH_4^+ (mean = 5.53 ± 0.54 mg/L), DIN (mean = 5.55 ± 0.54 mg/L), and TN (mean = 6.12 ± 0.55 mg/L) were significantly higher in Powell Creek than the other watersheds. Further, SRP (mean = 0.25 ± 0.3 mg/L) and TP (mean = 0.31 ± 0.03 mg/L) concentrations were also high. Powell Creek watershed had the

highest groundwater DIN:SRP (mean = 51.5 ± 2.6) and TN:TP (mean = 44.0 ± 1.8) ratios of the study, reflecting high N content from wastewater, even in relation to the high natural P concentrations in this watershed. This wastewater influence was further demonstrated by the presence of human chemical tracers. For example, while concentrations of sucralose in Powell Creek groundwater were lower than the other watersheds in this study with septic systems (mean = 579.5 ± 349), they were still in the moderate to significant range. The pharmaceuticals carbamazepine (mean = 0.28 ± 0.28 ng/L), acetaminophen (0.96 ± 0.96 ng/L), and primidone (5.50 ± 2.2 ng/L) were also detected in low concentrations supporting the influence of wastewater in this watershed. Interestingly, the herbicide bentazon (mean = 7.56 ± 4.08 ng/L) was detected in Powell Creek groundwater, which demonstrates the rapid percolation from the ground surface to the water table. Powell Creek groundwater $\delta^{15}\text{N-NH}_4^+$ values in the low end of the range for wastewater (mean = $+2.77 \pm 0.59$ ‰), while $\delta^{15}\text{N-NO}_3^-$ values were more enriched (mean = $+4.90 \pm 2.6$ ‰), indicating microbial fractionation associated with nitrification and denitrification.

It is noteworthy that during year 1 of the project, the property at 127 Dow Ln. (GW7 and GW8) was vacant for three months following Hurricane Irma on September 10, 2017, so this septic system was not used during the wet season sampling events. Also, the property at 131 Dow Ln. (GW9) was occupied only about 50% of the time during the dry season. These absences could explain the relatively low levels of sucralose observed in Powell Creek groundwater in year 1. Despite the low occupancy rates, however these groundwater wells still showed the influence of wastewater in year 1.

Powell Creek surface waters also showed the influence of wastewater from septic systems, stormwater runoff, and bird populations on water quality. Powell Creek surface water FIB abundance often exceeded FDEP surface water standards for both enterococci (mean = 767 ± 162 MPN/100mL) and *E. coli* (mean = 893 ± 147 MPN/100mL), however BOD concentrations did not (mean = 0.96 ± 0.08 mg/L). NH_4^+ concentrations were high in Powell Creek surface water (mean = 0.04 ± 0.002 mg/L), while NO_x concentrations were elevated compared to the other watersheds (mean = 0.06 ± 0.004 mg/L). Thus, DIN concentrations were also high in Powell Creek (mean = 0.10 ± 0.005 mg/L). SRP concentrations were high (mean = 0.11 ± 0.004 mg/L) and decreased from upstream to downstream in Powell Creek. TN concentrations (mean = 0.75 ± 0.03 mg/L) exceeded the FDEP surface water standard for the Lower Caloosahatchee (>0.5 mg/L), while TP concentrations (mean = 0.15 ± 0.01 mg/L) exceeded the FDEP standard for the Lower Caloosahatchee (>0.04 mg/L) and peninsular streams (>0.12 mg/L). Powell Creek surface water DIN:SRP (mean = 1.24 ± 0.09) and TN:TP (mean = 16.4 ± 1.3) ratios were very low, indicating strong N-limitation. Detections of the human marker HF183 were found at each site in Powell Creek during both seasons and were among the highest observed relative to the other watersheds (mean = 177.2 ± 76 GEU/100mL), suggesting the influence of wastewater on surface water quality. GFD was also detected at all sites (mean = $242.5 \pm$

80 TSC/100mL), indicating that bird populations also influence water quality in the Powell Creek watershed. Human chemical tracers further revealed the influence of wastewater. Surface water concentrations of sucralose (mean = 749 ± 66 ng/L) were typically higher than other sites in the study area with sucralose concentrations always exceeding “moderate” and sometimes exceeding “significant” classifications. Pharmaceuticals were also detected in Powell Creek surface waters, including carbamazepine (mean = 11.9 ± 1.1 ng/L), primidone (mean = 3.23 ± 0.66 ng/L), and acetaminophen (mean = 3.18 ± 1.6 ng/L). Chemical tracers of surficial runoff were found in Powell Creek surface water samples, including the herbicides bentazon (mean = 3.16 ± 0.41 ng/L), fluridone (mean = 4.13 ± 0.62 ng/L), imazapyr (mean = 32.3 ± 3.2 ng/L), and 2,4-D (mean = 2.12 ± 0.96 ng/L) and the pesticide imidacloprid (mean = 4.87 ± 0.80 ng/L), indicating that stormwater runoff is also affecting surface water quality. Furthermore, surface water POM $\delta^{15}\text{N}$ values were within the range for wastewater (mean = $+4.05 \pm 0.31$ ‰). C:N ratios in POM samples exceeded 6.6 during the wet season (mean = 8.83 ± 0.17) and N:P ratios were lower than 16 (mean = 10.1 ± 0.44), indicating N-limitation. POM C:P ratios were less than 106 (mean = 88.0 ± 4.19), indicating P-enrichment.

5. Overall Discussion

This targeted sampling program provided insight to identify the contributing sources of FIB and nutrients in the North Fort Myers watershed. This year 2 study corroborated the conclusions of the year 1 study and demonstrated these sources remain relatively consistent over time with location generally being the most influential factor. While there were differences between the three watersheds serviced by septic systems, in each watershed there were multiple lines of evidence that indicated leachate from septic systems was adversely affecting water quality. Notably, shallow water tables were consistently observed in the study area, suggesting that septic system drainfields likely do not have the physical separation required by FAC Rule 64E-6 for adequate treatment of wastewater prior to reaching the groundwater. This was most evident in Powell Creek which had the highest water tables and greatest evidence of septic system contamination. Further, evidence of wastewater contamination included elevated groundwater and surface water DIN concentrations (especially NH_4^+ in Powell Creek and NO_x in Hancock Creek), high surface water FIB levels, the presence of human wastewater tracers in groundwater and surface water, including sucralose, carbamazepine, primidone, acetaminophen, and the molecular marker HF183, as well as enriched $\delta^{15}\text{N}$ values of groundwater and POM within the range of wastewater. The presence of the chemical tracers 2,4-D, bentazon, diuron, fluridone, imazapyr, and imidacloprid provided evidence that stormwater runoff was also adversely affecting surface water quality. There were also depleted $\delta^{15}\text{N}$ values observed within the range of synthetic fertilizers, suggesting these may be another source of nutrients in North Fort Myers. However, research has indicated ~80-90% uptake of applied fertilizers within the turf thatch with minimal amounts reaching the groundwater and surface waters, even

in the wet season (Hochmuth et al. 2009; Shaddox and Unruh 2018). Finally, the presence of multiple bird molecular tracers indicate that avian populations may affect water quality at some sites in North Fort Myers, particularly in Hancock Creek. The results of this multi-year study indicate that septic systems are not protective of water quality in North Fort Myers watersheds, which has also been found for other urbanized waterfront residential areas, such as Michigan's Lower Peninsula (Verhougstraete et al. 2015) and North Carolina (Humphrey et al. 2011). Many of these issues in North Fort Myers arise from aging septic systems that are installed in too great of a density ($> 1/\text{acre}$; Bicki and Brown 1991) with shallow water tables (Bicki and Brown 1990).

The results of this study also support the findings of previous studies in impaired coastal waters of Florida that improperly located and aging septic systems contribute to the pollution of groundwater and adjacent surface waters. For example, shallow seasonal high water tables that interfere with the functioning of septic systems (Bicki and Brown 1990) have also been observed in Jupiter (Lapointe and Krupa 1995), St. George Island (Corbett et al. 2002), Dania Beach (Meeroff et al. 2008), and Port St. Lucie (Lapointe et al. 2020). Similar issues with shallow water tables interfering with the performance of septic systems have been observed in many other waterfront communities, such as in North Carolina (Humphrey et al. 2011), Rhode Island (Cox et al. 2020), and Texas (Forbis-Stokes et al. 2016). Further, sucralose concentrations and aqueous stable nitrogen isotopes in the study area were similar to concentrations observed in groundwaters and surface waters in Indian River (Tarnowski 2014), Martin, Charlotte, and St. Lucie Counties (Lapointe et al. 2016, 2017, 2018, 2020). POM $\delta^{15}\text{N}$ values observed in this study were also similar to those observed in Indian River County and Port St. Lucie POM and macrophyte samples collected from canal developments affected by aging septic system leachate (Tarnowski 2014; Lapointe et al. 2020).

A conservative estimate of N-loading from North Fort Myers septic systems to the Caloosahatchee River can be easily estimated. In the North Fort Myers study area, there are 2,164 "likely" septic systems. Based on 2010 US Census data, the average household in the study area has 2.21 residents. Each resident contributes a conservatively estimated 9.0 lbs / year of nitrogen (Lapointe and Herren 2016). These data suggest approximately 43,041 lbs / year of nitrogen are loaded into the Caloosahatchee River from septic systems within the study area. Phosphorus loading can be estimated similarly at a rate of 0.83 lb / person / year (Lapointe and Herren 2016) resulting in an estimate of 3,969 lbs of phosphorus loaded to this system annually from septic systems, though some of this will be lost through adsorption to the soil during transport through the vadose zone. This calculation does assume 100% occupancy and it is likely there are many seasonal residents in North Fort Myers, so a determination of occupancy rates would be useful for a more accurate calculation of nutrient loading from the study area. Regardless, this estimate

demonstrates that the nutrient contribution to the Caloosahatchee River from septic system leachate in North Fort Myers is not negligible.

This study has many implications regarding the downstream effects of nutrient enrichment from the local North Fort Myers basin on the recurring HAB events, such as those that occurred in Lee County following Hurricane Irma in 2017-2018. Surface water collected in residential canals of nearby Cape Coral during these blooms on July 19, 2018 had very high concentrations of NH_4^+ (29.5 mg/L) and SRP (4.58 mg/L) resulting in low DIN:SRP ratios (14.3) with enriched POM $\delta^{15}\text{N}$ values (+7.18 ‰). In the estuarine and coastal environment adjacent to Lee County, *M. aeruginosa* and *K. brevis* blooms thrive in low N:P environments (Ketchum and Keen 1948; Odum 1953; Lapointe et al. 2006; Yentsch et al. 2008; Lapointe et al. 2012; 2017). During wet periods and following tropical storms or hurricanes, the Caloosahatchee River and Estuary can be seeded with *M. aeruginosa* from Lake Okeechobee discharges, as well as local sources if salinity values are adequately low. These blooms can experience exponential growth when they encounter high levels of DIN, especially NH_4^+ (Lapointe et al. 2017), such as what was found in the North Fort Myers watershed in this study. The mean $\delta^{15}\text{N}$ value of *M. aeruginosa* (+6.93 ‰) following multiple hurricanes in 2004 and 2005 were enriched similarly to the mean value of POM for the surface water sites (+4.69 ‰) and mean groundwater $\delta^{15}\text{N}$ - NH_4^+ (+3.90 ‰) and $\delta^{15}\text{N}$ - NO_3^- (+6.36 ‰) in the present study. Furthermore, the $\delta^{15}\text{N}$ value of *K. brevis* (+3.85 ‰) in 2018 was in the range of sewage nitrogen (> +3 ‰), similar to the enriched values of red drift macroalgae from Lee County coastal reefs (+4.86 ‰; Lapointe and Bedford 2007) and *K. brevis* from Sanibel in the 2005 bloom (+7.83 ‰; Yentsch et al. 2008). Therefore, these types of HABs are expected to be exacerbated following periods of heavy stormwater runoff to the estuarine and coastal environment which has a naturally high background P (Odum 1953). Reduced salinity is known to favor red tide blooms (Slobodkin 1953; Brand and Compton 2007; Medina et al. 2020), so following heavy, prolonged rain events and stormwater runoff these blooms are likely to develop and persist longer. These combined data further support the linkage between increasing localized nutrient enrichment from wastewater and worsening HABs in Lee County.

This multi-year study indicates that septic systems are not protective of water quality in North Fort Myers watersheds. Similar findings have been reported by other researchers in England (Withers et al. 2011, 2014) and Cape Cod, MA (Howes et al. 2004). Therefore, Lee County has a unique opportunity to significantly improve the local watershed by reducing dependence upon aging septic systems in North Fort Myers. As these sources of cumulative wastewater pollution are eliminated along Hancock and Powell Creeks, as well as in the central drainage watershed, water quality should improve. Additionally, implementing structures to better manage stormwater runoff could further improve water quality. Finally, the susceptibility of these systems to localized HABs may be reduced by

balancing the ecological stoichiometry of the watershed to achieve a nutrient load and N:P ratio where these microalgae will not thrive.

6. Recommendations

The results of this study strongly suggest that improved wastewater infrastructure and management in North Fort Myers, including advanced wastewater treatment (nutrient removal), would be beneficial for local water quality. This is especially true for nitrogen, as septic systems are a major source of N-loading and their removal represents a direct approach to nitrogen reduction, which would qualify for BMAP credits. Stormwater management projects would also help reduce pollutant loading via surficial runoff. Continued monitoring of the study area is recommended to track changes in water quality as improvements to infrastructure are implemented. Further, the development of a comprehensive nutrient budget and / or watershed model would allow for greater evaluation of the role of septic systems, stormwater runoff, and other sources of bacteria and nutrients in the North Fort Myers study area. This information will allow watershed managers to identify the most effective projects and abatement strategies to improve water quality in North Fort Myers and allow for continued, sustainable growth in Lee County.

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8. References

- Anderson, D.M., Hoagland, P., Kaoru, Y., and White, A.W. 2000. Estimated annual economic impacts from harmful algal blooms (HABs) in the United States (No. WHOI-2000-11). National Oceanic and Atmospheric Administration Norman OK National Severe Storms Lab.
- Aspila, K.I., Agemian, H., and Chau, A.S.Y. 1976. A semi-automated method for the determination of inorganic, organic and total phosphate in sediments. *Analyst*, 101(1200), 187-197.
- Atkinson, M.J., and Smith, S.V. 1983. C: N: P ratios of benthic marine plants. *Limnology and Oceanography*, 28(3), 568-574.
- Bateman, A.S., and Kelly, S.D. 2007. Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health studies*, 43(3), 237-247.

- Bicki, T.J. 1984. Impact of on-site sewage disposal systems on surface and ground water quality. University of Florida, Soil Science Department, for Florida Department of Health and Rehabilitative Services.
- Bicki, T.J., and Brown, R.B. 1990. On-Site Sewage Disposal: The importance of the wet season water table. *Journal of Environmental Health*, 52(5), 277-279.
- Bicki, T.J., and Brown, R.B. 1991. On-site sewage disposal: the influence of system density on water quality. *Journal of Environmental Health*, 39-42.
- Boehm, A.B., Van de Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D., and Weisberg, S.B., 2013. Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research*, 47(18), 6812-6828.
- Brand, L.E., and Compton, A. 2007. Long-term increase in *Karenia brevis* abundance along the Southwest Florida Coast. *Harmful Algae*, 6(2), 232-252.
- Buerge, I.J., Buser, H.R., Kahle, M., Muller, M.D., and Poiger, T. 2009. Ubiquitous occurrence of the artificial sweetener acesulfame in the aquatic environment: an ideal chemical marker of domestic wastewater in groundwater. *Environmental Science and Technology*, 43(12), 4381-4385.
- Byappanahalli, M.N., Nevers, M.B., Korajkic, A., Staley, Z.R., and Harwood, V.J. 2012. Enterococci in the environment. *Microbiology and Molecular Biology Reviews*, 76(4), 685-706.
- Corbett, D.R., Dillon, K., Burnett, W., and Schaefer, G. 2002. The spatial variability of nitrogen and phosphorus concentration in a sand aquifer influenced by onsite sewage treatment and disposal systems: a case study on St. George Island, Florida. *Environmental Pollution*, 117(2), 337-345.
- Costanzo, S.D., O'donohue, M.J., Dennison, W.C., Loneragan, N.R., and Thomas, M. 2001. A new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin*, 42(2), 149-156.
- Cox, A.H., Surabian, D., Loomis, G.W., Turenne, J.D., and Amador, J.A. 2020. Temporal Variability in the Vertical Separation Distance of Septic System Drainfields Along the Southern Rhode Island Coast. *Water, Air, and Soil Pollution*, 231(3), 1-17.
- D'Elia, C.F., Connor, E.E., Kaumeyer, N.L., Keefe, C.W., Wood, K.V., and Zimmerman, C.F. 1997. Nutrient analytical services: standard operating procedures. Technical Report Series No. 158-97.
- Dong, M.M., Trenholm, R., and Rosario-Ortiz, F.L. 2015. Photochemical degradation of atenolol, carbamazepine, meprobamate, phenytoin and primidone in wastewater effluents. *Journal of Hazardous Materials*, 282, 216-223.
- Florida Administrative Code. Rule 62E-6, Standards for Onsite Sewage Treatment and Disposal Systems.
- Florida Administrative Code. Rule 62-302, Surface Water Quality Standards.
- Florida Administrative Code. Rule 62-304.800, Caloosahatchee River Basin TDMLs.
- Florida Department of Environmental Protection. 2014. South Fork St. Lucie Estuary and River Microbial Source Tracking Study. Division of Environmental Assessment and Restoration. Tallahassee, FL.
- Forbis-Stokes, A., Munster, C., Karthikeyan, R., Mohanty, B.P., and Boulanger, B. 2016. Modeling onsite wastewater treatment systems in a coastal Texas watershed. *Water, Air, and Soil Pollution*, 227(12), 1-12.

- Green, H.C., and Field, K.G. 2012. Sensitive detection of sample interference in environmental qPCR. *Water Research*, 46(10), 3251-3260.
- Hochmuth, G.J., Nell, T., Sartain, J., Unruh, J.B., Martinez, C., Trenholm, L., and Cisar, J. 2009. Urban water quality and fertilizer ordinances: Avoiding unintended consequences: a review of the scientific literature. *EDIS*, 2009(2).
- Heaton, T.H. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology: Isotope Geoscience Section*, 59, 87-102.
- Hinkle, S.R., Böhlke, J.K., and Fisher, L.H. 2008. Mass balance and isotope effects during nitrogen transport through septic tank systems with packed-bed (sand) filters. *Science of the Total Environment*, 407(1), 324-332.
- Howes, B., Kelley, S., Ramsey, J., Samimy, R., Eichner, E., Schlezinger, D., and Wood, J. 2004. Linked Watershed-Embayment Model to Determine Critical Nitrogen Loading Thresholds for Popponesset Bay, Mashpee and Barnstable, Massachusetts. Commonwealth of Massachusetts, Department of Environmental Protection, Massachusetts Estuaries Project.
- Humphrey Jr, C.P., O'Driscoll, M.A., and Zarate, M.A. 2011. Evaluation of on-site wastewater system *Escherichia coli* contributions to shallow groundwater in coastal North Carolina. *Water Science and Technology*, 63(4), 789-795.
- Katz, B.G., Griffin, D.W., McMahon, P.B., Harden, H.S., Wade, E., Hicks, R.W., and Chanton, J.P. 2010. Fate of effluent-borne contaminants beneath septic tank drainfields overlying a karst aquifer. *Journal of Environmental Quality*, 39(4), 1181-1195.
- Ketchum, B. H., and Keen, J. 1948. Unusual phosphorus concentrations in the Florida "red tide" sea water. *Journal of Marine Research*, 7(1), 17-21.
- Kramer, B.J., Davis, T.W., Meyer, K.A., Rosen, B.H., Goleski, J.A., Dick, G.J., Oh, G., and Gobler, C.J. 2018. Nitrogen limitation, toxin synthesis potential, and toxicity of cyanobacterial populations in Lake Okeechobee and the St. Lucie River Estuary, Florida, during the 2016 state of emergency event. *PloS One*, 13(5), e0196278.
- Lapointe, B.E. 1985. Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: interactions between concentration and frequency of nutrient pulses. *Journal of Experimental Marine Biology and Ecology*, 93(3), 211-222.
- Lapointe, B.E. 1987. Phosphorus-and nitrogen-limited photosynthesis and growth of *Gracilaria tikvahiae* (Rhodophyceae) in the Florida Keys: an experimental field study. *Marine Biology*, 93(4), 561-568.
- Lapointe, B.E., and Herren, L.W. 2016. 2015 Martin County Watershed to reef septic study. Final Report for the Martin County Board of County Commissioners, Stuart, FL, 79.
- Lapointe, B.E., and Krupa, S. 1995. Tequesta Peninsula septic tank/water quality investigation. Final Report to the Loxahatchee River Environmental Control District, Jupiter, FL, 93.
- Lapointe, B.E., O'Connell, J.D., and Garrett, G.S. 1990. Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. *Biogeochemistry*, 10(3), 289-307.
- Lapointe, B.E., Bedford, B., Brand, L., and Yentsch, C.S. 2006. Harmful Algal Blooms in Coastal Waters of Lee County, FL: Bloom Dynamics and Identification of Land-Based Nutrient Sources.

- Lapointe, B.E., and Bedford, B.J. 2007. Drift rhodophyte blooms emerge in Lee County, Florida, USA: Evidence of escalating coastal eutrophication. *Harmful Algae*, 6, 421-437.
- Lapointe, B.E., Herren, L.W., and Bedford, B.J. 2012. Effects of hurricanes, land use, and water management on nutrient and microbial pollution: St. Lucie Estuary, Southeast Florida. *Journal of Coastal Research*, 28(6), 1345-1361.
- Lapointe, B.E., Herren, L.W., Debortoli, D.D., and Vogel, M. 2015. Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. *Harmful Algae*, 43, 82-102.
- Lapointe, B.E., Herren, L.W., Paule, A.L., Sleeman, A., and Brewton, R.A. 2016. Charlotte County Water Quality Assessment, Phase I: Data Analysis and Recommendations for Long-term Monitoring. Final Report to Charlotte County, FL Board of County Commissioners.
- Lapointe, B.E., Herren, L.W. and Paule, A.L. 2017. Septic systems contribute to nutrient pollution and harmful algal blooms in the St. Lucie Estuary, Southeast Florida, USA. *Harmful Algae*, 70, 1-22.
- Lapointe, B.E., Brewton, R.A., and Wilking, L.E. 2018. Microbial Source Tracking of Bacterial Pollution in the North Fork of the St. Lucie River. Final Report to the City of Port St. Lucie Utilities Department.
- Lapointe, B.E., Brewton, R.A., and Baladi, D. 2020. Septic system – groundwater – surface water couplings in the North Fork of the St. Lucie River: MST Phase II. Final Report to the City of Port St. Lucie Utilities Department.
- Lee, M.S., Qin, G., Nakanishi, K., and Zagorski, M.G. 1989. Biosynthetic studies of brevetoxins, potent neurotoxins produced by the dinoflagellate *Gymnodinium breve*. *Journal of the American Chemical Society*, 111(16), 6234-6241.
- Lim, F.Y., Ong, S.L., and Hu, J. 2017. Recent advances in the use of chemical markers for tracing wastewater contamination in aquatic environment: a review. *Water*, 9(2), 143-168.
- Matthews, D. 2016. Microbial source tracking: interpretation of qPCR results [Powerpoint slides]. Florida Department of Environmental Protection (FDEP). Retrieved from https://fldeploc.dep.state.fl.us/appdata/status/SolZ/Q_meetings/march2016/08-microbial-source-tracking-03302016.pdf.
- Medina, M., Huffaker, R., Jawitz, J.W., and Muñoz-Carpena, R. 2020. Seasonal dynamics of terrestrially sourced nitrogen influenced *Karenia brevis* blooms off Florida's southern Gulf Coast. *Harmful Algae*, 98, 101900.
- Meeroff, D.E., Bloetscher, F., Bocca, T., and Morin, F. 2008. Evaluation of water quality impacts of on-site treatment and disposal systems on urban coastal waters. *Water, Air, and Soil Pollution*, 192(1), 11-24.
- National Research Council (NRC). 2000. Clean coastal waters: understanding and reducing the effects of nutrient pollution. National Academies Press.
- Odum, H. T. 1953. Dissolved phosphorus in Florida waters. Report of Investigations, 9, 1-40.
- Oppenheimer, J., Eaton, A., Badrazzaman, M., Haghani, A., and Jacangelo, J. 2011. Occurrence and suitability of suitability of sucralose as an indicator compound of wastewater loading of surface waters in urbanized regions. *Water Research*, 45, 4019-4027.

- Paerl, H.W., and Fogel, M.L. 1994. Isotopic characterization of atmospheric nitrogen inputs as sources of enhanced primary production in coastal Atlantic Ocean waters. *Marine Biology*, 119(4), 635-645.
- Peterson, B.J., and Fry, B. 1987. Stable Isotopes in Ecosystem Studies. *Annual Review of Ecology and Systematics*, 293-320.
- Phlips, E., Badylak, S., Hart, J., Haunert, D., Lockwood, J., O'Donnell, K., Sun, D., Viveros, P., and Yilmaz, M. 2012. Climatic influences on autochthonous and allochthonous phytoplankton blooms in a subtropical estuary, St. Lucie Estuary, Florida, USA. *Estuaries and Coasts*, 35, 335-352.
- Redfield, A.C. 1958. The biological control of chemical factors in the environment. *American Scientist*, 46(3), 230A-221.
- Reischer, G., David, C. K., Ralf, S., Robert, L. M., and Andreas, H. F. 2006. Quantitative PCR method for sensitive detection of ruminant fecal pollution in freshwater and evaluation of this method in alpine karstic regions. *Applied and Environmental Microbiology*, 72(8), 5610-5614.
- Risk, M.J., Lapointe, B.E., Sherwood, O.A., and Bedford, B.J. 2009. The use of $\delta^{15}\text{N}$ in assessing sewage stress on coral reefs. *Marine Pollution Bulletin*, 58(6), 793-802.
- Savoye, N., Aminot, A., Treguer, P., Fontugne, M., Naulet, N., and K  rouel, R. 2003. Dynamics of particulate organic matter $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France). *Marine Ecology Progress Series*, 255, 27-41.
- Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R., and Lukasik, J. 2002. Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology*, 68(12), 5796-5803.
- Sidstedt, M., Jansson, L., Nilsson, E., Noppa, L., Forsman, M., Radstrom, P., and Hedman, J. 2015. Humic substances cause fluorescence inhibition in real-time polymerase chain reaction. *Analytical Biochemistry*, 487, 30-37.
- Shaddox, T., and Unruh, J.B. 2018. The Fate of Nitrogen Applied to Florida Turfgrass. *EDIS*, 2018(1).
- Silvanima, J., Woeber, A., Sunderman-Barnes, S., Copeland, R., Sedlacek, C., and Seal, T. 2018. A synoptic survey of select wastewater-tracer compounds and the pesticide imidacloprid in Florida's ambient freshwaters. *Environmental Monitoring and Assessment*, 190(7), 435.
- Slobodkin, L.B. 1953. A possible initial condition for red tides on the coast of Florida. *Journal of Marine Research*, 12(1), 148-155.
- Snider, D.M., Roy, J.W., Robertson, W.D., Garda, D.I., and Spoelstra, J. 2017. Concentrations of artificial sweeteners and their ratios with nutrients in septic system wastewater. *Groundwater Monitoring and Remediation*, 37(3), 94-102.
- Stoner, B., Howard, B., Noel, S., and Arrington, D.A. 2016. Loxahatchee River Water Quality Monitoring, Task 2: Final Report Assessment of 2014-2015). Loxahatchee River Water Quality.
- Tarnowski, M. 2014. The effects of on-site sewage treatment and disposal systems on the relief canals of Indian River County, the St. Sebastian River, and the Central Indian River Lagoon. Master's Thesis. Florida Atlantic University, Boca Raton, Florida.
- Tran, N.H., Gin, K.Y.H., and Hgo, H.H. 2015. Fecal pollution source tracking toolbox for identification, evaluation and characterization of fecal contamination in receiving

- urban surface waters and groundwater. *Science of the Total Environment*, 538, 38-57.
- Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., and Foreman, K. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography*, 42(5part2), 1105-1118.
- Verhougstraete, M.P., Martin, S.L., Kendall, A.D., Hyndman, D.W., and Rose, J.B. 2015. Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. *Proceedings of the National Academy of Sciences*, 112(33), 10419-10424.
- Viso, E. and Zachariadis, G. 2018. Method Development of Phosphorus and Boron Determination in Inorganic fertilizers by ICP-AES. *Separations*, 5(3), p.36.
- W. Dexter Bender and Associates, Inc. 1995. North Fort Myers surface and ground water quality survey. Final Report to Lee County Department of Utilities.
- Wilcox, J.D., Bahr, J.M., Hedman, C.J., Hemming, J.D., Barman, M.A., and Bradbury, K.R. 2009. Removal of organic wastewater contaminants in septic systems using advanced treatment technologies. *Journal of Environmental Quality*, 38(1), 149-156.
- Withers, P. J. A., Jarvie, H. P., and Stoate, C. 2011. Quantifying the impact of septic tank systems on eutrophication risk in rural headwaters. *Environment International*, 37(3), 644-653.
- Withers, P. J., Jordan, P., May, L., Jarvie, H. P., and Deal, N. E. 2014. Do septic tank systems pose a hidden threat to water quality? *Frontiers in Ecology and the Environment*, 12(2), 123-130.
- Yang, Y.Y., Toor, G.S., Wilson, P.C., and Williams, C.F. 2016. Septic systems as hot-spots of pollutants in the environment: Fate and mass balance of micropollutants in septic drainfields. *Science of the Total Environment*, 566, 1535-1544.
- Yentsch, C.E., Lapointe, B.E., Poulton, N., and Phinney, D.A. 2008. Anatomy of a red tide bloom off the southwest coast of Florida. *Harmful Algae*, 7, 817-826.

9. Appendix

Appendix 1. Detailed site information by water type and watershed, including study site names, site abbreviations, addresses, location descriptions, and GPS coordinates.

Water Type	Watershed	Site Name	Site Abbreviation	Address / Site Description	Latitude	Longitude
Surface Water	Powell Creek	HBOI-SW01	SW1	Bridge at East Mariana & Whidden Rd.	26.6784	-81.8781
		HBOI-SW02	SW2	Bridge at Brooks Rd. & Lavin Ln.	26.6715	-81.8789
		HBOI-SW03	SW3	Sunset Dr. cul-de-sac water access	26.6698	-81.8744
		HBOI-SW04	SW4	Bridge at Bayline Dr. & Powell Creek	26.6820	-81.8775
	Central Drainage	HBOI-SW05	SW5	Drainage feature on River Rd. near river	26.6623	-81.8817
	Hancock Creek	HBOI-SW06	SW6	North Shore Park	26.6572	-81.8827
		HBOI-SW07	SW7	Bridge @ Pondella & Hancock Creek	26.6674	-81.8966
		HBOI-SW08	SW8	Seawall @ corner of Craig St. & Thompson St.	26.6712	-81.9022
		HBOI-SW09	SW9	Bridge @ Pine Island Rd. & Yellow Fever Creek	26.6817	-81.9104
		16-3GR	16-3GR	Hancock Creek at Hancock Bridge Pkwy.	26.6600	-81.8977
Groundwater	Reference	NOFOPZ-10	GW10	1397 Orchid Rd.	26.6726	-81.9176
	Hancock Creek	NOFOPZ-01	GW1	1297 Parkview Ct., east (nearest parking lot)	26.6727	-81.8998
		NOFOPZ-02	GW2	1297 Parkview Ct., middle	26.6727	-81.9001
		NOFOPZ-03	GW3	1297 Parkview Ct., west (nearest creek)	26.6727	-81.9002
	Central Drainage	NOFOPZ-04	GW4	73/75 Cabana Ave.	26.6652	-81.8819
		NOFOPZ-05	GW5	57 Cypress St.	26.6664	-81.8819
		NOFOPZ-06	GW6	1104 Seventh Way	26.6642	-81.8819
	Powell Creek	NOFOPZ-07	GW7	127 Dow Ln., front yard	26.6710	-81.8762
		NOFOPZ-08	GW8	127 Dow Ln., back yard	26.6709	-81.8763
		NOFOPZ-09	GW9	131 Dow Ln., back yard	26.6711	-81.8765

Appendix 2. Overall means ($\pm$ standard error) of groundwater aqueous nitrogen isotope values of ammonium ($\delta^{15}\text{N-NH}_4^+$) and nitrate ($\delta^{15}\text{N-NO}_3^-$) by watershed (reference well, central drainage basin, Hancock Creek, and Powell Creek) and site.

Watershed	Well	Count	$\delta^{15}\text{N-NH}_4^+$ (‰)	Count	$\delta^{15}\text{N-NO}_3^-$ (‰)
Reference	Watershed Avg. / GW10	8	2.20 ± 1.10	6	-1.52 ± 2.80
Hancock	Watershed Avg.	22	4.51 ± 0.87	22	8.49 ± 2.70
	GW1	8	3.99 ± 1.83	8	13.81 ± 5.63
	GW2	7	5.80 ± 1.47	7	8.30 ± 2.72
	GW3	7	3.81 ± 1.07	7	2.61 ± 4.09
Central	Watershed Avg.	24	5.54 ± 0.59	23	4.65 ± 1.19
	GW4	8	2.53 ± 0.58	7	4.09 ± 1.23
	GW5	8	6.53 ± 0.72	8	5.88 ± 3.41
	GW6	8	7.58 ± 0.85	8	3.91 ± 1.09
Powell	Watershed Avg.	24	2.77 ± 0.59	23	4.90 ± 2.59
	GW7	8	1.79 ± 0.79	7	9.36 ± 4.50
	GW8	8	2.25 ± 0.59	8	-0.19 ± 2.61
	GW9	8	4.29 ± 1.40	8	6.09 ± 5.67

Appendix 3. Monthly surface water fecal indicator bacteria abundance for enterococci and *Escherichia coli* (*E. coli*), biochemical oxygen demand (BOD), and color data (single value or mean $\pm$ standard error) by site for Hancock Creek sites.

Watershed	Site	Month	Count	Enterococci (MPN/100mL)	<i>E. coli</i> (MPN/100mL)	Count	BOD (mg/L)	Color (CU)
Hancock Creek	SW9	Oct-17	1	45	291	1	0.5	57
		Nov-17	1	461	365	1	BDL	43
		Dec-17	1	727	649	1	BDL	26
		Jan-18	1	238	548	1	BDL	27
		Feb-18	1	2420	291	1	1.3	31
		Mar-18	1	44	118	1	0.8	34
		Apr-18	1	77	613	1	1.5	67
		May-18	1	1414	299	1	0.6	79
	SW8	Oct-17	1	55	146	1	0.7	81
		Nov-17	1	32	59	1	0.6	73
		Dec-17	1	687	199	1	1.4	60
		Jan-18	1	299	236	1	1	56
		Feb-18	1	328	517	1	3.3	68
		Mar-18	1	59	461	1	0.9	65
		Apr-18	1	387	517	1	0.9	72
		May-18	1	1986	866	1	1.1	76
	SW7	Oct-17	1	192	548	1	1	105
		Nov-17	1	68	144	1	1	82
		Dec-17	1	770	206	1	0.8	80
		Jan-18	1	345	345	1	1.4	65
		Feb-18	1	548	299	1	2	64
		Mar-18	1	228	579	1	0.9	60
		Apr-18	1	2420	2420	1	1.4	55
		May-18	1	2420	1203	1	1.2	57
	16-3GR	Oct-17	2	343 $\pm$ 65	492 $\pm$ 40	2	1.10 $\pm$ 0.01	106 $\pm$ 5
		Nov-17	2	735 $\pm$ 132	850 $\pm$ 191	2	1.25 $\pm$ 0.35	99 $\pm$ 3
		Dec-17	2	677 $\pm$ 261	581 $\pm$ 23	2	1.45 $\pm$ 0.45	87 $\pm$ 3
		Jan-18	2	1812 $\pm$ 430	813 $\pm$ 165	2	1.85 $\pm$ 0.65	65 $\pm$ 3
		Feb-18	2	2420 $\pm$ 0	921 $\pm$ 0	2	1.35 $\pm$ 0.35	58 $\pm$ 3
		Mar-18	2	881 $\pm$ 297	669 $\pm$ 39	2	1.45 $\pm$ 0.45	52 $\pm$ 3
		Apr-18	2	1595 $\pm$ 583	752 $\pm$ 46	2	1.35 $\pm$ 0.15	51 $\pm$ 2
		May-18	1	2420	1120	1	1.7	50
	SW6	Oct-17	1	7	56	1	1.5	172
		Nov-17	1	17	130	1	1.5	165
		Dec-17	1	55	70	1	0.6	105
		Jan-18	1	26	91	1	0.6	75
		Feb-18	1	1986	276	1	1	55
		Mar-18	1	25	84	1	0.6	44
		Apr-18	1	101	74	1	1.9	47
		May-18	1	344	649	1	1	40

Appendix 4. Monthly surface water fecal indicator bacteria abundance for enterococci and *Escherichia coli* (*E. coli*), biochemical oxygen demand (BOD), and color data (single value or mean $\pm$ standard error) by site for Powell Creek and the central drainage feature surface water sites.

Watershed	Site	Month	Count	Enterococci (MPN/100mL)	<i>E. coli</i> (MPN/100mL)	Count	BOD (mg/L)	Color (CU)
Powell Creek	SW4	Oct-17	1	76	172	1	0.7	89
		Nov-17	1	98	186	1	0.7	63
		Dec-17	1	2420	2420	1	0.4	43
		Jan-18	1	2420	2420	1	BDL	36
		Feb-18	1	2420	2420	1	0.6	40
		Mar-18	1	2420	2420	1	1.2	29
		Apr-18	1	2420	2420	1	1.8	40
		May-18	1	2420	1300	1	0.5	57
	SW1	Oct-17	1	97	208	1	0.3	88
		Nov-17	1	155	201	1	0.8	63
		Dec-17	1	2420	2420	1	0.6	44
		Jan-18	1	2420	2420	1	BDL	35
		Feb-18	1	2420	2420	1	0.8	41
		Mar-18	1	2420	2420	1	1.5	37
		Apr-18	1	276	1203	1	1.4	59
		May-18	1	2420	921	1	0.6	68
	SW2	Oct-17	1	281	461	1	0.5	91
		Nov-17	1	548	517	1	1	68
		Dec-17	1	1300	1046	1	0.7	48
		Jan-18	1	2420	2420	1	0.4	49
		Feb-18	1	816	921	1	1.7	53
		Mar-18	1	687	770	1	1.2	52
		Apr-18	1	649	770	1	1.4	71
		May-18	1	2420	517	1	0.9	78
	SW3	Oct-17	1	488	1120	1	1	90
		Nov-17	1	133	261	1	0.9	71
		Dec-17	1	579	291	1	0.6	72
		Jan-18	1	921	921	1	0.4	63
		Feb-18	1	344	517	1	2.4	54
		Mar-18	1	411	299	1	1.4	50
		Apr-18	1	435	727	1	1.4	62
		May-18	1	1733	488	1	1.1	75
Central Drainage	SW5	Oct-17	1	921	272	1	0.6	158
		Nov-17	1	1986	517	1	1.8	147
		Dec-17	1	2967	1196	1	0.7	97
		Jan-18	1	2853	1890	1	0.6	77
		Feb-18	1	854	4840	1	4.4	73
		Mar-18	1	1036	1266	1	1	48
		Apr-18	1	2420	1733	1	1.3	51
		May-18	1	2420	1986	1	0.9	59

Appendix 5. Monthly surface water nutrient concentration data (single value or mean $\pm$ standard error) by site for Hancock Creek surface water sites; BDL = below detection limit.

Watershed	Site	Month	Count	Ammonium (mg/L)	Nitrate + Nitrite (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	Soluble Reactive Phosphorus (mg/L)	Total Kjeldahl Nitrogen (mg/L)	Total Dissolved Nitrogen (mg/L)	Total Dissolved Phosphorus (mg/L)	DIN:SRP	TDN:TDP
Hancock Creek	SW9	Oct-17	3	0.03 $\pm$ <0.01	0.06 $\pm$ <0.01	0.09 $\pm$ <0.01	0.13 $\pm$ <0.01	0.40 $\pm$ 0.01	0.46 $\pm$ 0.01	0.17 $\pm$ <0.01	0.67 $\pm$ <0.01	2.74 $\pm$ 0.08
		Nov-17	3	0.05 $\pm$ <0.01	0.07 $\pm$ <0.01	0.12 $\pm$ <0.01	0.14 $\pm$ <0.01	0.34 $\pm$ 0.01	0.41 $\pm$ 0.01	0.18 $\pm$ <0.01	0.84 $\pm$ 0.02	2.24 $\pm$ 0.05
		Dec-17	1	0.02	0.06	0.07	0.1	0.42	0.48	0.13	0.76	3.69
		Jan-18	1	0.04	0.04	0.08	0.14	0.33	0.37	0.16	0.54	2.31
		Feb-18	3	BDL	0.01 $\pm$ <0.01	0.01 $\pm$ <0.01	0.20 $\pm$ <0.01	0.32 $\pm$ 0.02	0.33 $\pm$ 0.02	0.23 $\pm$ <0.01	0.06 $\pm$ <0.01	1.44 $\pm$ 0.09
		Mar-18	3	0.04 $\pm$ <0.01	0.01	0.04 $\pm$ 0.01	0.24 $\pm$ <0.01	0.45 $\pm$ 0.02	0.45 $\pm$ 0.02	0.26 $\pm$ 0.01	0.18 $\pm$ 0.03	1.75 $\pm$ 0.12
		Apr-18	1	0.03	BDL	0.03	0.39	0.7	0.7	0.46	0.08	1.52
		May-18	1	0.05	0.06	0.1	0.41	0.99	1	0.42	0.25	2.38
	SW8	Oct-17	3	0.02	0.07 $\pm$ <0.01	0.08 $\pm$ 0.01	0.16 $\pm$ <0.01	0.63 $\pm$ 0.10	0.70 $\pm$ 0.10	0.22 $\pm$ <0.01	0.47 $\pm$ 0.04	3.14 $\pm$ 0.46
		Nov-17	3	0.03 $\pm$ <0.01	0.08 $\pm$ <0.01	0.11 $\pm$ <0.01	0.13 $\pm$ <0.01	0.51 $\pm$ 0.08	0.60 $\pm$ 0.09	0.17 $\pm$ <0.01	0.88 $\pm$ 0.01	3.45 $\pm$ 0.52
		Dec-17	1	BDL	BDL	BDL	0.15	0.95	0.95	0.22	<0.01	4.32
		Jan-18	1	BDL	BDL	BDL	0.15	0.81	0.81	0.24	<0.01	3.38
		Feb-18	3	BDL	BDL	0.00 $\pm$ <0.01	0.15 $\pm$ <0.01	0.97 $\pm$ 0.13	0.97 $\pm$ 0.13	0.25 $\pm$ <0.01	<0.01 $\pm$ <0.01	3.93 $\pm$ 0.57
		Mar-18	3	0.07 $\pm$ <0.01	0.02 $\pm$ <0.01	0.09 $\pm$ <0.01	0.18 $\pm$ <0.01	0.77 $\pm$ 0.05	0.79 $\pm$ 0.05	0.20 $\pm$ <0.01	0.49 $\pm$ 0.01	3.93 $\pm$ 0.24
		Apr-18	1	0.07	0.01	0.08	0.19	1	1	0.22	0.42	4.55
		May-18	1	0.02	0.01	0.03	0.2	1.4	1.4	0.22	0.14	6.36
	SW7	Oct-17	3	BDL	0.07 $\pm$ <0.01	0.07 $\pm$ <0.01	0.15 $\pm$ <0.01	0.68 $\pm$ 0.05	0.75 $\pm$ 0.05	0.21 $\pm$ 0.01	0.44 $\pm$ 0.01	3.54 $\pm$ 0.36
		Nov-17	3	0.01	0.01	0.02	0.10 $\pm$ <0.01	0.58 $\pm$ 0.02	0.58 $\pm$ 0.02	0.15 $\pm$ 0.01	0.08 $\pm$ 0.08	3.99 $\pm$ 0.25
		Dec-17	1	0.01	0.05	0.07	0.11	0.84	0.89	0.2	0.57	4.45
		Jan-18	1	BDL	0.02	0.02	0.09	1.2	1.2	0.15	0.25	8
		Feb-18	3	BDL	BDL	0.00 $\pm$ <0.01	0.09 $\pm$ <0.01	0.75 $\pm$ 0.04	0.75 $\pm$ 0.04	0.18 $\pm$ 0.02	0.00 $\pm$ <0.01	4.30 $\pm$ 0.21
		Mar-18	3	0.06 $\pm$ <0.01	0.01 $\pm$ <0.01	0.07 $\pm$ <0.01	0.13 $\pm$ <0.01	0.83 $\pm$ 0.02	0.85 $\pm$ 0.01	0.17 $\pm$ 0.01	0.59 $\pm$ 0.01	5.09 $\pm$ 0.21
		Apr-18	1	0.03	BDL	0.03	0.12	1	1	0.17	0.28	5.88
		May-18	1	BDL	BDL	0	0.15	1	1	0.2	0	5
	16-3GR	Oct-17	4	0.03 $\pm$ <0.01	0.07 $\pm$ <0.01	0.10 $\pm$ <0.01	0.15 $\pm$ <0.01	0.74 $\pm$ 0.08	0.80 $\pm$ 0.07	0.21 $\pm$ 0.01	0.67 $\pm$ 0.01	3.81 $\pm$ 0.29
		Nov-17	4	0.03 $\pm$ <0.01	0.03 $\pm$ 0.01	0.05 $\pm$ <0.01	0.08 $\pm$ <0.01	0.69 $\pm$ 0.07	0.72 $\pm$ 0.06	0.15 $\pm$ 0.01	0.66 $\pm$ 0.02	4.73 $\pm$ 0.37
		Dec-17	2	0.02 $\pm$ <0.01	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01	0.09 $\pm$ 0.01	0.81 $\pm$ 0.03	0.97 $\pm$ 0.03	0.13 $\pm$ 0.01	2.00 $\pm$ 0.30	7.49 $\pm$ 0.85
		Jan-18	2	0.01	0.07	0.08	0.06 $\pm$ 0.01	0.98 $\pm$ 0.02	1.00 $\pm$ <0.01	0.14 $\pm$ <0.01	0.64 $\pm$ 0.64	7.14 $\pm$ <0.01
		Feb-18	4	0.02	BDL	0.00 $\pm$ <0.01	0.08 $\pm$ 0.01	0.82 $\pm$ 0.06	0.82 $\pm$ 0.06	0.17 $\pm$ 0.01	0.05 $\pm$ 0.05	4.86 $\pm$ 0.46
		Mar-18	4	0.04 $\pm$ 0.01	0.01 $\pm$ <0.01	0.05 $\pm$ 0.01	0.10 $\pm$ 0.01	0.87 $\pm$ 0.02	0.88 $\pm$ 0.02	0.14 $\pm$ 0.01	0.53 $\pm$ 0.09	6.56 $\pm$ 0.33
		Apr-18	2	0.02	BDL	0.01 $\pm$ 0.01	0.11 $\pm$ 0.01	0.93 $\pm$ 0.02	0.93 $\pm$ 0.02	0.18 $\pm$ 0.03	0.10 $\pm$ 0.10	5.41 $\pm$ 0.66
		May-18	1	BDL	BDL	<0.01	0.14	0.91	0.91	0.19	<0.01	4.79
	SW6	Oct-17	3	0.02 $\pm$ <0.01	0.22 $\pm$ <0.01	0.24 $\pm$ <0.01	0.09 $\pm$ <0.01	0.93 $\pm$ 0.03	1.13 $\pm$ 0.03	0.14 $\pm$ 0.01	2.56 $\pm$ 0.03	8.34 $\pm$ 0.55
		Nov-17	3	0.03 $\pm$ <0.01	0.25 $\pm$ <0.01	0.28 $\pm$ <0.01	0.07 $\pm$ <0.01	0.81 $\pm$ 0.05	1.05 $\pm$ 0.05	0.11 $\pm$ <0.01	3.78 $\pm$ 0.06	9.58 $\pm$ 0.42
		Dec-17	1	0.03	0.27	0.3	0.09	0.85	1.1	0.14	3.46	7.86
		Jan-18	1	0.02	0.28	0.3	0.07	0.96	1.2	0.09	4.56	13.04
		Feb-18	3	BDL	BDL	<0.01 $\pm$ <0.01	0.02 $\pm$ <0.01	0.71 $\pm$ 0.02	0.71 $\pm$ 0.02	0.05 $\pm$ <0.01	0.00 $\pm$ <0.01	15.43 $\pm$ 0.68
		Mar-18	3	0.03 $\pm$ <0.01	BDL	0.03 $\pm$ <0.01	0.05 $\pm$ <0.01	0.79 $\pm$ 0.01	0.79 $\pm$ 0.01	0.09 $\pm$ 0.01	0.52 $\pm$ 0.02	8.51 $\pm$ 0.89
		Apr-18	1	BDL	BDL	<0.01	0.07	0.93	0.93	0.11	<0.01	8.45
		May-18	1	BDL	BDL	<0.01	0.09	1.1	1.1	0.26	<0.01	4.23

Appendix 6. Monthly surface water nutrient concentration data (single value or mean $\pm$ standard error) by site for Powell Creek and the central drainage feature; BDL = below detection limit.

Watershed	Site	Month	Count	Ammonium (mg/L)	Nitrate + Nitrite (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	Soluble Reactive Phosphorus (mg/L)	Total Kjeldahl Nitrogen (mg/L)	Total Dissolved Nitrogen (mg/L)	Total Dissolved Phosphorus (mg/L)	DIN:SRP	TDN:TDP
Powell Creek	SW4	Oct-17	3	0.07 $\pm$ 0.00	0.11 $\pm$ 0.00	0.18 $\pm$ 0.00	0.07 $\pm$ 0.00	0.77 $\pm$ 0.05	0.88 $\pm$ 0.05	0.13 $\pm$ 0.00	2.58 $\pm$ 0.02	6.94 $\pm$ 0.27
		Nov-17	3	0.04 $\pm$ 0.00	0.08 $\pm$ 0.00	0.12 $\pm$ 0.00	0.08 $\pm$ 0.00	0.70 $\pm$ 0.03	0.78 $\pm$ 0.03	0.12 $\pm$ 0.01	1.52 $\pm$ 0.04	6.62 $\pm$ 0.76
		Dec-17	1	0.03	0.08	0.11	0.12	0.64	0.72	0.15	0.97	4.8
		Jan-18	1	0.06	0.1	0.16	0.19	0.65	0.75	0.22	0.88	3.41
		Feb-18	3	0.05 $\pm$ 0.00	0.08 $\pm$ 0.00	0.12 $\pm$ 0.00	0.18 $\pm$ 0.00	0.21 $\pm$ 0.02	0.29 $\pm$ 0.02	0.22 $\pm$ 0.00	0.68 $\pm$ 0.00	1.31 $\pm$ 0.07
		Mar-18	3	0.03 $\pm$ 0.00	0.09 $\pm$ 0.00	0.12 $\pm$ 0.00	0.23 $\pm$ 0.00	0.49 $\pm$ 0.02	0.59 $\pm$ 0.02	0.32 $\pm$ 0.00	0.54 $\pm$ 0.02	1.86 $\pm$ 0.06
		Apr-18	1	BDL	0.02	0.02	0.42	0.58	0.6	0.57	0.05	1.05
		May-18	1	0.12	0.03	0.15	0.44	0.64	0.67	0.46	0.33	1.46
	SW1	Oct-17	3	0.07 $\pm$ 0.00	0.11 $\pm$ 0.00	0.18 $\pm$ 0.00	0.07 $\pm$ 0.00	0.97 $\pm$ 0.12	1.07 $\pm$ 0.12	0.13 $\pm$ 0.00	2.47 $\pm$ 0.04	8.23 $\pm$ 0.91
		Nov-17	3	0.04 $\pm$ 0.00	0.08 $\pm$ 0.00	0.12 $\pm$ 0.00	0.08 $\pm$ 0.00	0.56 $\pm$ 0.02	0.64 $\pm$ 0.01	0.11 $\pm$ 0.00	1.52 $\pm$ 0.03	5.85 $\pm$ 0.13
		Dec-17	1	0.03	0.09	0.12	0.11	0.57	0.66	0.14	1.08	4.71
		Jan-18	1	0.04	0.1	0.14	0.17	0.38	0.48	0.22	0.8	2.18
		Feb-18	3	0.04 $\pm$ 0.01	0.07 $\pm$ 0.00	0.10 $\pm$ 0.01	0.19 $\pm$ 0.00	0.23 $\pm$ 0.03	0.30 $\pm$ 0.03	0.24 $\pm$ 0.01	0.55 $\pm$ 0.05	1.23 $\pm$ 0.10
		Mar-18	3	0.02	0.04 $\pm$ 0.00	0.05 $\pm$ 0.01	0.23 $\pm$ 0.00	0.52 $\pm$ 0.02	0.56 $\pm$ 0.02	0.30 $\pm$ 0.01	0.21 $\pm$ 0.02	1.87 $\pm$ 0.02
		Apr-18	1	0.06	0.01	0.07	0.44	0.72	0.73	0.52	0.17	1.4
		May-18	1	0.14	0.06	0.2	0.36	0.87	0.93	0.37	0.56	2.51
	SW2	Oct-17	3	0.08 $\pm$ 0.00	0.13 $\pm$ 0.00	0.21 $\pm$ 0.00	0.07 $\pm$ 0.00	0.75 $\pm$ 0.05	0.88 $\pm$ 0.05	0.12 $\pm$ 0.01	2.90 $\pm$ 0.02	7.37 $\pm$ 0.72
		Nov-17	3	0.07 $\pm$ 0.00	0.10 $\pm$ 0.00	0.16 $\pm$ 0.00	0.07 $\pm$ 0.00	0.66 $\pm$ 0.05	0.76 $\pm$ 0.05	0.11 $\pm$ 0.00	2.37 $\pm$ 0.00	6.91 $\pm$ 0.42
		Dec-17	1	0.05	0.1	0.14	0.08	0.66	0.76	0.14	1.8	5.43
		Jan-18	1	0.11	0.14	0.26	0.11	0.66	0.8	0.18	2.29	4.44
		Feb-18	3	BDL	BDL	0.00 $\pm$ 0.00	0.15 $\pm$ 0.00	0.37 $\pm$ 0.03	0.37 $\pm$ 0.03	0.21 $\pm$ 0.01	0.00 $\pm$ 0.00	1.75 $\pm$ 0.14
		Mar-18	3	0.07 $\pm$ 0.00	0.03 $\pm$ 0.00	0.10 $\pm$ 0.00	0.16 $\pm$ 0.00	0.83 $\pm$ 0.03	0.86 $\pm$ 0.03	0.22 $\pm$ 0.01	0.61 $\pm$ 0.02	3.90 $\pm$ 0.14
		Apr-18	1	0.07	0.02	0.08	0.28	1	1	0.35	0.29	2.86
		May-18	1	0.1	0.04	0.14	0.27	0.89	0.93	0.29	0.52	3.21
	SW3	Oct-17	3	0.06 $\pm$ 0.00	0.13 $\pm$ 0.00	0.19 $\pm$ 0.00	0.07 $\pm$ 0.00	0.73 $\pm$ 0.01	0.86 $\pm$ 0.01	0.12 $\pm$ 0.00	2.83 $\pm$ 0.02	7.01 $\pm$ 0.26
		Nov-17	3	0.05 $\pm$ 0.00	0.10 $\pm$ 0.00	0.15 $\pm$ 0.00	0.06 $\pm$ 0.00	0.68 $\pm$ 0.06	0.78 $\pm$ 0.06	0.11 $\pm$ 0.01	2.33 $\pm$ 0.06	6.88 $\pm$ 0.20
		Dec-17	1	0.03	0.16	0.19	0.07	0.85	1	0.12	2.54	8.33
		Jan-18	1	0.08	0.18	0.26	0.1	0.77	0.95	0.14	2.69	6.79
		Feb-18	3	0.01	BDL	0.00 $\pm$ 0.00	0.10 $\pm$ 0.00	0.41 $\pm$ 0.04	0.41 $\pm$ 0.04	0.15 $\pm$ 0.00	0.00 $\pm$ 0.00	2.69 $\pm$ 0.21
		Mar-18	3	0.04 $\pm$ 0.00	0.02 $\pm$ 0.00	0.06 $\pm$ 0.00	0.10 $\pm$ 0.00	0.84 $\pm$ 0.01	0.86 $\pm$ 0.01	0.13 $\pm$ 0.00	0.57 $\pm$ 0.01	6.48 $\pm$ 0.16
		Apr-18	1	0.03	BDL	0.03	0.14	0.9	0.9	0.17	0.2	5.29
		May-18	1	0.03	0.01	0.04	0.18	0.83	0.84	0.25	0.22	3.36
Central Drainage	SW5	Oct-17	3	0.29 $\pm$ 0.00	0.09 $\pm$ 0.00	0.38 $\pm$ 0.00	0.18 $\pm$ 0.00	1.23 $\pm$ 0.09	1.33 $\pm$ 0.09	0.24 $\pm$ 0.00	2.15 $\pm$ 0.01	5.47 $\pm$ 0.29
		Nov-17	3	0.20 $\pm$ 0.00	0.19 $\pm$ 0.00	0.38 $\pm$ 0.00	0.10 $\pm$ 0.00	1.03 $\pm$ 0.09	1.23 $\pm$ 0.09	0.23 $\pm$ 0.00	3.76 $\pm$ 0.02	5.77 $\pm$ 0.26
		Dec-17	1	0.07	0.22	0.29	0.09	0.99	1.2	0.14	3.24	8.57
		Jan-18	1	0.09	0.13	0.22	0.06	0.92	1.1	0.1	3.73	11
		Feb-18	3	0.02 $\pm$ 0.00	0.01	0.02 $\pm$ 0.01	0.06 $\pm$ 0.00	0.82 $\pm$ 0.14	0.82 $\pm$ 0.14	0.17 $\pm$ 0.00	0.35 $\pm$ 0.08	4.75 $\pm$ 0.88
		Mar-18	3	0.04 $\pm$ 0.00	0.02 $\pm$ 0.00	0.06 $\pm$ 0.00	0.06 $\pm$ 0.00	0.97 $\pm$ 0.07	0.98 $\pm$ 0.06	0.10 $\pm$ 0.01	1.03 $\pm$ 0.01	9.87 $\pm$ 0.37
		Apr-18	1	0.05	0.01	0.06	0.1	0.81	0.82	0.18	0.61	4.56
		May-18	1	0.11	0.15	0.26	0.19	0.98	1.1	0.22	1.37	5

Appendix 7. Overall means ($\pm$ standard error) of surface water particulate organic matter (POM), a proxy for phytoplankton, showing stable isotope values of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$), as well as elemental composition (C:N:P) by watershed (Hancock Creek, Powell Creek, the central drainage feature, *Microcystis aeruginosa* from Davis Ramp and SW6, and *Karenia brevis* from coastal red tide sites) and site; NA = data not available.

Watershed / Sample Type	Site	Count	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	C:N	C:P	N:P
Hancock Creek	Watershed Avg.	120	-30.14 $\pm$ 0.3	5.30 $\pm$ 0.22	8.49 $\pm$ 0.12	97.7 $\pm$ 3.57	11.8 $\pm$ 0.47
	SW9	24	-32.17 $\pm$ 0.40	2.86 $\pm$ 0.65	8.93 $\pm$ 0.38	60.6 $\pm$ 5.02	6.95 $\pm$ 0.50
	SW8	24	-31.45 $\pm$ 0.34	4.42 $\pm$ 0.37	8.23 $\pm$ 0.23	85.9 $\pm$ 4.88	10.85 $\pm$ 0.83
	SW7	24	-31.07 $\pm$ 0.38	5.98 $\pm$ 0.26	8.07 $\pm$ 0.18	110.3 $\pm$ 7.03	14.04 $\pm$ 1.10
	16-3GR	24	-29.57 $\pm$ 0.42	5.98 $\pm$ 0.25	8.20 $\pm$ 0.14	99.5 $\pm$ 7.40	12.26 $\pm$ 0.92
	SW6	24	-26.44 $\pm$ 0.42	7.25 $\pm$ 0.19	9.01 $\pm$ 0.33	132.1 $\pm$ 7.23	15.04 $\pm$ 0.96
Central Drainage	Watershed Avg. / SW5	24	-28.13 $\pm$ 0.57	4.18 $\pm$ 0.56	8.54 $\pm$ 0.19	114.7 $\pm$ 4.99	13.60 $\pm$ 0.65
Powell Creek	Watershed Avg.	95	-30.70 $\pm$ 0.29	4.05 $\pm$ 0.31	8.83 $\pm$ 0.17	88.0 $\pm$ 4.21	10.09 $\pm$ 0.44
	SW4	24	-30.92 $\pm$ 0.58	4.02 $\pm$ 0.69	8.76 $\pm$ 0.41	77.6 $\pm$ 10.51	9.35 $\pm$ 1.25
	SW1	23	-31.63 $\pm$ 0.72	5.39 $\pm$ 0.63	8.56 $\pm$ 0.36	78.3 $\pm$ 6.03	9.37 $\pm$ 0.71
	SW2	24	-30.83 $\pm$ 0.67	3.50 $\pm$ 0.54	8.96 $\pm$ 0.30	93.1 $\pm$ 8.55	10.31 $\pm$ 0.78
	SW3	24	-29.38 $\pm$ 0.38	3.37 $\pm$ 0.39	9.04 $\pm$ 0.24	103.1 $\pm$ 7.17	11.33 $\pm$ 0.62
Blue-green Algae	Davis Ramp	3	-29.92 $\pm$ 0.19	6.93 $\pm$ 0.81	7.41 $\pm$ 0.35	22.90 $\pm$ 1.45	3.13 $\pm$ 0.34
	SW6	3	-28.79 $\pm$ 0.02	8.79 $\pm$ 0.04	6.37 $\pm$ 0.17	NA	NA
Red Tide	Bonita	1	-9.36	3.83	6.12	50.80	8.32
	Lighthouse	1	-9.83	3.33	6.07	27.26	4.50
	Lovers Key	1	-12.17	4.23	6.11	30.85	5.06
	Lynn Hall	1	-19.29	4.72	2.91	20.18	6.94
	South Seas	1	-10.10	3.73	6.10	31.43	5.16
	Tarpon	1	-7.10	3.26	7.09	53.28	7.53

