Caloosahatchee River – North Fort Myers Nutrient and Bacteria Source Identification Study

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Executive Summary

Nutrient and bacteria 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 have resulted in closures of water bodies for recreational use. One of the major sources of freshwater in Southwest Florida, the Caloosahatchee River, is impaired for nutrients, chlorophyll *a*, dissolved oxygen, 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 dissolved oxygen 602-304.800 F.A.C. (effective August 7, 2009). Along the river in Lee County, North Fort Myers has experienced persistent bacterial pollution at North Shore Park. To address this human health issue, Lee County Division of Natural Resources, Florida Department of Environmental Protection (FDEP), and Harbor Branch Oceanographic Institute-Florida Atlantic University conducted a microbial source tracking (MST) study to determine contributing factors to this persistent bacterial pollution.

This study involved both groundwater and surface water sampling. Surface water samples were collected from ten sites within the major drainage basins in North Fort Myers: Hancock Creek, Powell Creek, and a central drainage feature between the US-41 and US-41 Business bridges. These samples were analyzed for dissolved nutrient concentrations and bacterial abundance, as well as the presence of bacterial markers and chemical tracers. Particulate organic matter was collected at each site, as a proxy for phytoplankton, and analyzed to determine stable nitrogen and carbon isotope values, as well as elemental composition, which can be interpreted to indicate the nutrient sources fueling primary production. POM samples were also collected opportunistically during harmful algal bloom events (HABs) that occurred in Lee County during the study period and analyzed as above. Additionally, ten shallow groundwater monitoring wells were installed and sampled within the North Fort Myers area for dissolved nutrient concentrations, bacterial abundance, chemical tracer presence, and aqueous stable nitrogen isotope values. Surface water and groundwater samples were collected twice during the 2017 wet season (October and November) and the 2018 dry season (March and April). Additional surface water samples were collected bimonthly for continued monitoring of dissolved nutrients and bacterial abundance.

Surface water samples had elevated nutrient levels, with the highest ammonium and soluble reactive phosphorus (SRP) concentrations observed at the central drainage feature, and the highest nitrate concentrations observed at North Shore Park. Furthermore, total phosphorus (TP) concentrations almost always exceeded FDEP criteria for the peninsular stream region (0.12 mg/L). Ratios of nitrogen:phosphorus (N:P) are ecologically relevant and useful in understanding the occurrence of HABs. Surface water sites had very low (< 5) dissolved inorganic nitrogen to SRP (DIN:SRP) ratios in both the wet and dry seasons, which indicated strong nitrogen-limitation for primary production (e.g. algae blooms and plant growth). This is critical for addressing blooms of the red tide organism (*Karenia brevis*) and some blue-green algae species (*Microcystis aeruginosa*) that thrive where N:P ratios are low. Stable nitrogen isotope values of phytoplankton at study sites were within the range of wastewater (+4.54 ‰) and were similar to values from *M. aeruginosa* collected at Davis Boat Ramp (+6.93 ‰) in Fort Myers and *K. brevis* from coastal areas (+3.85 ‰). These combined data show the linkage of the local watershed to downstream HABs.

Surface water sampling confirmed that there are recurring bacterial issues within the North Fort Myers study area. Enterococci concentrations often exceeded the FDEP Ten Percent Threshold Value (TPTV) of ≥ 130 MPN/100 mL and *Escherichia coli (E. coli*) concentrations often exceeded the FDEP TPTV of ≥ 410 MPN/100 mL. 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 North Shore Park. Additionally, sucralose, an artificial sweetener used as tracer of human wastewater, was detected at every surface water site during the study period, usually in "moderate" concentrations (0.1 - 1 µg/L) and sometimes in "significant" concentrations (> 1 µg/L). Generally, sites near septic systems had the highest sucralose concentrations. Pharmaceuticals, including carbamazepine and primidone, were detected at many sites and were especially high at the Powell Creek sites. The prevalence of these chemical tracers suggests that wastewater is ubiquitously present in surface waters throughout the study area. Additionally, the detection of herbicides and pesticides indicate that stormwater runoff and chemical macrophyte control also affect surface water quality. The combined data suggest that some of the water quality issues in the study area are related to contamination by human wastewater and surface runoff.

Groundwater monitoring confirmed the influence of human wastewater within the North Fort Myers study area. For example, ammonium and SRP concentrations in groundwater were much higher than in surface water. Ammonium was especially high in Powell Creek, an area that is heavily reliant on septic systems. DIN:SRP ratios within the Powell Creek basin were also elevated, indicating phosphorus-limitation of algal growth. This further reflects septic tank effluent moving through the groundwater, where phosphorus is selectively removed by adsorption in soils relative to nitrogen. Aqueous isotopes of ammonia and nitrate were within the range expected for wastewater (+5.57 ‰ and +6.82 ‰, respectively). Water levels in the study area were high, with an overall average depth to water table of 2.7 ft. This suggests septic systems may not always meet the minimum Florida Administrative Code requirements for separation of the drainfield and high water table (~3.5 ft). Groundwater samples had lower fecal indicator bacteria than observed in surface water, however, concentrations of enterococci and *E. coli* sometimes exceeded FDOH surface water criteria for "moderate" or "poor" conditions. Sucralose was detected at every site and usually exceeded "moderate" levels and was often present in "significant" concentrations. Carbamazepine and primidone were also frequently detected, particularly in the Hancock Creek and central drainage feature basins, indicating the presence of wastewater. Other chemical tracers were typically not present in groundwater, reflecting the influence of stormwater runoff on surface water quality.

The multiple lines of evidence in this study indicated a strong influence of human wastewater on local water quality. Due to the lack of reuse water application or wastewater treatment plant effluent discharge in the study area, septic systems are the only available wastewater source. To improve water quality and reduce nutrient loading to local watersheds and the downstream Caloosahatchee River, we recommend decreasing reliance on septic systems within North Fort Myers. Further, stormwater runoff may also contribute to the degradation of this area, therefore, the addition of stormwater management structures to help decrease the influence of surficial runoff would also be important for restoring this watershed. Finally, an assessment of the methods used to manage 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 collected during the Caloosahatchee – North Fort Myers Nutrient and Bacteria Source Identification Study showing relative levels of analytes, summarized for surface water and groundwater; a dash (-) indicates the substance was below detection limits or did not amplify, "NA" indicates the substance was not analyzed at that site, green shading indicates trace concentrations or a low value relative to applicable standards (not all analytes have numerical standards), yellow shading indicates a value above background levels or approaching the standard, and red shading indicates exceedance of surface quality water standards or a significant presence. There are no numerical standards for reactive nutrients, so classifications were based on an estimated percent contribution of the FDEP surface water standard for the peninsular stream region (TN=1.54 mg/L and TP=0.12 mg/L): ammonium and nitrate were considered elevated at 10% of the total nitrogen (TN) standard, DIN was considered elevated at 20% of the TN standard, and phosphate (SRP) was considered elevated at 20% of the total phosphorus standard; the numerical classifications and units for each parameter are listed in the legend.

			Bacteria							Chemical Tracers										Dissolved Nutrients					
Type	**Drainage Basin**	**Site**	**Enterococci**	***E. coli***	**BacR-qPCR**	**GFD-purified-qPCR**	**GULL2-qPCR**	**HF183-qPCR**	**BOD**	**2,4-D**	**Acetami-nophen**	**Bentazon**	**Carbama-zepine**	**Primidone**	**Sucralose**	**Diuron**	**Fluridone**	**Imazapyr**	**Imidacloprid**	**Ammonium**	**Nitrate + Nitrite**	**DIN**	**SRP**	**TN**	**TP**
Surface Water	**Hancock Creek**	SW9								-	-			-		-			-						
		SW8			-						-														
		SW7									-														
		16-3GR									-														
		SW6						-			-														
	Powell Creek	SW4			-		-			-	-					-									
		SW1			-					-	-					-									
		SW2			-											-									
		SW3			-											-									
	Central Drainage	SW5					-							-											
Ground-water	**Hancock Creek**	GW1		-	NA	NA	NA	NA		-	-	-				-	-	-	-						
		GW2		-	NA	NA	NA	NA	-	-	-	-				-	-	-	-						
		GW3		-	NA	NA	NA	NA		-	-	-		-		-	-	-	-						
	Powell Creek	GW7		-	NA	NA	NA	NA		-		-	-	-		-	-	-	-						
		GW8		-	NA	NA	NA	NA		-	-	-	-	-		-	-	-	-						
		GW9			NA	NA	NA	NA		-	-		-			-	-	-	-						
	Central Drainage	GW4			NA	NA	NA	NA	-	-	-					-	-	-	-		-				
		GW5			NA	NA	NA	NA		-	-	-		-		-	-	-	-						
		GW6		-	NA	NA	NA	NA		-	-	-		-		-	-	-	-						
	Reference	GW10		-	NA	NA	NA	NA		-	-		-	-		-		-	-						
Legend	Low		<35	<126	MDL-9,999	MDL-9,999	MDL-9,999	MDL-9,999	<1.0	MDL-0.009	MDL-0.009	MDL-0.09	MDL-0.009	MDL-0.009	MDL-0.009	MDL-0.009	MDL-0.009	MDL-0.009	MDL-0.009	<0.02	<0.02	<0.04	<0.005	<0.25	<0.01
	Moderate		35-129	126-409	10,000-99,999	10,000-99,999	10,000-99,999	10,000-99,999	1.0-2.3	0.01-0.99	0.01-0.99	0.1-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.01-0.99	0.02-0.153	0.02-0.153	0.04-0.309	0.005-0.023	0.25-1.53	0.01-0.12
	Significant		≥ 130	≥ 410	≥ 100,000	≥ 100,000	≥ 100,000	≥ 100,000	≥2.4	≥1.0	≥1.0	≥1.0	≥1.0	≥1.0	≥1.0	≥1	≥1	≥1	≥1	≥0.154	≥0.154	≥0.308	≥0.024	≥1.54	≥0.12
	Parameter Units		MPN/100mL	MPN/100mL	TSC/100mL	TSC/100mL	TSC/100mL	GEU/100mL	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L

Exceeds water quality standards or a significant presence
Approaching standards
Low value relative to standards
- Below detection limit
NA Not analyzed for

Table of Contents

List of Tables

List of Figures

List of Appendices

Acronyms Used in the Text

BDL	below detection level
BOD	biochemical oxygen demand, biological oxygen demand
BMAP	Basin Management Action Plan
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
GF/F	glass fiber filters
HAB	harmful algal bloom
HBOI-FAU	Harbor Branch Oceanographic Institute-Florida Atlantic University
LCDNR	Lee County Division of Natural Resources
LCEL	Lee County Environmental Lab
MDL	minimum detection limit
mg/L	milligrams per liter
MGM	monthly geometric mean
MPN	most probable number
MST	microbial source tracking
NH_4	ammonium
NNC	Numeric Nutrient Criteria
NO_3	nitrate + nitrite
PQL	practical quantitation limit
SE	standard error
SM	standard method
SRP	soluble reactive phosphorus, phosphate
TMDL	Total Maximum Daily Load
TN	total nitrogen
TKN	total Kjeldahl nitrogen
TP	total phosphorus
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

Water quality is an ongoing, evolving issue for urbanized areas 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) 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 of microbial pollution. The objective of this study was to gain a better understanding of the bacterial prevalence in relation to source tracking parameters, water quality, and land-use in this watershed to determine the sources of the pollution in North Fort Myers.

Water quality in North Fort Myers is relatable to downstream effects, such as harmful algal blooms (HABs). Blooms of the dinoflagellate *Karenia brevis* have been historically reported off the coast of Lee County. *K. brevis* blooms are commonly referred to as “red tides” and contain high concentrations of brevetoxins, a type of neurotoxin that can cause fish kills, shellfish contamination, and negatively affect 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 coast of Lee County at the turn of the century. These blooms have been associated with contributions from sewage sources, as well as rainfall and agricultural fertilizers (Lapointe and Bedford, 2007). In multiple years, including 2005 and 2018, blooms of 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 in the St. Lucie Estuary in 2016 and were attributed to algal “seeding” from Lake Okeechobee combined with nitrogen-loading from the local watershed (Kramer et al., 2018). These HABs can negatively impact local economies through 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, 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 isotopes 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 stable nitrogen isotope values of macroalgae collected in the Caloosahatchee River were within the range of sewage nitrogen and that these values increased heading downstream from Ortona Lock to Franklin Lock. Interestingly, stable nitrogen isotope 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 drainage basin to minimize the risk of worsening harmful algal blooms in Lee County waters (**Fig. 1**).

Figure 1. Images reflecting a degraded ecosystem, including a) a harmful algal bloom 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 on August 3, 2018.

1.2 Study Site

Lee County encompasses 1,212 square miles and is bisected by a section of the Caloosahatchee River. With a population of 722,336 as of 2016, according to US Census data, it is the 8th largest county in the state. 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, from 618,754 in 2010 to 722,336 in 2016 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.

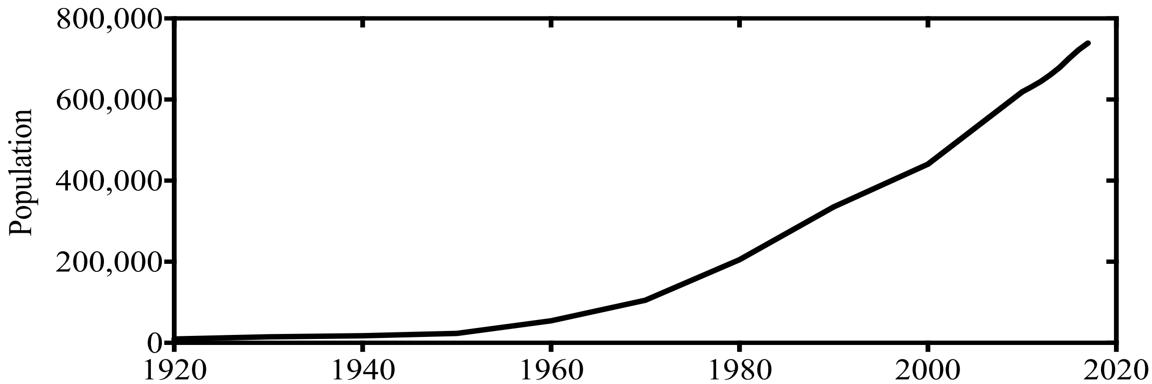

Figure 2. Population growth in Lee County from 1920 projected to 2020, showing rapid growth since 1970.

The Caloosahatchee 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 the daily average percent dissolved oxygen (% DO) saturation must not be below 42% in the Lower Caloosahatchee. Biological 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.

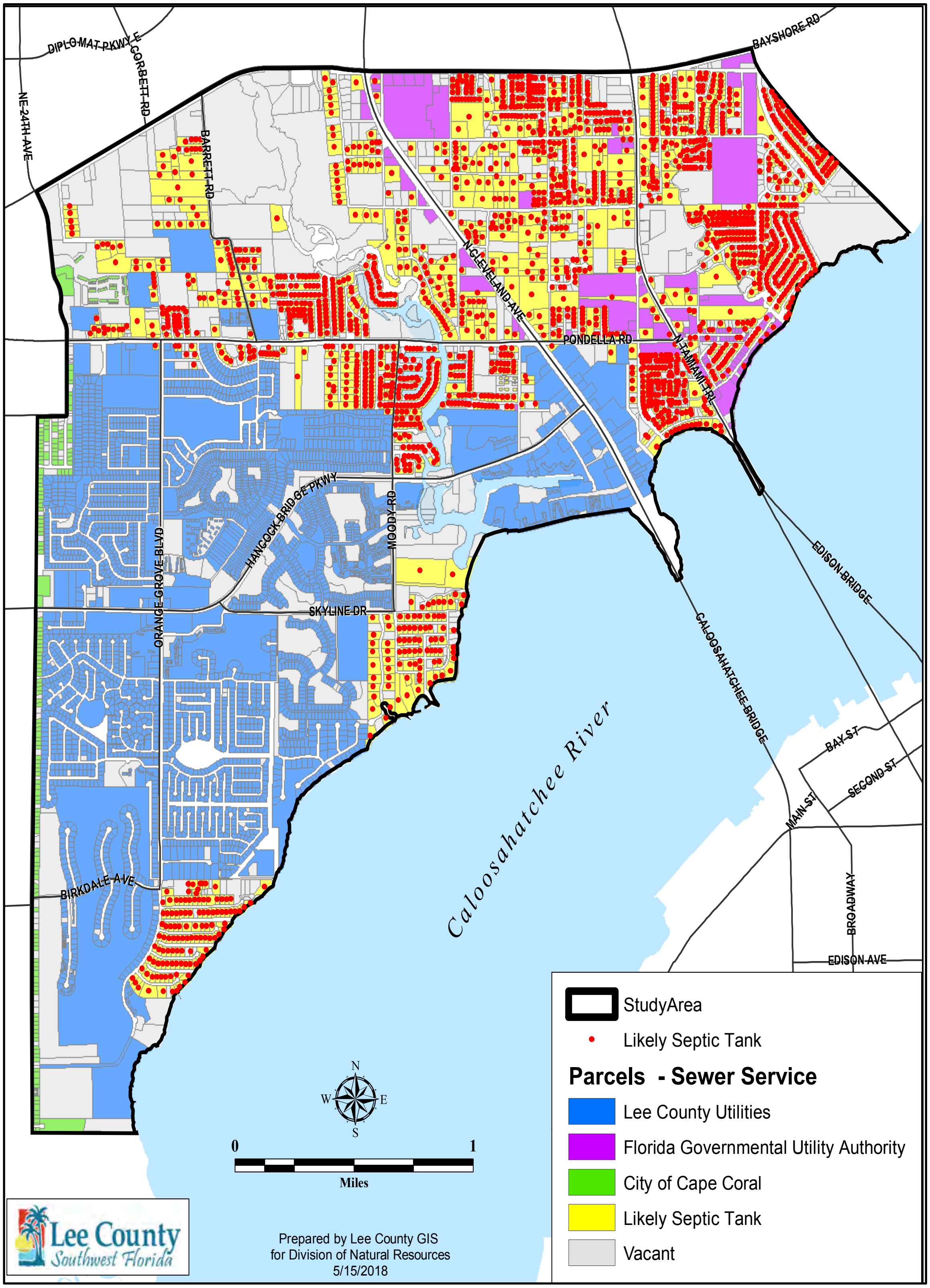

Figure 3. Wastewater service map showing the study area within North Fort Myers with parcels that are "likely" septic tanks and parcels that are connected to Lee County Utilities, Florida Governmental Utility Authority, or the City of Cape Coral Utilities.

The urban areas of North Fort Myers were developed along canals and creeks that ultimately flow into the Caloosahatchee River. The eastern side of North Fort Myers is situated on Powell Creek, the western side is drained by Hancock Creek, and there is a central drainage feature between US-41 and US-41 Business. 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 parcels there are no data available on wastewater disposal (FDOH). In other counties in South Florida, including Monroe, Palm Beach, Martin, Volusia, Indian River, Brevard, Charlotte, and St. Lucie, septic systems have contributed to the degradation of adjacent surface waters through groundwater discharge and tidal pumping, leading to increased dissolved nutrient and bacterial concentrations (Lapointe and Krupa, 1995; Lapointe et al., 1990; 2012; 2015; 2016; 2017; 2018). Further, many septic systems in Southwest Florida may not meet FAC requirements due to elevated seasonal high water tables (Lapointe et al., 2016). A minimum of six inches of cover is required on top of a septic system drainfield, the required drainfield depth is approximately one foot (may be less in some soil types), and the required separation from the bottom of the drainfield to the high water table is two feet (FAC Rule 62E-6). Therefore at least 3.5 feet of separation is needed from the ground surface to the water table to meet the minimum requirements. In some areas with high water levels, "mounding" has been used to help meet these requirements by adding additional separation. As such, understanding interactions between septic systems and ground 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 NNC 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.

1.3 Microbial Source Tracking

The presence and magnitude of certain fecal indicator bacteria (FIB) can be used as a measure of the safety and suitability of the water for various recreational uses. Surface water bacteria can originate from point sources (wastewater treatment plant discharge) and non-point sources (septic tanks, 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 it is important to understand there is high variability involved, as well as dependence on weather conditions, rainfall catchment, and drainage infrastructure (Tran et al., 2015).

Fecal coliforms are bacteria that are 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 is thought to be a better indicator of human health risk than fecal coliforms by the USEPA. Enterococci are a subgroup within the fecal streptococcus group that occur within human digestive systems. While *E. coli* has a low salinity tolerance, enterococci are able to survive in salt water. 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) MPN 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 multiple lines of evidence and a suite of source tracking tools can address these uncertainties and be helpful in clarifying FIB presence.

Molecular markers can be valuable in determining the source of bacterial impairment (Scott et al., 2002). The use of the analytical technique, quantitative polymerase chain reaction (qPCR), allows for the amplification, identification, and quantification of genetic material (DNA). Specific genetic markers can determine if the source of the bacteria is most likely avian (GFD, Gull2) or human (HF183). The avian GFD marker is an unclassified *Helicobacter sp*. and is indicative of many bird species including gulls, goose, chicken, pigeon, egret, crow, and others. Gull2 is associated with the bacteria species *Catellicoccus marimammalium*, which is found in the feces of seagulls and other coastal birds (measured in Target Sequence Copies per 100 mL; TSC/100 mL). The human marker HF183 is in the *Bacteroides* genus and does not survive well in oxygenated conditions, but can remain present and detectable. 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 tank effluent exhibits a “moderate” signal, occurring in the hundreds of thousands GEU/100 mL. Treated wastewater is highly variable, ranging from “low” signals of non-detectable or 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. If detectable, these markers allow for discrimination between human and avian bacteria sources within a body of water. However, there are limitations to these molecular analyses. 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 the molecular marker results, chemical tracers are often useful in MST studies to illuminate 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 used in source tracking 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 studies along the Loxahatchee River (Loxahatchee River District), Indian River County canals and St. Sebastian River (Tarnowski, 2014), and Martin County (Lapointe et al., 2017) to pinpoint areas where septic systems are leaching into surface water. Treated wastewater and reclaimed water have been found to have sucralose concentrations ranging from 10 to 40 µg/L (FDEP, 2014), while septic tank effluent concentrations are more variable and can range from 2 to 67 µg/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 and variability, chemical tracers of human waste 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 surface waters, most notably in rivers, but has also been documented in groundwater (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 nitrogen isotopes of water and algal tissue represent another method of nutrient source tracking. Algae integrate nutrients over days to weeks, so the chemical signature is representative of longer-term nutrient availability (Lapointe, 1985). As such, stable nitrogen isotopes in primary producers, such as phytoplankton or macroalgae, are often used to discriminate between natural and anthropogenic nutrient sources (Risk et al., 2009). The reported stable nitrogen isotope values ($\delta^{15}N$) for synthetic fertilizers range from -2 ‰ to + 2‰ (Bateman and Kelly, 2007), while human wastewater exhibit more enriched ratios ranging from +3 ‰ to +19 ‰ (Heaton, 1986; Costanzo et al., 2001). Further, aqueous stable nitrogen isotopes from a water sample in the form of ammonium ($\delta^{15}N$-NH_4) and nitrate ($\delta^{15}N$-NO_3) can be used to distinguish between sources of dissolved inorganic nitrogen (DIN). Ammonium is the predominant species of nitrogen in septic system effluent (Bicki et al., 1984; Lapointe et al., 1990; Valiela et al., 1997) with $\delta^{15}N$ values ranging from +4 ‰ to +5 ‰ (Lapointe and Krupa, 1995; Hinkle et al., 2008; Katz et al., 2010). The resulting stable isotope values provide insight to the source of the nitrogen (i.e. atmospheric, fertilizer, or wastewater).

This MST effort in North Fort Myers considered the above mentioned dissolved nutrient criteria, bacterial concentrations, chemical indicators, stable isotopes, and environmental parameters in relation to 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 a central drainage feature located between US-41 and US-41 Business (**Fig. 4**). The primary land-use for each of these basins 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 to 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 slight estuarine influence nearest to the Caloosahatchee River.

Ten surface water sites were selected: five in the Hancock Creek watershed, four on Powell Creek, and one in the central drainage feature. Ten groundwater sites were also included: three sites in Hancock Creek, three sites in Powell Creek, three sites in the central drainage feature, and a reference site within a less developed area (**Fig. 4**). For detailed information about the geographic location of surface and groundwater sites, see **Appendix 1**.

2.1.1 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 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. 5**). 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 in Hancock Creek adjacent to Craig St. and Thompson St. in a residential area and was collected from a private seawall (**Fig. 5**). Downstream was HBOI-SW07 (SW7), which was collected from Hancock Creek upstream of the Pondella Rd. Bridge (**Fig. 5**). An established LCDNR sampling site, 16-3GR, was the next downstream site. 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. 5**). 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. 5**). At SW6, samples were collected by wading out approximately ten yards from shore with a sampling pole.

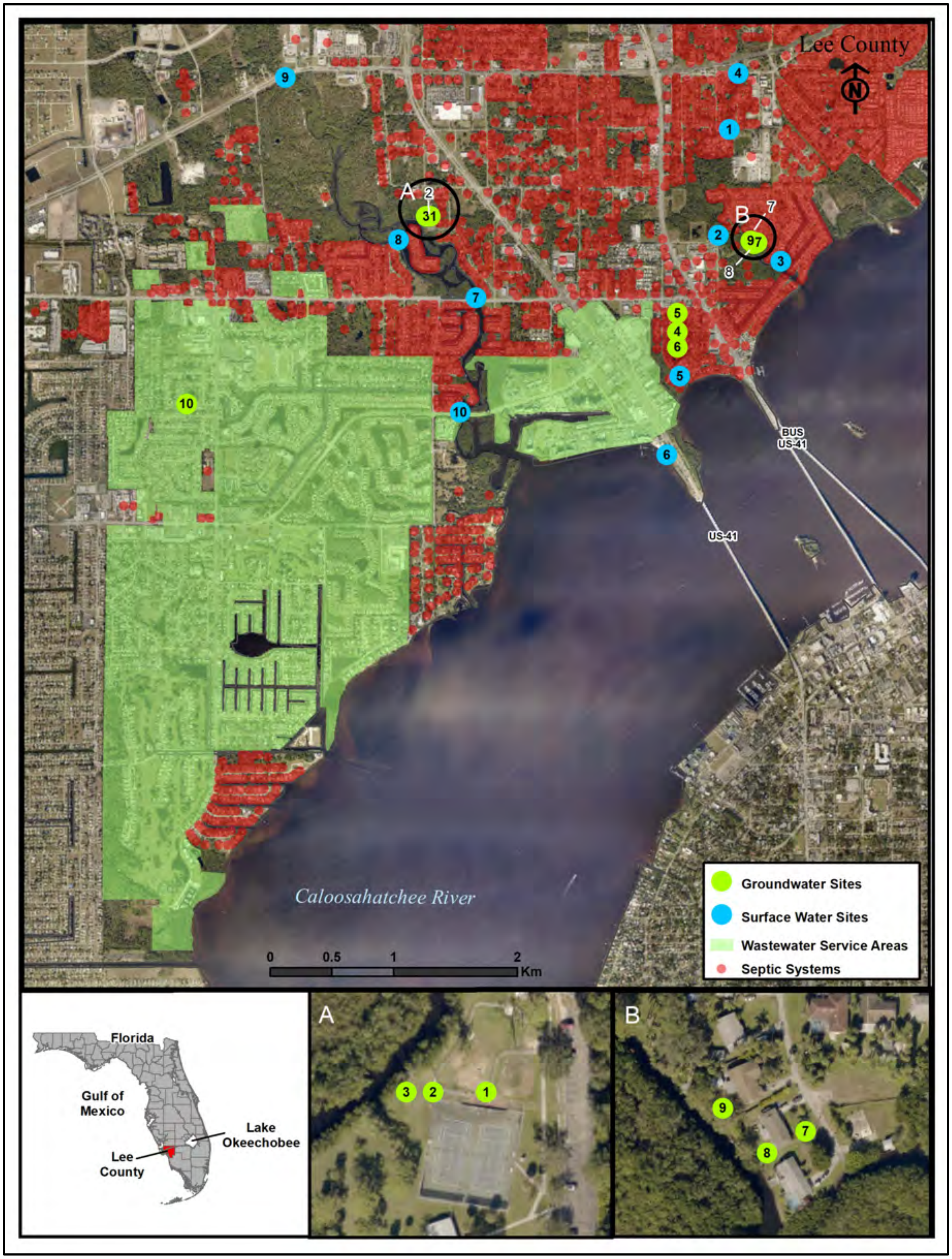

Figure 4. Study area in North Fort Myers, showing locations of groundwater sites (GW 1-10, green circles) and surface water sites (SW 1-10, blue circles), as well as areas connected to sewer for wastewater disposal (green shading) and parcels with septic systems (red shading). Inset A shows a close up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

Figure 5. 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).

Figure 6. Upstream (left) and downstream (right) images of surface water sites within the Powell Creek watershed from the most upstream site (SW4) to where the creek meets the Caloosahatchee River (SW3).

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 Powell Creek, HBOI-SW04 (SW4), receives flow from an upstream residential area, but was fairly 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. 6**). The next site, HBOI-SW01 (SW1), is located 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. 6**). 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. 6**). The final site in Powell Creek 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. 6**).

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. 7**).

Figure 7. Upstream (left) and downstream (right) images of the central drainage feature between US-41 and US-41 Business.

2.1.2 Groundwater Sites

Shallow groundwater wells (7.25 ft.) 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 sites were located in the Hancock Creek watershed, all of which were located within Judd Community Park in a primarily residential area. The first site, 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.**

8). The second site, NOFOPZ-02 (GW2), was adjacent to the north side of the tennis court and west of GW1 (**Fig. 8**). The last site, NOFOPZ-03 (GW3), was located west of GW2, nearest to a canal feeding into Hancock Creek (**Fig. 8**).

Figure 8. 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 Powell Creek watershed included three groundwater sites from within a densely populated residential area. Two sites 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. 9**). The third site, NOFOPZ-09 (GW9), was also near Powell Creek and located in the back yard of 131 Dow Ln. (**Fig. 9**).

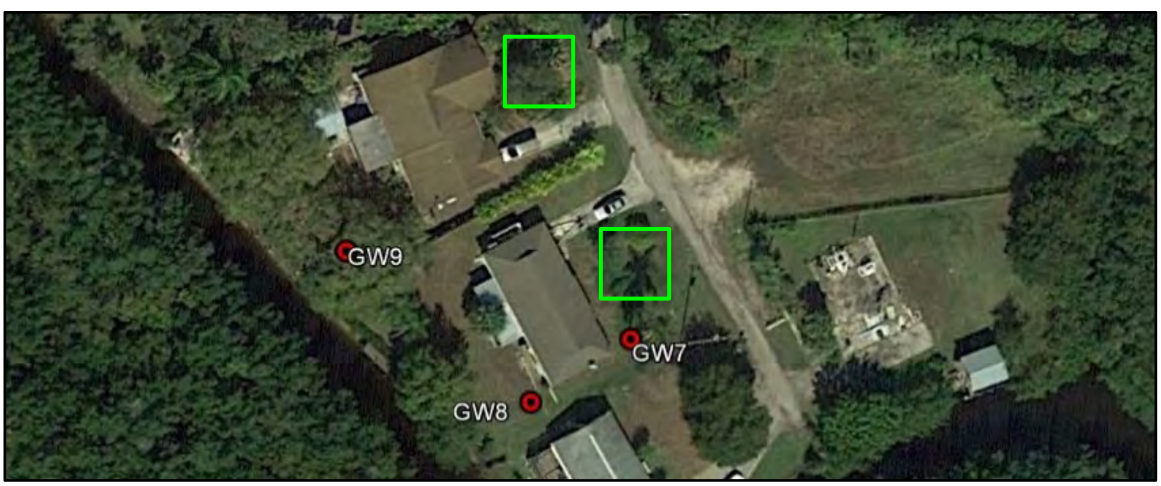

Figure 9. Groundwater monitoring wells (GW7-GW9) located in Powell Creek watershed on the eastern side of North Fort Myers, FL; the approximate location of the septic tank drainfields are indicated by the green squares.

The central drainage feature contained three groundwater sites, all of which were located in dense residential areas. The sites in this watershed were not as closely aggregated as those in the Hancock and Powell Creek watersheds. The northern most site was NOFOPZ-05 (GW5), which was located at 57 Cypress St. near a septic system drainfield (**Fig. 10**). Just south of GW5 was NOFOPZ-04 (GW4) at 73/75 Cabana Ave. (**Fig. 10**). GW4 was located close to two septic system drainfields (**Fig. 10**). NOFOPZ-06 (GW6) was the most southern site, located at 1104 Seventh Way, and had a septic system drainfield near the well (**Fig. 10**). It is noteworthy that not all septic system drainfields in this location are not represented on **Fig. 10**, only locations that had visible mounding.

Figure 10. Groundwater monitoring wells (GW4-GW6) located near the "central drainage feature" in North Fort Myers, FL; the approximate location of some septic tank drainfields are indicated by the green squares. Not all drainfields in this area are represented in this map due to a lack of information.

The reference site, NOFOPZ-10 (GW10), was located in a relatively undeveloped area in the western part of North Fort Myers off of Pine Island Rd. at 1397 Orchid Dr. (**Fig. 11**). Several sewered businesses and homes were located near this site, but land-use was less densely occupied relative to the other basins.

Figure 11. Groundwater monitoring well (GW10) located in a less developed area of western North Fort Myers, FL.

2.2 Rainfall

Rainfall data over the study period (September 2017 – April 2018) was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (https://www.ncdc.noaa.gov/data-access). The selected station, Fort Myers 0.8N, FL US, (GHCND: US1FLLE0037) was located just southeast of the study area (26.6064°, -81.8773°) and had fairly complete coverage over the study period (90%). Daily total precipitation was plotted to indicate seasonal rainfall inputs relative to sampling events.

2.3 Sample Collection

Surface and groundwater sampling were conducted by HBOI-FAU and LCEL following established FDEP standard operating procedures (SOPs) for the collection of surface water and groundwater (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 twice during the wet season (October 17-18, 2017 and November 14-15, 2017) and twice during the dry season (February 13-14, 2018 and March 13-14, 2018). In addition to the above sampling events, surface water samples were also collected monthly between the wet and dry season sampling events and through May 2018 for nutrient, BOD, color, and bacterial concentration analyses.

2.3.1 Surface Water Sampling

Environmental parameters were measured at all surface water sites using calibrated multiparameter probes. Temperature and DO (mg/L and % saturation) were measured using a YSI ProODO, and pH, conductivity, and salinity were measured using a YSI Pro1030. Water clarity was assessed visually using a secchi disk when possible. Water samples for dissolved nutrient analysis were collected in triplicate into clean high-density polyethylene (HDPE) bottles at ten surface water sites (**Fig. 12**), immediately submerged in ice in a dark cooler, and delivered to LCEL. Dissolved nutrient analyses included ammonium (NH_4), nitrate + nitrite (NO_3), SRP, total Kjeldahl nitrogen (TKN), TN, and TP. TKN is the sum of organic N and NH_4, while DIN is the sum of NH_4 and NO_3. Therefore, TKN and DIN are not discussed in this report as they can be inferred from the data presented. A single water sample at each site was also collected into clean HDPE bottles for BOD, color, and determination of enterococci and *E. coli* concentrations, which were stored on ice in a dark cooler and delivered to LCEL for analysis.

Chemical tracers were collected in 1 L clean amber glass bottles for analysis of 2,4-Dichlorophenoxyacetic acid (2,4-D), acetaminophen, bentazon, carbamazepine, mCPP, primidone, sucralose, and triclopyr. Additional tracers were tested for during the March 2018 sampling event, including diuron, fenuron, fluridone, imazapyr, imidacloprid, linuron, and pyraclostrobin. Sucralose detections were classified as "moderate" from 0.1-1.0 µg/L and "significant" at > 1.0 µg/L (FDEP, 2014). Molecular marker samples were collected in clean HDPE bottles and included analyses for the general bacteria marker BacR, the bird markers GFD and Gull2, and the human marker HF138. Chemical and microbial tracer samples were stored on ice in a dark cooler and shipped overnight to the FDEP Central Laboratory for determination of chemical concentrations and molecular markers, following standard methods available on the FDEP website at: https://floridadep.gov/dear/florida-dep-laboratory/content/dep-laboratory-quality-assurance-manual-and-sops.

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, as 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 47 mm glass fiber filters (GF/F) using a vacuum pump. The volume filtered was recorded and the filter was wrapped in aluminum foil and stored at -20°C until analysis. The POM filters were analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, as well as elemental composition (%C, %N, and %P) at the University of Georgia, Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL). At UGA-SIEL, samples were analyzed for $\delta^{13}C$, $\delta^{15}N$, %C, and %N 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). %P was analyzed following the methodology of Asplia et al. (1976) on a Technicon Autoanalyzer II with an IBM-compatible, Labtronics, Inc. DP500 software data collection system (D'Elia et al., 1997). 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 red tide (*K. brevis*; March 2018) and analyzed similarly for stable isotope values and elemental composition.

2.3.2 Groundwater Sampling

Environmental parameters including, temperature, %DO, DO (mg/L), pH, conductivity, salinity, and depth to water table were measured at groundwater sites by LCDNR or LCEL staff using calibrated multiparameter probes (**Fig. 12**). Depth to water was measured during sample collection and at additional times during the project period. Per FDEP protocols, the well volume was calculated and triple the well volume was purged before sampling. Groundwater samples were collected for analyses of environmental parameters, dissolved nutrient concentrations, bacteria levels, and human source chemical tracers as described previously. Molecular marker analysis was not conducted on groundwater samples.

Groundwater was also collected for analysis of nitrogen isotopic composition through determination of $\delta^{15}N-NH_4$ and $\delta^{15}N-NO_3$ aqueous stable nitrogen isotope values. These samples were collected into 1 L HDPE bottles and immediately stored on ice in the field. In the lab samples were vacuum filtered through 47 mm GF/F (**Fig. 12**) and the volume filtered was recorded. Filters were wrapped in aluminum foil, labeled, and frozen until shipment to UGA-SIEL for analysis. At UGA-SIEL the water samples were run through ammonia diffusion. This involved increasing the pH of the dissolved sample to convert ammonium to gaseous ammonia, which was captured on an acidified filter in the bottle headspace. NO_3-specific N was quantified by first boiling-off the volatile ammonia, adding a reducing agent to convert oxidized N to NH_4, then proceeding with the standard diffusion and ammonia capture on an acidified filter. The filter was then analyzed as a typical solid sample on a Carlo Erba Isotope Ratio Mass Spectrometer for $\delta^{15}N-NH_4$ and $\delta^{15}N-NO_3$.

2.4 Data Analysis

Environmental parameters, dissolved nutrient concentrations, bacterial prevalence, molecular markers, chemical tracers, and stable isotope values were compared using overall site and seasonal averages. Any results flagged as below detection levels ("U") or less than the criterion of detection ("T") were excluded from data analysis. However, results flagged as between the laboratory detection limit and the practical quantitation limit ("I") and estimated values ("J") were included in data analysis. In the case of samples flagged with a "U" and either "I" or "J" the results were excluded from analysis. Relative concentrations of these parameters were then compared between sites to examine the relationships of variables. In conjunction with land-use, these data were considered together to infer possible sources of bacterial contamination and water quality issues. 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. Bacterial standards for enterococcus apply to marine waters and those for *E. coli* are applicable to freshwaters. Bacterial standards do not apply to groundwater. However, while water quality standards for both *E. coli* and enterococcus are not applicable at all sites, they are mentioned to serve as points of reference. Results were interpreted with an eye towards potential solutions to this chronic public health issue and to make recommendations for future studies.

Figure 12. Images from the Caloosahatchee – North Fort Myers Nutrient and Bacteria Source Identification 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.

3. Results

3.1 Rainfall

All sampling events were conducted on days with little to no precipitation (0 to 0.12 inches; **Fig 13**). During the wet season approximately 30 inches of rain fell in the two months prior to the first sampling event. The local station recorded approximately ten inches of precipitation during Hurricane Irma on September 10-11, 2017, however there was a week-long gap in rainfall data following the event. In the week prior to the first wet season sampling event, approximately four inches of precipitation was recorded. Rainfall was lower during the dry season, with approximately two inches of recorded rainfall in the two months prior to the first dry season sampling event and 0 to 0.3 inches of recorded rainfall in the week prior to each dry season sampling event.

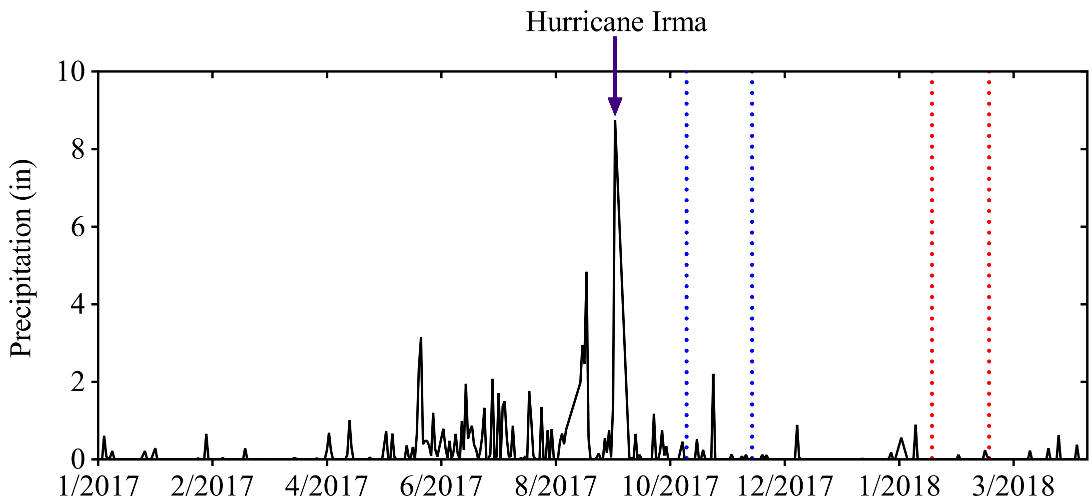

Figure 13. Daily rainfall (in) observed in North Fort Myers, FL from January 2017 through May 2018, showing Hurricane Irma (purple arrow), the wet season sampling events (blue lines) in October and November 2017, and the dry season sampling events (red lines) in February and March 2018.

3.2 Surface Water

3.2.1 Environmental Parameters

There was slight variability in environmental parameters of surface water between sites (**Table 1**). Salinity and conductivity were lowest at upstream sites and increased going downstream, while pH was similar between sites (**Table 1**). The lowest overall salinity was observed upstream at Powell Creek at SW4 (0.43 ± 0.19), while the highest salinity was observed downstream at Hancock Creek at SW6 (5.73 ± 3.29; **Table 1**). Downstream sites often had higher DO, although a clear upstream to downstream trend was not always present (**Table 1**). DO concentrations varied from 3.13 ± 0.60 mg/L at the upstream site in Hancock Creek to 7.12 ± 0.90 mg/L at the downstream Hancock Creek site (**Table 1**). A few sites were below the surface water criteria of 42% for DO, including SW9 in Hancock Creek, SW2 in Powell Creek, and SW5 in the central drainage feature. BOD and color were often lower at upstream sites compared to downstream sites. The lowest BOD concentrations were observed upstream at Powell Creek (0.80 ± 0.14 mg/L) and the highest were observed at the downstream site in Powell Creek (1.43 ± 0.34 mg/L) and the central drainage feature site (1.95 ± 0.85 mg/L; **Table 1**). Color ranged from 41 ± 6 CU at the upstream site in Hancock Creek to 109 ± 35 CU at the downstream site in Hancock Creek (**Table 1**).

Table 1. Environmental parameters (overall average ± standard error) observed by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage feature, and a reference area), and site; BDL = below detection level; SE presented to show the variability in physical conditions at each site over the study period.

Water Type	Drainage Basin	Site	Count	pH	Salinity	Temperature (°C)	% Dissolved Oxygen	Dissolved Oxygen (mg/L)	Conductivity (µS)	Biochemical Oxygen Demand (mg/L)	Color (CU)
Surface Water	Hancock Creek	SW9	4	7.38±0.14	1.23±0.93	23.23±1.41	35.55±6.03	3.13±0.60	2,280±1,659	0.87±0.20	41±6
		SW9	4	7.42±0.09	4.20±2.29	24.10±1.63	52.30±6.52	4.50±0.62	6,618±3,689	1.38±0.64	72±3
		SW7	4	7.46±0.12	4.65±2.54	24.28±1.77	57.00±5.45	4.85±0.54	8,012±4,310	1.23±0.26	78±10
		16-3 GR	4	7.42±0.13	4.85±2.71	24.23±1.68	62.20±9.68	5.29±0.86	8,307±4,570	1.18±0.18	78±12
		SW6	4	7.62±0.16	5.73±3.29	24.25±1.78	83.38±8.82	7.12±0.90	9,606±5,478	1.15±0.22	109±35
	Powell Creek	SW4	4	7.19±0.07	0.43±0.19	23.23±1.55	59.58±4.85	5.09±0.26	869±392	0.80±0.14	55±13
		SW1	4	7.20±0.10	0.63±0.39	23.15±1.56	55.33±4.39	4.78±0.34	1,224±742	0.85±0.25	57±12
		SW2	4	7.24±0.12	2.55±1.74	22.83±1.81	38.53±11.88	3.42±1.07	4,561±3,014	1.10±0.25	66±9
		SW3	4	7.34±0.16	3.70±2.29	22.98±1.75	59.83±9.03	5.22±0.81	6,393±3,872	1.43±0.34	66±9
	Central Drainage	SW5	4	7.23±0.14	4.95±2.82	22.85±1.78	40.78±11.69	3.61±1.11	8,453±4,751	1.95±0.85	107±27
Groundwater	Hancock Creek	GW1	4	6.59±0.04	0.58±0.08	24.54±1.00	21.30±8.40	1.80±0.72	1,161±145	0.400	133±13
		GW2	4	6.60±0.06	1.15±0.42	25.26±1.32	24.08±7.36	2.00±0.61	2,215±780	BDL	106±23
		GW3	4	6.49±0.01	0.94±0.28	24.96±1.26	6.00±2.49	0.51±0.22	1,384±622	0.300	92±11
	Powell Creek	GW7	4	6.76±0.03	0.45±0.04	24.09±1.15	16.60±4.15	1.40±0.35	908±72	2.93±0.57	93±60
		GW8	4	6.85±0.07	0.83±0.11	24.74±1.25	31.13±11.07	2.53±0.84	1,653±196	2.73±0.55	112±38
		GW9	4	6.71±0.05	1.22±0.05	23.69±0.97	5.43±2.30	0.47±0.20	2,367±88	2.40±0.58	138±19
	Central Drainage	GW4	4	6.76±0.04	0.65±0.01	26.06±1.26	4.25±1.11	0.35±0.10	1,300±15	BDL	210±162
		GW5	4	6.28±0.04	0.37±0.02	25.98±1.37	3.13±0.99	0.26±0.09	756±39	1.10±0.26	166±18
		GW6	4	6.62±0.03	0.54±0.03	25.81±1.12	2.63±0.96	0.22±0.08	1,090±50	1.30±0.44	159±85
	Reference	GW10	4	6.94±0.07	0.31±0.03	23.96±0.63	3.50±0.47	0.30±0.04	628±53	0.50±0.14	71±18

Seasonal differences were observed for some environmental parameters (**Appendix 2**). During the wet season, salinity and conductivity were similar between sites and were much lower overall than values observed during the dry season (**Appendix 2**). Dry season salinity and conductivity increased from upstream to downstream (**Appendix 2**). During the wet season salinity ranged from 0.20 ± < 0.01 to 0.35 ± 0.05 compared to a range of 0.65 ± 0.35 to 11.25 ± 1.95 during the dry season (**Appendix 2**). DO was higher during the dry season, ranging from 45.95 ± 0.45 to 93.15 ± 4.05 %, relative to the wet season, which spanned from 22.70 ± 18.10 to 73.60 ± 16.10 % (**Appendix 2**). During both seasons upstream sites often had lower DO concentrations that increased downstream, with the exception of Powell Creek during the wet season (**Appendix 2**). BOD generally increased from upstream to downstream in both Hancock Creek and Powell Creek during the wet season, with concentrations ranging from 0.50 to 1.50 ± < 0.01 mg/L, while dry season concentrations were slightly higher, ranging from 0.80 ± 0.20 to 2.70 ± 1.70 mg/L (**Appendix 2**). Color varied from 50 ± 7 to 169 ± 169 CU during the wet season and from

33 ± 1 to 66 ± 1 CU during the dry season, with higher levels typically observed at downstream sites during both seasons (**Appendix 2**). For monthly BOD and color data from the additional sampling events see **Appendix 3** and **Appendix 4**.

3.2.2 Dissolved Nutrient Concentrations

In Hancock Creek, overall NH_4 concentrations were slightly higher upstream than downstream, ranging from 0.03 ± < 0.01 mg/L at SW6 to 0.05 ± 0.01 mg/L at SW7 (**Appendix 5**). A clear upstream to downstream trend was observed in overall SRP concentrations, with the highest concentration observed upstream at SW9 (0.18 ± 0.01 mg/L) and the lowest at SW6 (0.06 ± 0.01 mg/L; **Appendix 5**). Conversely, NO_3 concentrations were relatively low at most sites within Hancock Creek (< 0.07 mg/L), with the exception of SW6, which had an overall average concentration of 0.23 ± 0.01 mg/L (**Appendix 5**). TN increased from upstream to downstream, ranging from 0.41 ± 0.0.2 mg/L at SW9 to 0.92 ± 0.05 mg/L at SW6, while TP decreased from upstream to downstream, ranging from 0.10 ± 0.01 mg/L at SW6 to 0.21 ± 0.01 mg/L at SW9 (**Appendix 5**). All sites in Hancock Creek exceeded the FDEP standard in the Lower Caloosahatchee for TP (≥ 0.04 mg/L) and all sites, except SW9, exceeded the FDEP standard in the Lower Caloosahatchee for TN (≥ 0.5 mg/L). Additionally, every site in Hancock Creek, except SW6, exceeded the FDEP TP standard for the peninsular stream region (≥ 0.12 mg/L). DIN:SRP and TN:TP ratios were generally low, indicating slight N-limitation, with the highest ratios at SW6 (1.71 ± 0.46 and 10.47 ± 0.92, respectively; **Appendix 5**).

Seasonal differences were also observed at Hancock Creek. NH_4 was generally lower during the wet season compared to the dry season, while NO_3 concentrations were higher during the wet season (**Fig. 14a,b**). Wet season SRP concentrations were relatively consistent throughout Hancock Creek, with a slight decline from upstream to downstream, while dry season concentrations exhibited a distinct upstream to downstream trend (**Fig. 14c**). TN was more variable during the wet season and increased from upstream to downstream, while dry season concentrations were relatively similar between sites, except for SW9, which was much lower (**Fig. 15a**). During both seasons, SW9 was the only site with concentrations below the standard for TN in the Lower Caloosahatchee. All sites were below the standard for TN in the peninsular stream region. In contrast, TP was relatively similar between sites during the wet season, with slightly lower values observed downstream at SW6. During the dry season concentrations were generally lower and exhibited a decline from upstream to downstream (**Fig. 15b**). TP concentrations always exceeded the standard for both the Lower Caloosahatchee and the peninsular stream region for each site, regardless of season. During the wet season, DIN:SRP and TN:TP ratios were higher at SW6 relative to the other sites in Hancock Creek (**Fig. 15c,d**). The highest TN:TP was also observed at SW6 during the dry season, while DIN:SRP was relatively similar between sites during the dry season (**Fig. 15c,d**). TN:TP increased from upstream to downstream during both seasons and values did not vary greatly between seasons. These values consistently indicated N-limitation. DIN:SRP was lower during the dry season compared to the wet season, and no clear spatial pattern was evident in either season (**Fig. 15c,d**). For monthly dissolved nutrient data from the additional sampling events, see **Appendix 3**.

In Powell Creek, overall NH_4 concentrations were generally similar between sites, ranging from 0.05 ± 0.01 mg/L at SW1 to 0.07 ± < 0.01 mg/L at SW2 (**Appendix 5**). NO_3 concentrations also had low overall variability between sites and varied from 0.08 ± 0.01 mg/L at SW1 to 0.09 ± < 0.01 mg/L at SW4 (**Appendix 5**). Overall, SRP concentrations were generally higher upstream in Powell Creek, ranging from 0.08 ± 0.01 mg/L at SW3 to 0.14 ± 0.02 mg/L at SW1 (**Appendix 5**). TN concentrations were slightly lower at upstream sites relative to downstream sites, while TP decreased from upstream to downstream. TN fluctuated from 0.64 ± 0.07 mg/L at SW4 to 0.73 ± 0.06 mg/L at SW3, and TP spanned from 0.13 ± < 0.01 mg/L at SW3 to 0.20 ± 0.02 mg/L at SW4 (**Appendix 5**). Overall concentrations for TN and TP exceeded FDEP standards for the Lower Caloosahatchee at every site in Powell Creek, and TP concentrations exceeded FDEP standards for the peninsular stream region as well. DIN:SRP and TN:TP ratios were generally low, indicating N-limitation, and were relatively similar between sites. DIN:SRP ranged from 1.19 ± 0.27 at SW1 to 1.47 ± 0.36 at SW2, and TN:TP varied from 4.18 ± 0.80 at SW4 to 5.77 ± 0.55 at SW3 (**Appendix 5**).

Seasonal effects were observed at Powell Creek. NH_4 concentrations were higher during the wet season relative to the dry season. During both seasons concentrations were relatively similar between sites with the highest concentrations consistently observed at SW2 (**Fig. 14a**). NO_3 concentrations were also higher during the wet season and were relatively similar between sites, while during the dry season concentrations declined from upstream to downstream (**Fig. 14b**). In contrast, SRP concentrations were much lower during the wet season compared to the dry season (**Fig. 14c**). Wet season SRP concentrations had little variation between sites, while dry season concentrations declined from upstream to downstream (**Fig. 14c**). TN concentrations were higher during the wet season relative to the dry season. Wet season TN concentrations were generally similar between sites, while dry season concentrations were higher downstream (**Fig. 15a**). Wet season TN concentrations were always elevated above the standard, while during the dry season only the two most downstream sites had concentrations higher than the standard for the Lower Caloosahatchee. TP concentrations were higher during the dry season and decreased from upstream to downstream. Wet season concentrations were lower and did not vary much between sites (**Fig. 15b**). During both seasons TP concentrations exceeded the standard for the Lower Caloosahatchee and the peninsular stream region at every site. DIN:SRP and TN:TP were higher during the wet season compared to the dry season (**Fig. 15c,d**). During the wet season, DIN:SRP was slightly higher downstream, while during the dry season DIN:SRP declined slightly from upstream to downstream (**Fig. 15c**). TN:TP increased from upstream to downstream during the dry season, while TN:TP during the wet season were relatively consistent between sites and indicated N-limitation (**Fig. 15d**). For monthly dissolved nutrient data from the additional sampling events, see **Appendix 4**.

In the central drainage feature, high overall nutrient concentrations were often observed. NH_4 concentrations averaged 0.14 ± 0.03 mg/L, which was the highest of all the surface water sites (**Appendix 5**). NO_3 concentrations were often higher than other surface water sites at 0.09 ± 0.02 mg/L, while SRP concentrations were 0.10 ± 0.01 mg/L (**Appendix 5**). The highest TN (1.09 ± 0.07 mg/L) concentrations were also observed at the central drainage site and were well above the standard for the Lower Caloosahatchee. TP concentrations were moderately high (0.18 ± 0.02 mg/L) relative to other sites and exceeded the FDEP standard for both the Lower Caloosahatchee and the peninsular stream region (**Appendix 5**). DIN:SRP (1.82 ± 0.39) and TN:TP (6.53 ± 0.67) were low at the central drainage site, indicating N-limitation (**Appendix 5**).

Dissolved nutrient concentrations at the central drainage site were also seasonally influenced. NH_4, NO_3, and SRP concentrations were all higher during the wet season compared to the dry season (**Fig. 14**). Furthermore, NH_4, NO_3, and SRP concentrations during the wet season were among the highest observed relative to the other surface water sites, while during the dry season concentrations were among the lowest observed (**Fig. 14**). Similarly, TN and TP concentrations were higher during the wet season and were the highest observed concentrations among the surface water sites (**Fig. 15a,b**). TN concentrations were among the highest compared to the other surface water sites, while TP concentrations were among the lowest (**Fig. 15a,b**). TN and TP concentrations consistently exceeded FDEP standards for the Lower Caloosahatchee during the wet and dry seasons, and TP concentrations consistently exceeded FDEP standards for the peninsular stream region during both seasons. DIN:SRP ratios at the central drainage site were low but were among the highest observed during both seasons relative to the other surface water sites. DIN:SRP was higher during the wet season compared to the dry season (**Fig. 15c**). Conversely, TN:TP ratios were higher during the dry season relative to the wet season at the central drainage site, but still indicated N-limitation (**Fig. 15d**). For monthly dissolved nutrient data from the additional sampling events see **Appendix 4**. For seasonal dissolved nutrient concentrations averages, see **Appendix 6**.

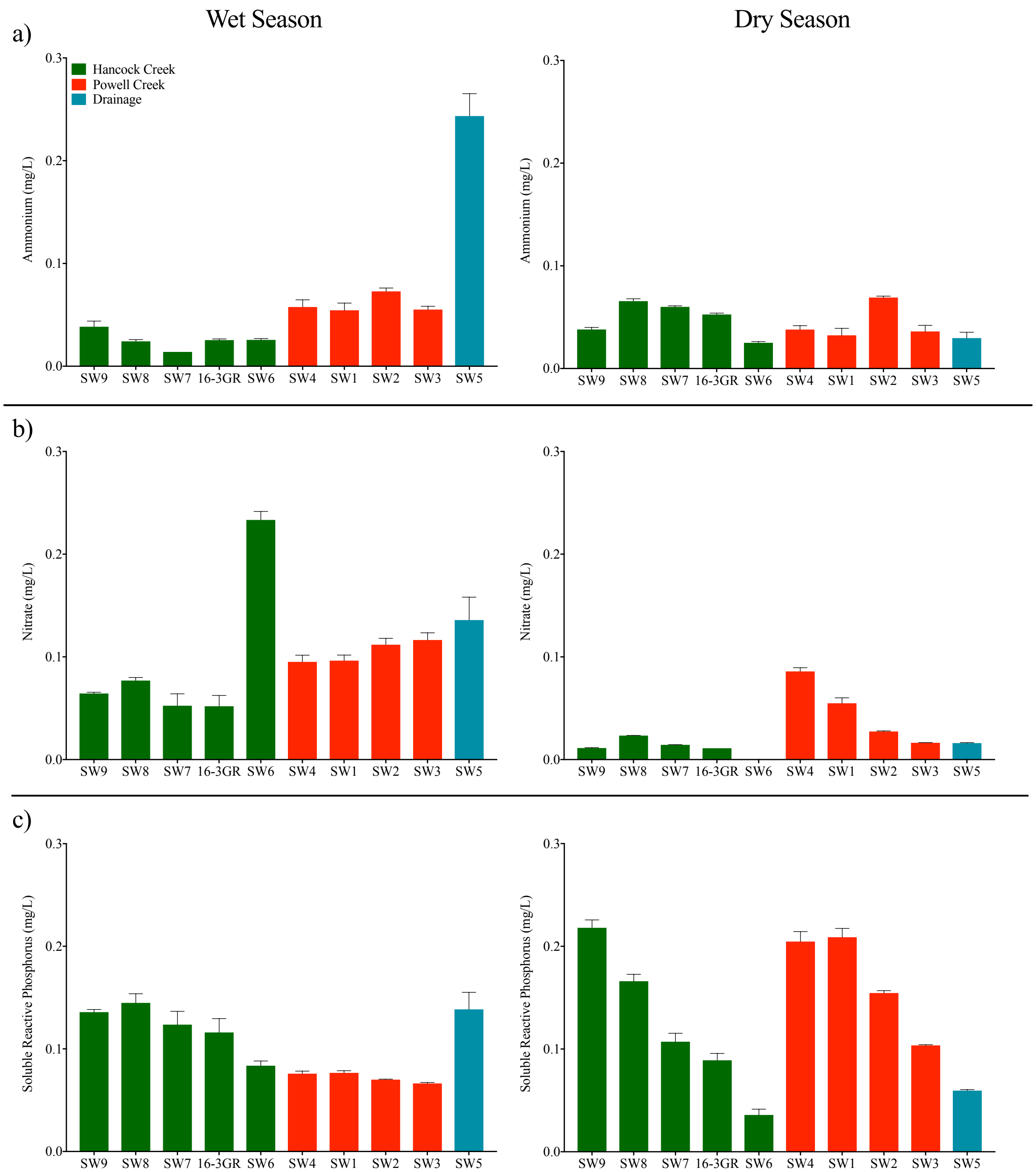

Figure 14. Surface water dissolved reactive nutrient concentrations (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, and a central drainage feature), and season (wet 2017 and dry 2018), including a) ammonium (NH_4), b) nitrate + nitrite (NO_3), and c) soluble reactive phosphorus (SRP).

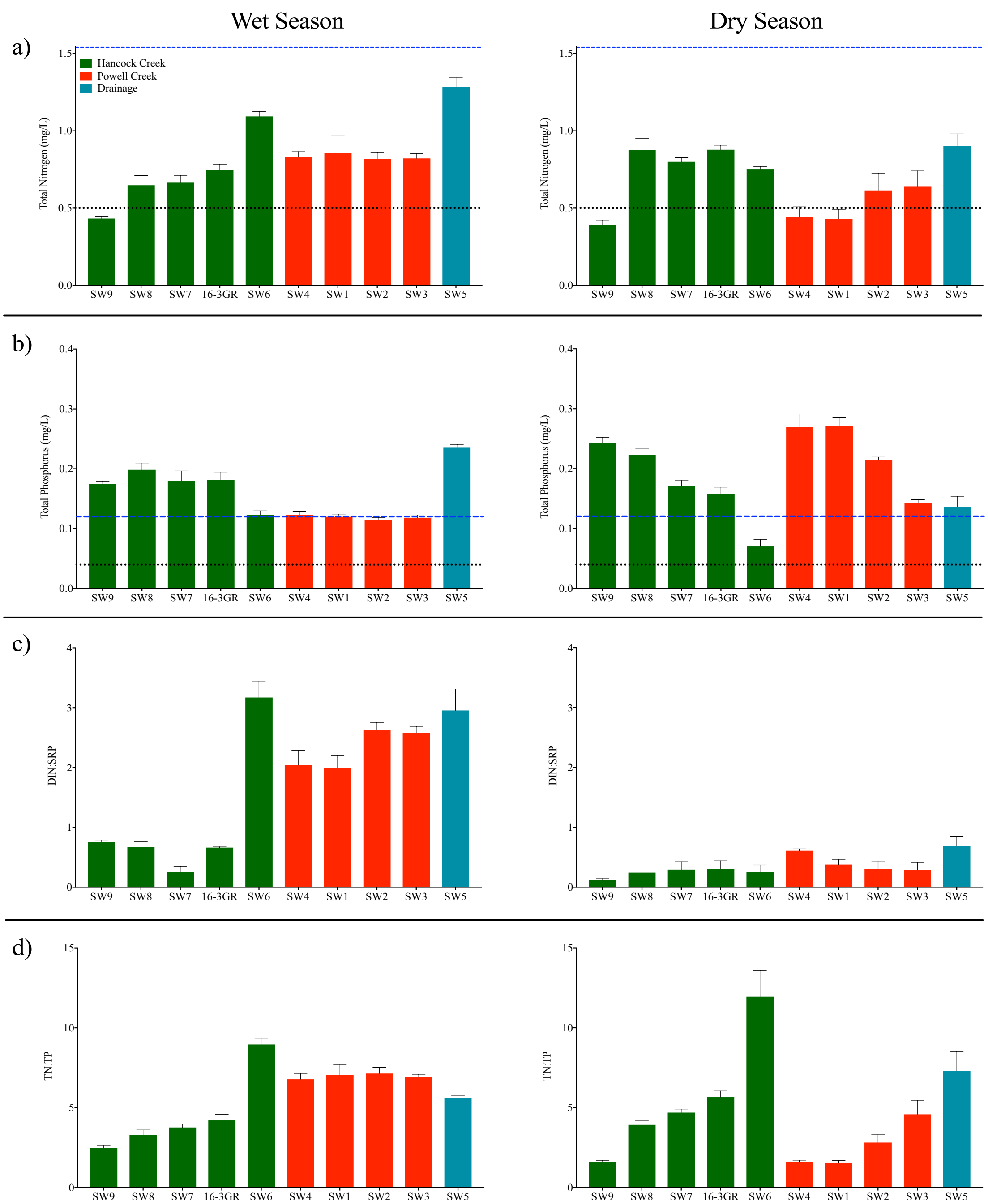

Figure 15. Surface water dissolved total nutrient concentrations and ratios (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, and a central drainage feature), and season (wet 2017 and dry 2018), including, a) total nitrogen, with a black 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), b) total phosphorus, with a black 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), c) dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) and d) total nitrogen to total phosphorus (TN:TP).

3.2.3 Bacterial Prevalence

In Hancock Creek, overall enterococci concentrations were highly variable, ranging from 119 ± 70 MPN/100 mL at SW8 to 1,269 ± 423 MPN/100 mL at 16-3GR (**Appendix 7**). SW8 was the only site below the TPTV threshold of 130 MPN/100 mL. Average overall *E. coli* concentrations spanned from 137 ± 49 MPN/100 mL at SW6 to 693 ± 85 MPN/100 mL at 16-3GR (**Appendix 7**), the latter of which was the only site to exceed the TPTV threshold of 410 MPN/100 mL. Dry season enterococci concentrations were higher than wet season concentrations, ranging from 194 ± 135 MPN/100 mL at SW8 to 1,860 ± 560 MPN/100 mL at 16-3GR during the dry season and from 44 ± 12 MPN/100 mL at SW8 to 678 ± 243 MPN/100 mL at 16-3GR during the wet season (**Fig. 16a**). All dry season enterococci concentrations exceeded the TPTV threshold. However, during the wet season only SW9, SW7, and 16-3GR had concentrations above the threshold. *E. coli* concentrations were also generally higher during the dry season, spanning from 180 ± 96 MPN/100 mL at SW6 to 823 ± 99 MPN/100 mL at 16-3GR. Wet season concentrations varied from 93 ± 37 MPN/100 mL at SW6 to 564 ± 116 MPN/100 mL at 16-3GR (**Fig. 16b**). During the dry season, SW8, SW7, and 16-3GR had *E. coli* concentrations above the TPTV threshold, while during the wet season 16-3GR was the only site to exceed the threshold. For monthly bacterial concentration data from the additional sampling events see **Appendix 8**.

Overall bacteria concentrations in Powell Creek were relatively high compared to other surface water drainage basins. Enterococci concentrations always exceeded the TPTV threshold and fluctuated from 344 ± 76 MPN/100 mL at SW3 to 1,273 ± 662 MPN/100 mL at SW1 (**Appendix 7**). *E. coli* concentrations were also above the TPTV threshold at every site and spanned from 549 ± 198 MPN/100 mL at SW3 to 1,312 ± 640 MPN/100 mL at SW1 (**Appendix 7**). Levels of enterococci and *E. coli* were highest at upstream sites and declined at downstream sites (**Appendix 7**). Bacterial concentrations at Powell Creek varied seasonally. Both enterococci and *E. coli* concentrations were higher during the dry season relative to the wet season (**Fig. 16**). Furthermore, wet season bacterial concentrations generally increased from upstream to downstream, while dry season bacterial concentrations decreased from upstream to downstream (**Fig. 16**). The highest surface water enterococci concentrations were observed during the dry season at SW4 and SW1 (2,420 ± < 1 MPN/100 mL; **Fig. 16a**). During the wet season, SW2 and SW3 bacteria concentrations were above the TPTV threshold for both enterococci and *E. coli*, while during the dry season every site was over the enterococci threshold and every site except SW3 exceeded the *E. coli* threshold. For monthly bacterial concentration data from the additional sampling events see **Appendix 9**.

The central drainage site had some of the highest average bacterial concentrations observed in this study. Enterococci concentrations averaged 1,199 ± 265 MPN/100 mL, well above the TPTV threshold. *E. coli* concentrations were the highest observed in the study and exceeded the TPTV threshold at 1,724 ± 1,060 MPN/100 mL (**Appendix 7**). During the wet season, enterococci concentrations were higher than the dry season concentrations at the central drainage site (1,454 ± 533 MPN/100 mL and 945 ± 91 MPN/100 mL, respectively) but always exceeded the TPTV threshold regardless of season (**Fig. 16a**). Conversely, *E. coli* concentrations were lower during the wet season (395 ± 123 MPN/100 mL) and below the TPTV threshold. However, dry season concentrations (3,053 ± 1,787 MPN/100 mL) were well above the threshold (**Fig. 16b**). Dry season *E. coli* concentrations were the highest among the surface water sites (**Fig. 16b**). For monthly bacterial concentration data from the additional sampling events see **Appendix 9**. For seasonal averages of bacterial concentrations see **Appendix 10**.

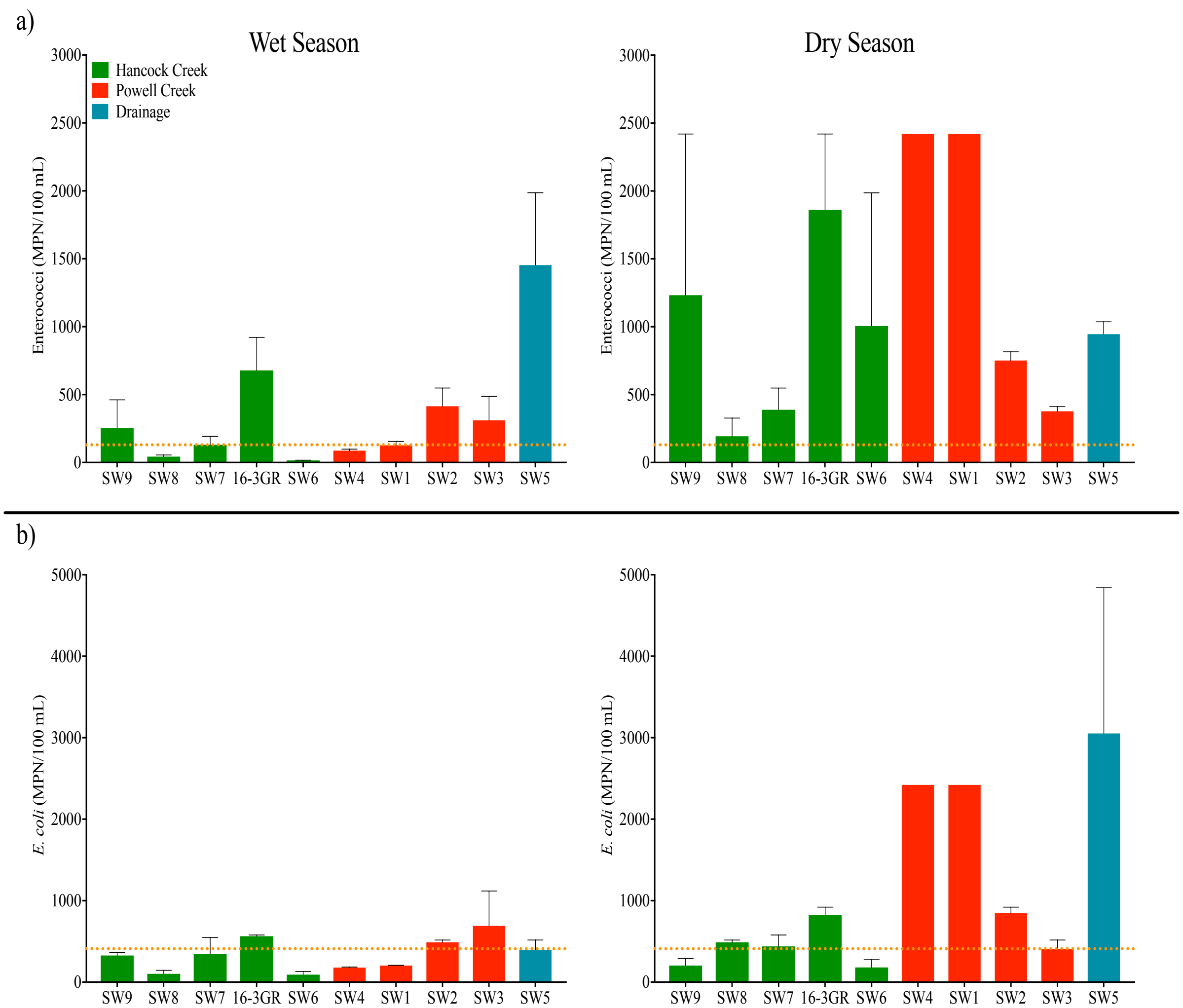

Figure 16. Surface water bacterial concentrations (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, and a central drainage feature), and season (wet 2017 and dry 2018) of a) enterococci and b) *Escherichia coli* (*E. coli)* concentrations; dotted lines indicate FDEP Ten Percent Threshold Value (TPTV) criteria for enterococci (marine water ≥ 130 MPN/100 mL) and *E. coli* (fresh water ≥ 410 MPN/100 mL); marine standards do not apply to the study area and are shown for reference only.

3.2.4 Microbial Source Tracking: Molecular Markers

In Hancock Creek, BacR detections ranged from 130 TSC/100 mL at SW9 during the dry season to 710 TSC/100 mL at SW6 during the wet season. The BacR marker was detected once at each site in the Hancock Creek watershed, with the exception of SW8 (**Appendix 7**). Detections of the GFD marker fluctuated from 157 ± 70 TSC/100 mL at SW9 to 7,025 ± 444 TSC/100 mL at 16-3GR, which was the highest observed overall concentration (**Appendix 7**). Overall Gull2 detections were also highest at Hancock Creek, ranging from 620 TSC/100 mL at SW8 to 31,400 ± 25,227 TSC/100 mL at SW6 (**Appendix 7**). Much higher levels of the Gull2 marker were observed downstream in Hancock Creek relative to upstream sites (**Appendix 7**). Detections of the human marker HF183 were relatively low compared to the other drainage basins, spanning from below detection level (BDL) at SW6 to 113 ± 73 GEU/100 mL at 16-3GR, with no apparent spatial trend (**Appendix 7**).

Seasonal differences were observed in Hancock Creek for some of the molecular markers. Detections of BacR were observed at the downstream sites during the wet season, while only the upstream site had detectable levels of BacR during the dry season (**Appendix 10**). GFD detections were usually higher during the wet season, with the highest detections occurring at 16-3GR during the wet season (**Fig. 17a**). The Gull2 marker was detected at all Hancock Creek sites during the dry season, with much higher detection levels at the two most downstream sites, 16-3GR and SW6 (**Fig. 17b**). During the wet season, Gull2 detections were often lower than those observed during the dry season, and the two most upstream sites had no detectable levels present (**Fig. 17b**). HF183 detections during the wet season were typically slightly lower than dry season detections (**Fig. 17c**).

In Powell Creek, the BacR marker was not detected at any site during the study period (**Appendix 7**). Overall GFD detections were generally lower than Hancock Creek sites, ranging from 89 ± 48 TSC/100 mL at SW2 to 453 ± 358 TSC/100 mL at SW1 (**Appendix 7**). The Gull2 marker was only detected once at each site, except SW4, which did not have any detections (**Appendix 7**). Gull2 detections spanned from 240 TSC/100 mL at SW1 to 1,000 TSC/100 mL at SW3 (**Appendix 7**). Overall HF183 detections were relatively higher than Hancock Creek detections and ranged from 117 ± 59 GEU/100 mL at SW1 to 493 ± 443 GEU/100 mL at SW3 (**Appendix 7**).

Seasonal differences were apparent at Powell Creek. Dry season detections of the GFD marker were much higher than wet season detections. During both seasons detections were generally lower at downstream sites (**Fig. 17a**). The Gull2 marker was detected sporadically during the study, with only one detection in the wet season at SW1, and higher detections during the dry season at the two most downstream sites, SW2 and SW3 (**Fig. 17b**). During the wet season, the most downstream site had much higher detection levels of HF183 compared to the sites upstream. However, during the dry season the highest detection occurred at the most upstream site and the lowest detection occurred at the most downstream site (**Fig. 17c**). Dry season HF183 detections were generally higher than those observed during the wet season (**Fig. 17c**).

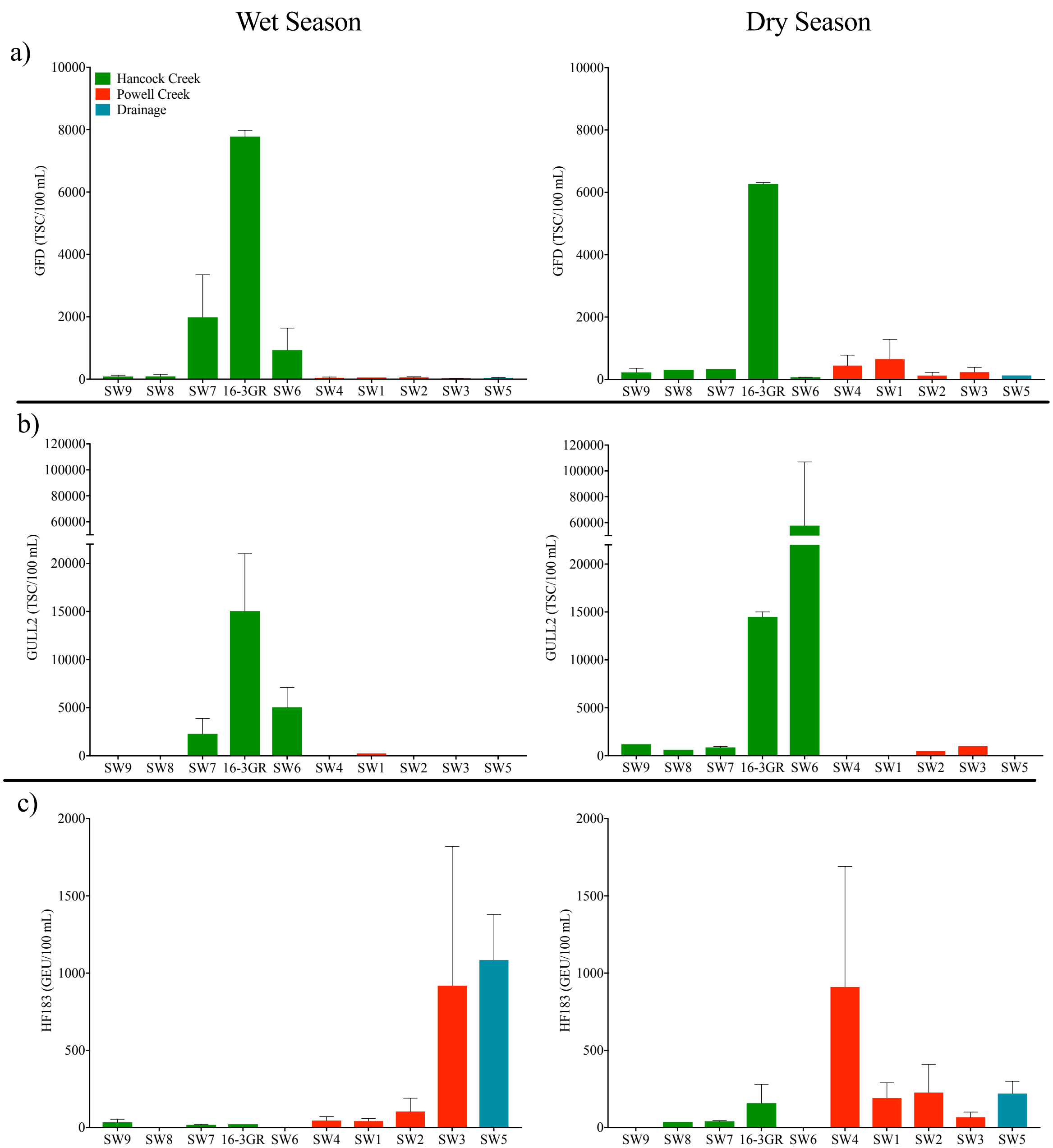

Figure 17. Surface water molecular markers (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, and a central drainage feature), and season (wet 2017 and dry 2018), including the avian markers a) GFD and b) Gull2, as well as the human marker c) HF183.

At the central drainage site, the BacR marker was only detected once during the wet season (330 TSC/100 mL), and the Gull2 marker was not detected during the study period

(**Appendix 7**). Overall GFD detections at the drainage site were the lowest among the surface water sites (67 ± 29 TSC/100 mL; **Appendix 7**). However, overall HF183 levels were highest at the drainage site (653 ± 279 GEU/100 mL; **Appendix 7**).

Wet season GFD detections at the central drainage site were lower than those observed during the dry season, and during both seasons were some of the lowest levels observed relative to the other sites (**Fig. 17a**). Conversely, HF183 detections during the wet season were the highest observed among the study sites and were much higher than detections at this site during the dry season (**Fig. 17c**). For seasonal averages of molecular markers see **Appendix 10**.

3.2.5 Microbial Source Tracking: Chemical Tracers

The chemical tracers mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any surface water sites during the study. In Hancock Creek, overall sucralose concentrations were generally lower than those observed in Powell Creek, but were always "moderate," ranging from 0.215 ± 0.017 μg/L at SW9 to 0.790 ± 0.183 μg/L at SW8 (**Appendix 11**). Acetaminophen was not detected at any sites in Hancock Creek during the study period, while carbamazepine was detected in relatively low concentrations at every site, fluctuating from 0.001 ± < 0.001 μg/L at SW9 and SW6 to 0.004 ± < 0.001 μg/L at SW8 (**Appendix 11**). Primidone was detected at most sites within Hancock Creek with the exception of SW9, ranging in overall concentration from 0.004 μg/L at SW6 to 0.008 ± 0.001 μg/L at SW8 (**Appendix 11**). Similarly, 2,4-D was detected at every Hancock Creek site except SW9, with overall concentrations spanning from 0.007 ± 0.003 μg/L at SW8 to 0.030 ± 0.012 μg/L at SW6 (**Appendix 11**). Overall, concentrations of bentazon were detected at every site in Hancock Creek and varied from 0.002 ± < 0.001 μg/L at SW9 to 0.010 ± 0.003 μg/L at SW7 and 16-3GR (**Appendix 11**).

Seasonal differences were observed at Hancock Creek. Sucralose concentrations were consistently "moderate", but were generally higher during the dry season compared to the wet season (**Fig. 18a**). During both seasons, sucralose concentrations were relatively low at SW9, while concentrations at the remaining sites declined from upstream to downstream during the wet season and remained fairly constant during the dry season (**Fig. 18a**). Carbamazepine concentrations were similar between the wet and dry seasons, with a slight decline in concentration upstream to downstream from SW8 to SW6, while the Yellow Fever Creek site, SW9, had the lowest concentrations during both seasons (**Fig. 18b**). Primidone was only detected once during the wet season at SW8 and was detected at all sites, except SW9, during the dry season (**Fig. 18c**). During the dry season, primidone concentrations declined from upstream to downstream (**Fig. 18c**). 2,4-D and bentazon concentrations were higher during the wet season relative to the dry season and were generally higher than concentrations in the other drainage basins during both seasons (**Appendix 12**). Surface water samples were analyzed for diuron, fluridone, imazapyr, and imidacloprid once during the second dry season sampling event. Diuron concentrations were similar within Hancock Creek, ranging from 0.002 to 0.004 µg/L, with no detection at SW9 (**Appendix 12**). Fluridone concentrations were relatively low at SW9 and declined from upstream to downstream at the remaining sites, ranging from 0.003 µg/L at SW6 to 0.095 µg/L at SW8 (**Appendix 12**). Imazapyr concentrations were higher at all upstream sites, including SW9, and fluctuated from 0.016 µg/L at 16-3GR to 0.036 µg/L at SW8 (**Appendix 12**). Imidacloprid was not detected at SW9 and ranged from 0.004 to 0.009 µg/L within the rest of Hancock Creek (**Appendix 12**). See **Appendix 12** for seasonal averages for all chemical markers in Hancock Creek.

In Powell Creek, the highest overall sucralose concentrations observed in the study were found at the upstream sites. Concentrations varied from 0.673 ± 0.130 µg/L at SW3 to 0.968 ± 0.283 µg/L at SW1, which are all within "moderate" levels (**Appendix 11**). Acetaminophen was detected once during the wet and dry seasons at SW3 and once during the dry season at SW2, while the remaining sites did not have detectable levels (**Appendix 11**). Overall carbamazepine concentrations were highest at upstream sites, ranging from 0.009 ± 0.003 µg/L at SW3 to 0.015 ± 0.001 µg/L at SW4 (**Appendix 11**). Similarly, primidone concentrations were highest at upstream sites and ranged from 0.005 ± 0.001 µg/L at SW3 to 0.010 ± 0.003 µg/L at SW1 (**Appendix 11**). 2,4-D was only detected at the two most downstream sites during the wet season, while bentazon was present in relatively low overall concentrations, spanning from 0.002 ± < 0.001 to 0.005 ± 0.001 µg/L (**Appendix 11**).

Seasonal variability was seen in Powell Creek chemical tracer results. During the wet season, sucralose concentrations were similar between sites and lower than those observed during the dry season, which declined from upstream to downstream sites (**Fig. 18a**). Wet season sucralose concentrations were all "moderate." During the dry season most sites had "significant" levels of sucralose and only the downstream site was "moderate". Acetaminophen was only detected at the two most downstream sites and was present in lower levels during the wet season compared to the dry season (**Appendix 12**). Carbamazepine concentrations were relatively constant during the wet season and higher than dry season concentrations, which declined from upstream to downstream (**Fig. 18b**). Primidone was not detected at any sites during the wet season and dry season concentrations generally declined from upstream to downstream (**Fig. 18c**). 2,4-D was also not detected during the wet season and was only detected at the two most downstream sites during the dry season (**Appendix 12**). Bentazon detections were similar between the wet and dry seasons and concentrations were relatively constant within Powell Creek (**Appendix 12**). Diuron was not detected during the single sampling event it was measured, while fluridone and imazapyr were present and ranged from 0.003 to 0.006 µg/L and 0.023 to 0.038 µg/L, respectively (**Appendix 12**). Imidacloprid concentrations spanned from

0.003 to 0.007 µg/L (**Appendix 12**). See **Appendix 12** for seasonal averages for all chemical markers in Powell Creek.

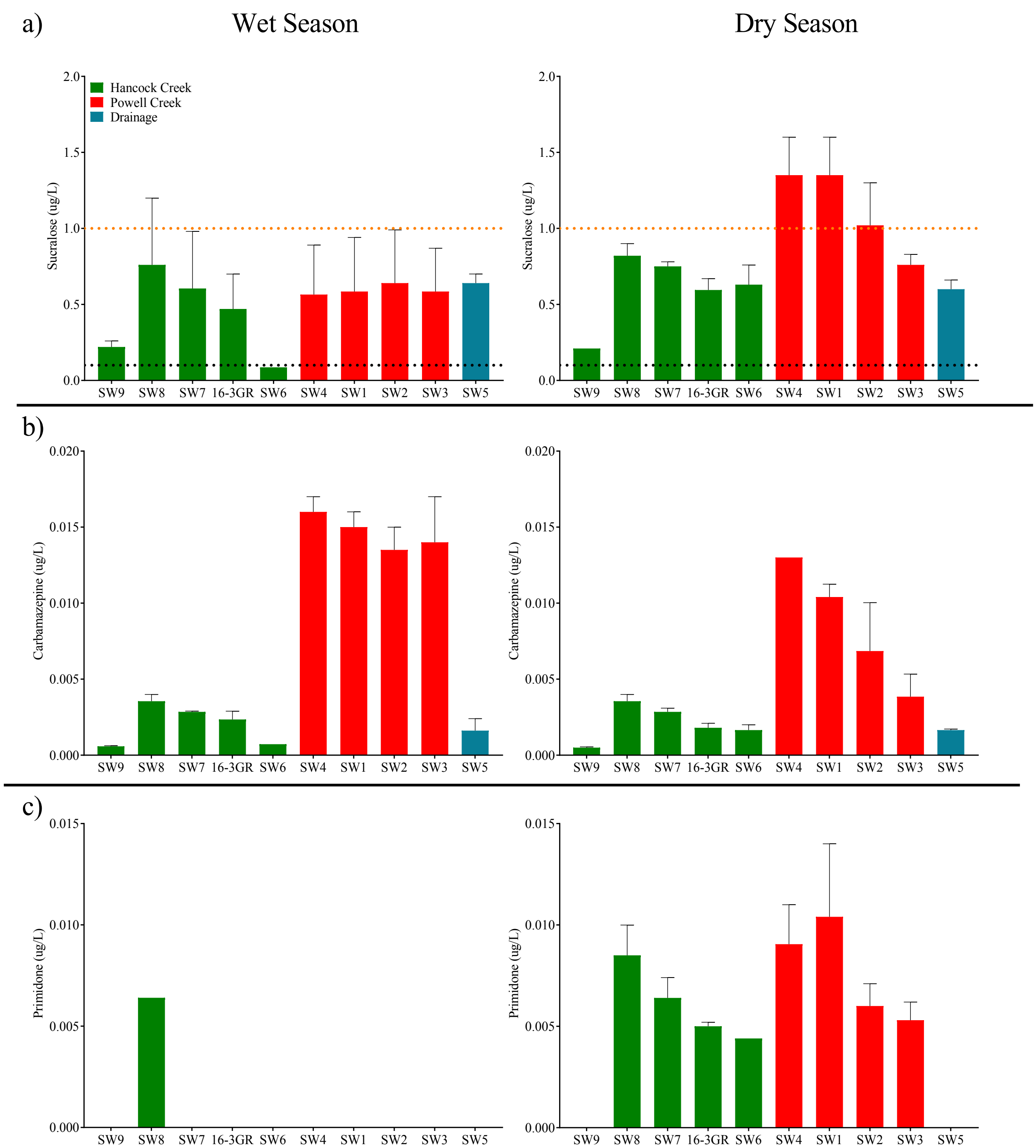

Figure 18. Surface water human chemical tracers concentrations (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, and a central drainage feature), and season (wet 2017 and dry 2018), including a) the artificial sweetener sucralose, with a black dotted line indicating "moderate" and an orange dotted line indicating "significant" concentrations, as well as the anticonvulsant pharmaceuticals b) carbamazepine and c) primidone.

Within the central drainage feature overall sucralose concentrations were "moderate" at 0.620 ± 0.037 µg/L, while acetaminophen averaged 0.012 ± < 0.001 µg/L (**Appendix 11**). Overall carbamazepine concentrations were 0.002 ± < 0.001 µg/L within the central drainage feature, while primidone was not detected during the study period (**Appendix 11**). 2,4-D and bentazon overall concentrations were 0.019 ± 0.013 µg/L and 0.009 ± 0.003 µg/L, respectively, and were relatively high compared to other sites (**Appendix 11**).

Some seasonal differences were observed for chemical tracers in the central drainage feature. However, sucralose and carbamazepine concentrations did not vary much between the wet and dry season (**Fig. 18a,b**). During both seasons sucralose concentrations were within "moderate" criteria. Acetaminophen was also detected during the wet season. Both 2,4-D and bentazon were present in higher concentrations during the wet season relative to the dry season (**Appendix 12**). See **Appendix 12** for seasonal averages for all chemical markers in the central drainage feature.

3.2.6 Stable Isotope and Elemental Composition Analyses

In Hancock Creek stable carbon isotope signatures were enriched, with a mean of -30.80 ± 0.40 ‰, reflecting terrestrial influence (**Appendix 13**). The overall mean stable nitrogen isotope value for Hancock Creek was +5.50 ± 0.35 ‰ (**Appendix 13**), which is within the range documented for wastewater. The mean C:N ratio was 7.69 ± 0.33, indicating weak N-limitation (**Appendix 13**). Mean C:P was 20.65 ± 1.07, which suggests high P-availability (**Appendix 13**). The N:P ratio averaged 3.11 ± 0.24, also supporting that primary production in Hancock Creek is N-limited (**Appendix 13**).

Slight seasonal variation was observed in Hancock Creek for both carbon and nitrogen isotope values. $\delta^{13}C$ ranged from -37.24 ± 0.21 to -26.26 ± 0.13 ‰ (**Fig. 19a**). In both seasons, $\delta^{13}C$ was most enriched at the downstream site (SW6). The most upstream site, SW9, was the most variable ranging from -37.24 ± 0.21 ‰ in the dry season to -30.44 ± 0.18 ‰ in the wet season (**Fig. 19a**). $\delta^{15}N$ was more variable between seasons, with the highest averages in the wet season, however SW9 was similar for both seasons (**Fig. 19b**). In the wet season, every site, except SW9, were within the range expected for wastewater (+3 ‰) and increased heading downstream, with the heaviest value at SW6 (Northshore Park). In the dry season, the pattern was similar to the wet season, but the values were slightly lighter and had less within site variability (i.e. the SE was smaller; **Fig. 19b**).

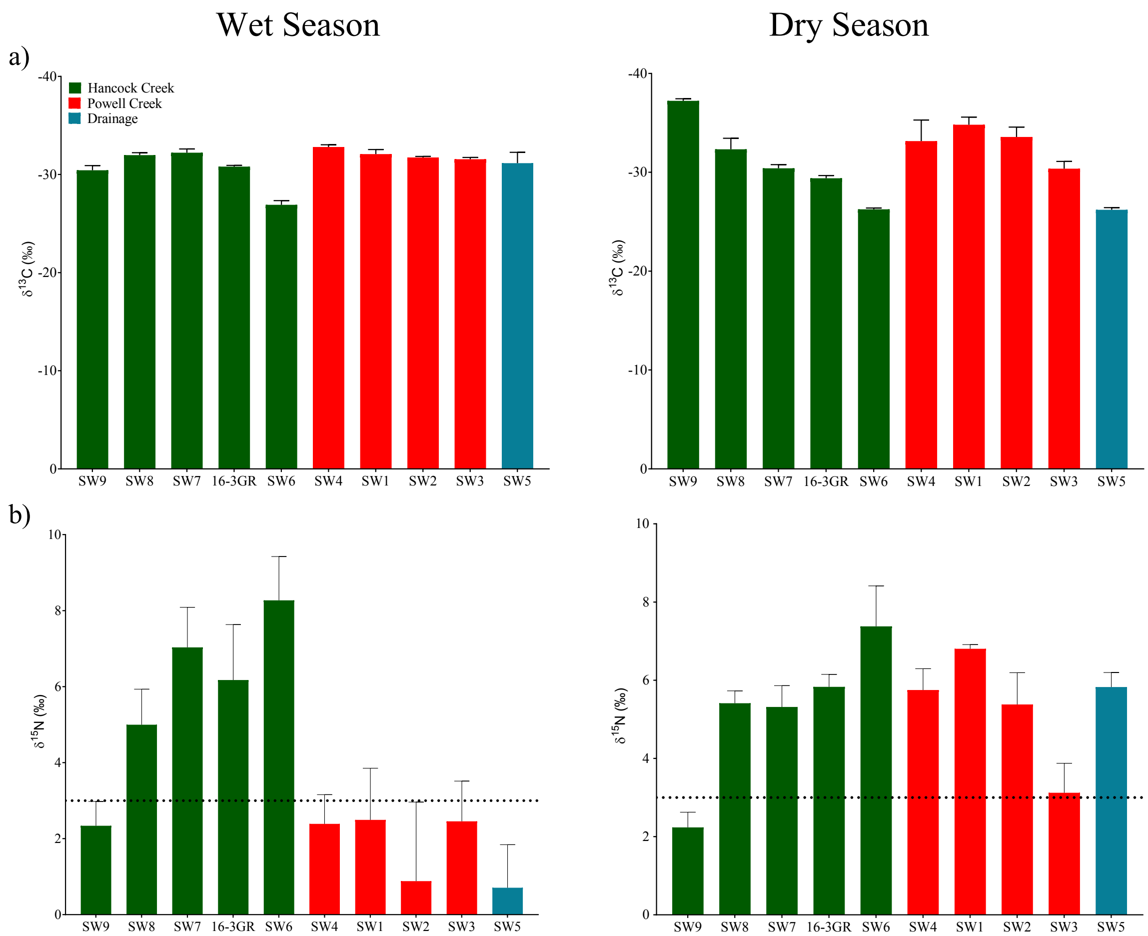

Figure 19. Stable isotope values (mean ± standard error) of particulate organic matter, a proxy for phytoplankton, collected from surface water sites in North Fort Myers by season (wet 2017 and dry 2018), drainage basin (Hancock Creek, Powell Creek, and a central drainage feature), showing values for a) carbon ($\delta^{13}C$) and b) nitrogen ($\delta^{15}N$) with a dotted line to represent the average value for unprocessed wastewater (+ 3‰), such as septic tank effluent.

Hancock Creek sites also exhibited seasonal effects in elemental composition of POM samples. C:N ratios were consistently higher during the wet season and above 6.6 at every site, indicating N-limitation, while during the dry season C:N ratios were below 6.6 at every site (**Fig. 20a**). With the exception of SW6, C:P ratios were higher during the dry season, although during both seasons ratios were well below 106, suggesting P-enrichment (**Fig. 20b**). N:P ratios were always below 16, further supporting evidence of P-enrichment, and ratios were higher at all sites during the dry season (**Fig. 20c**).

Powell Creek had the most enriched stable carbon isotope values of all the drainage basins in the study, with an overall mean of -32.52 ± 0.36 ‰, reflecting a high terrestrial influence (**Appendix 13**). Mean stable nitrogen isotope values in Powell Creek (+3.66 ± 0.45 ‰)

were lower than Hancock Creek, but higher than the central drainage site, and within the range for wastewater (**Appendix 13**). The mean C:N ratio was 7.49 ± 0.35, suggesting weak N-limitation (**Appendix 13**). C:P was 18.19 ± 1.04 and the N:P ratio was 2.59 ± 0.13 (**Appendix 13**), both indicating phosphorus enrichment. The low N:P ratio also suggest that primary production in Powell Creek is N-limited, similar to Hancock Creek.

Seasonal variability was observed in carbon and nitrogen isotopes at Powell Creek. In the wet season, $\delta^{13}C$ was relatively equal between sites, however in the dry season there was a trend of becoming more enriched from upstream to downstream (**Fig. 19a**). Mean $\delta^{15}N$ in the wet season was below the value observed in wastewater, however the standard error was within range at each site (**Fig. 19b**). This may reflect contributions of runoff of fertilizers to the nutrient budget. In the dry season mean $\delta^{15}N$ at all sites were within the range expected for wastewater (**Fig. 19b**).

Powell Creek also exhibited seasonal differences in the elemental composition of phytoplankton. The mean C:N ratio was > 6.6 for all sites in the wet season, indicating N-limitation (**Fig. 20a**). However, in the dry season, the means for all sites were below 6.6, suggesting more N-availability (**Fig. 20a**). Both seasons had mean C:P and N:P ratios that suggest P-enrichment (**Fig. 20b,c**). Contrary to the pattern observed in Hancock Creek during the wet season, the mean C:P ratio in Powell Creek displayed a trend of decreasing from upstream to downstream (**Fig. 20b**). C:P ratios were generally lower in the dry season and increased from upstream to downstream (**Fig. 20b**). Seasonal variability was limited in mean N:P ratios and averages were < 16 at all sites, suggesting N-limitation (**Fig. 20c**).

The central drainage feature was the most depleted basin in the study, with a mean stable carbon isotope value of -28.68 ± 0.92 ‰ (**Appendix 13**). This watershed also had the lightest stable nitrogen isotope value, with a mean of +3.27 ± 0.96 ‰, **Appendix 13**, which is still within the range for wastewater but may reflect mixed nutrient sources, such as the influence of fertilizers. The C:N ratio was also lowest at the central drainage feature (7.33 ± 0.72, **Appendix 13**), indicating less available nitrogen than the other basins. The C:P and N:P ratios were highest in this watershed (26.71 ± 1.51 and 4.46 ± 0.82 respectively, **Appendix 13**) and indicated high P-availability. The low N:P ratio also suggests this watershed is N-limited.

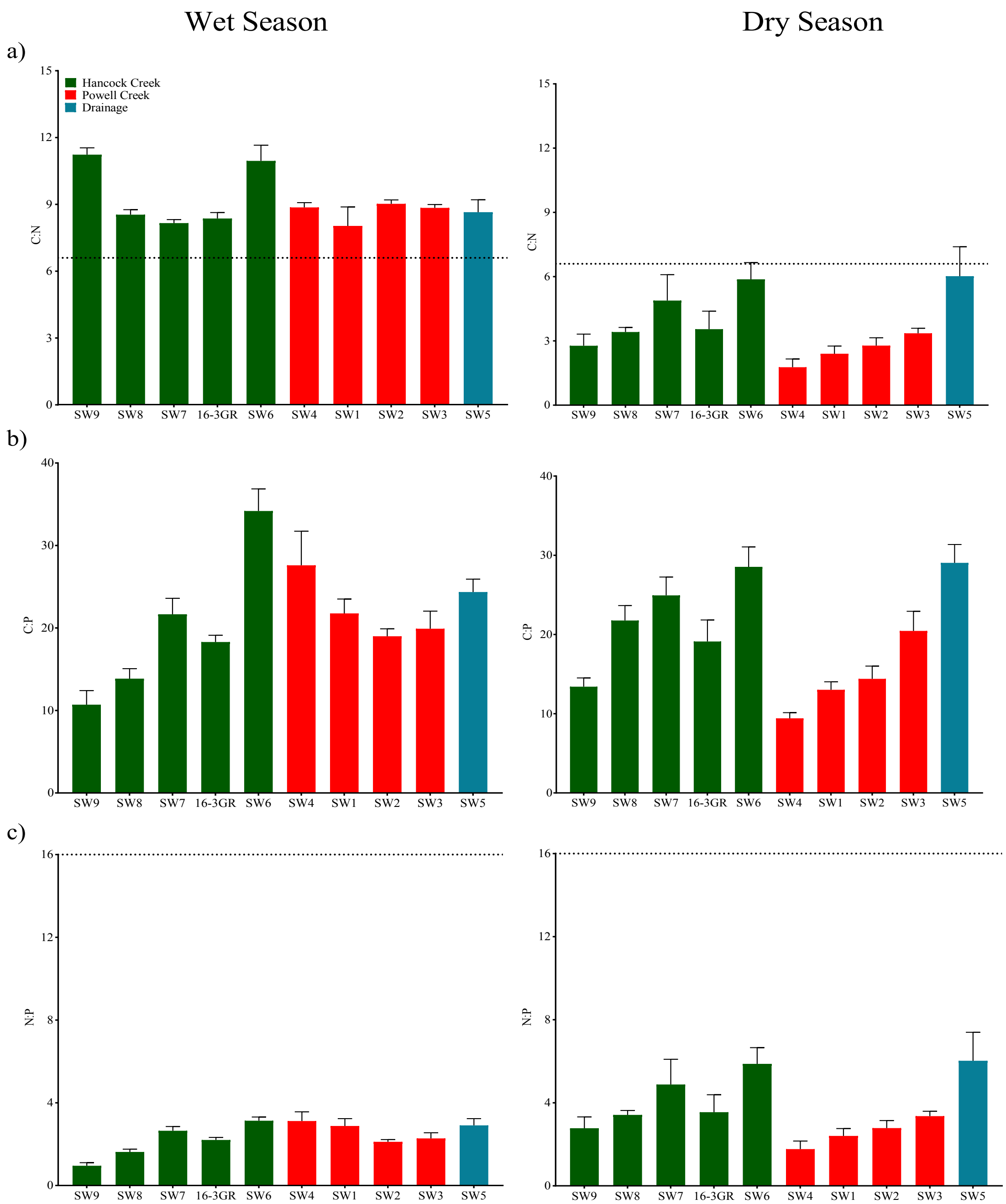

Figure 20. Elemental composition (mean ± standard error) of particulate organic matter, a proxy for phytoplankton, by season (wet 2017 and dry 2018) collected from surface water sites in North Fort Myers, including Hancock Creek, Powell Creek, and a central drainage feature, showing ratios of a) carbon 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).

Seasonally, the central drainage feature exhibited some differences in stable nitrogen and carbon isotopes. $\delta^{13}C$ was more depleted in the wet season than the dry season (**Fig. 19a**). $\delta^{15}N$ was lighter in the wet season and the mean was in the range expected fertilizer (**Fig. 19b**). However, in the dry season $\delta^{15}N$ was within the range expected for wastewater (**Fig. 20b**). There was subtle seasonality in the elemental composition of phytoplankton collected at the central drainage feature. The mean C:N ratio exceeded 6.6 in the wet season, suggesting N-imitation (**Fig. 20a**). Mean C:P was lower in the wet season and higher in the dry season (**Fig. 20b**). All C:P were lower than 106, representing P-enrichment. The greatest seasonal variability was observed in mean N:P, which was lower in the wet season, whereas the highest mean N:P of the study (6.02 ± 1.37) was observed in the dry season at the central drainage feature (**Fig. 20c**). Despite this N:P being a relatively high ratio for this study, this value still indicates N-limitation similar to the other sites.

HAB POM samples were collected in areas of Lee County during blue-green algae blooms in October 2017 and red tide events in March 2018. Blue-green algae POM samples from Davis Boat Ramp in the Caloosahatchee River had similar $\delta^{13}C$ signatures to those from the study area. Stable nitrogen isotope values of these POM samples were enriched similar to the study area and within the range of wastewater, with $\delta^{15}N$ values pf +6.94 ± 0.81 ‰. The elemental composition of the blue-green algae bloom was similar to those found for phytoplankton in the study area (**Appendix 13**). Red Tide samples collected in coastal areas of Lee County had lighter carbon isotope signature than the samples collected in the study area. This is reflective of the more enriched marine dissolved inorganic carbon found in coastal areas compared to freshwater canals. The stable nitrogen isotope values of red tide samples averaged +3.85 ± 0.23 ‰ in the coastal area, showing the linkage with upstream wastewater sources. Red tide samples had C:N ratios below 6.6, while C:P and N:P ratios were always below 106 and 16, respectively, indicating the red tide blooms were enriched with phosphorus and N-limited. See **Appendix 13** for overall and seasonal averages stable isotope values and elemental composition of blue-green algae and red tide samples.

3.3 Groundwater

3.3.1 Depth to Water Table

Water tables in the study area were generally high, with an overall average of 2.7 ft depth to groundwater. GW9 had the highest water table, with an average depth to water of 2.0 ft. The reference site had the lowest water table, with an average depth of 4.4 ft. Seasonal differences were observed in depth to water table, but some sites remained high throughout the study period (**Fig. 21**). The highest water tables were observed in October 2017 (1.6 ft overall average). In the dry season, the lowest overall average water table levels were observed (3.2 ft in December 2017 and 3.5 ft in January 2018). Throughout the study period water levels were observed that suggest many septic systems may not have adequate separation between the bottom of the drainfield and the high water table without additional mounding. See **Appendix 14** for monthly averages of depth to water at groundwater wells.

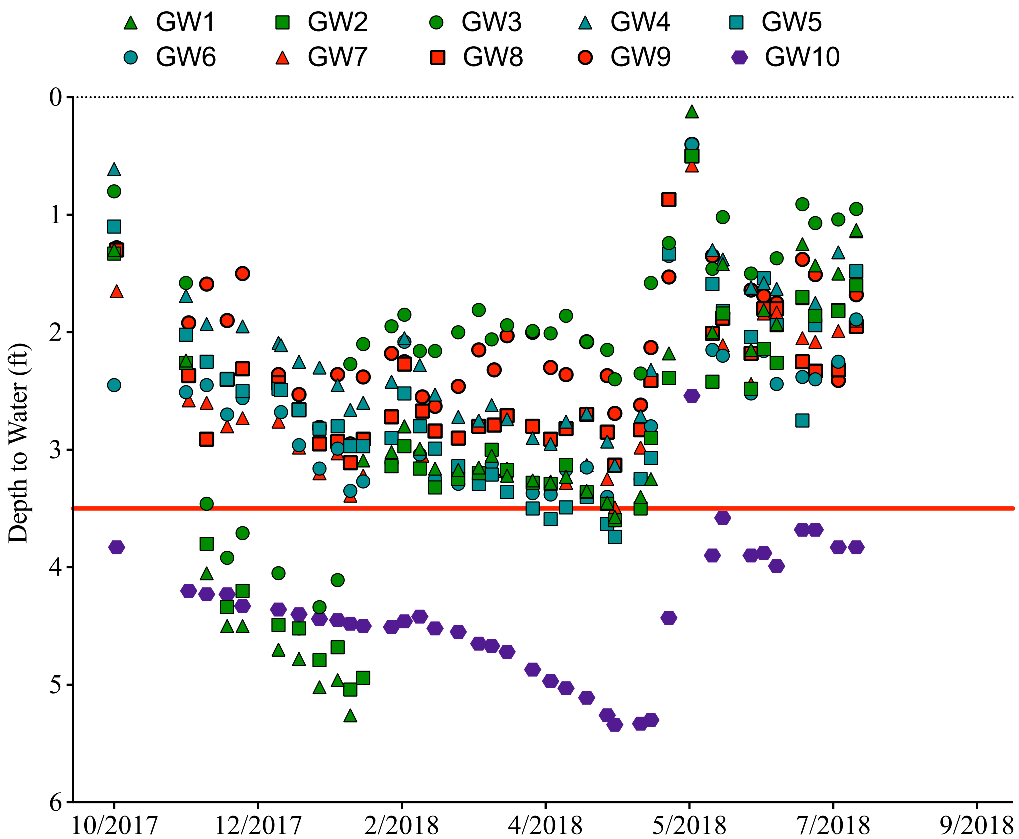

Figure 21. Depth to water table observed in North Fort Myers, FL by site, drainage basin (Hancock Creek = green, Powell Creek = red, a central drainage feature = teal, and a reference area = purple); the red line indicates the approximate minimum separation required from the ground surface (at zero) to the water table required by FAC Rule 62E-6, values above this line indicate septic systems in this area may not be compliant with current requirements for new septic systems. General required separation is 6" of cover, a 1' 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.

3.3.2 Environmental Parameters

Overall, slight variability in environmental parameters was observed between sites. Salinity, conductivity, and DO were generally lower in the central drainage feature and reference site relative to sites in Hancock and Powell Creeks (**Table 1**). Salinity ranged from 0.31 ± 0.03 at GW10 to 1.22 ± 0.05 at GW9, while conductivity spanned from 628 ± 53 at GW10 to 2,367 ± 88 at GW9 (**Table 1**). DO was lowest at GW6 (2.63 ± 0.96 %) and highest at GW8 (31.13 ± 11.07 %; **Table 1**). The lowest overall pH was observed at GW5

(6.28 ± 0.04) and the highest was observed at GW10 (6.94 ± 0.07; **Table 1**). Overall BOD was typically lowest in Hancock Creek and the reference site, ranging from BDL at GW2 and GW4 to 2.93 ± 0.57 mg/L at GW7 (**Table 1**). The lowest overall color was observed at GW10 (71 ± 18 CU) and the highest was observed at GW4 (210 ± 162 CU; **Table 1**).

Seasonal differences were observed for most environmental parameters. Salinity, conductivity, and DO were generally lower during the wet season compared to the dry season, particularly in the Hancock and Powell Creek watersheds (**Appendix 2**). During the wet season, salinity varied from 0.35 ± 0.02 to 1.19 ± 0.02 and conductivity ranged from 716 ± 43 to 2,310 ± 48. During the dry season salinity spanned from 0.27 ± < 0.01 to 1.88 ± < 0.01 and conductivity ranged from 541 ± 11 to 3,563 ± 12 (**Appendix 2**). DO varied from 1.00 ± 0.50 to 42.25± 21.95 % during the wet season and from 4.25 ± 0.15 to 34.05 ± 7.85 % during the dry season (**Appendix 2**). Wet season pH values fluctuated from 6.35 ± 0.01 to 6.91 ± 0.07 and from 6.22 ± 0.03 to 7.06 ± 0.02 during the dry season (**Appendix 2**). BOD was often higher during the dry season, while color was typically higher during the wet season (**Appendix 2**). During the wet season, BOD ranged from BDL to 2.75 ± 1.35 mg/L and from BDL to 3.60 ± 0.70 mg/L during the dry season (**Appendix 2**). Color varied from 21 ±3 to 382 ± 314 CU during the wet season and from 38 ± 3 to 165 ± 105 CU during the dry season (**Appendix 2**).

3.3.3 Dissolved Nutrient Concentrations

In Hancock Creek, overall NH_4 concentrations varied from 0.25 ± 0.03 to 0.49 ± 0.03 mg/L and were relatively low compared to the other basins (**Appendix 5**). Conversely, NO_3 and SRP concentrations were generally higher overall than the other basins (**Appendix 5**). NO_3 spanned from 0.01 ± < 0.01 to 1.42 ± 0.43 mg/L, and SRP ranged from 0.41 ± 0.03 to 0.96 ± 0.13 mg/L (**Appendix 5**). Overall TN concentrations were relatively low, fluctuating between 1.02 ± 0.11 to 2.46 ± 0.43 mg/L, while TP concentrations were relatively high, spanning from 0.44 ± 0.03 to 1.62 ± 0.34 mg/L (**Appendix 5**). DIN:SRP and TN:TP ratios were generally low in Hancock Creek, indicating N-limitation. Overall DIN:SRP ratios ranged from 0.58 ± 0.10 to 2.01 ± 0.64 and TN:TP ratios varied from 0.81 ± 0.14 to 2.76 ± 0.66 (**Appendix 5**).

Seasonal effects were often observed within the Hancock Creek watershed. NH_4 and SRP were consistently higher during the wet season relative to the dry season (**Fig. 22a,c**). Compared to the other basins NH_4 concentrations were low and comparable to the reference site during both seasons (**Fig. 22a**). SRP concentrations were generally high compared to the other basins, especially during the wet season (**Fig. 22c**). Conversely, NO_3 concentrations at Hancock Creek were the highest observed among the groundwater sites during the dry season and were higher than those observed during the wet season, with the exception of GW3 (**Fig. 22b**). TN concentrations were highest during the wet season while TP concentrations were highest during the dry season, except at GW1 (**Fig. 23a,b**). While low overall, DIN:SRP ratios were higher during the dry season, with the exception of GW3. Conversely, TN:TP ratios were higher during the wet season, with the exception of GW1 (**Fig. 23c,d**).

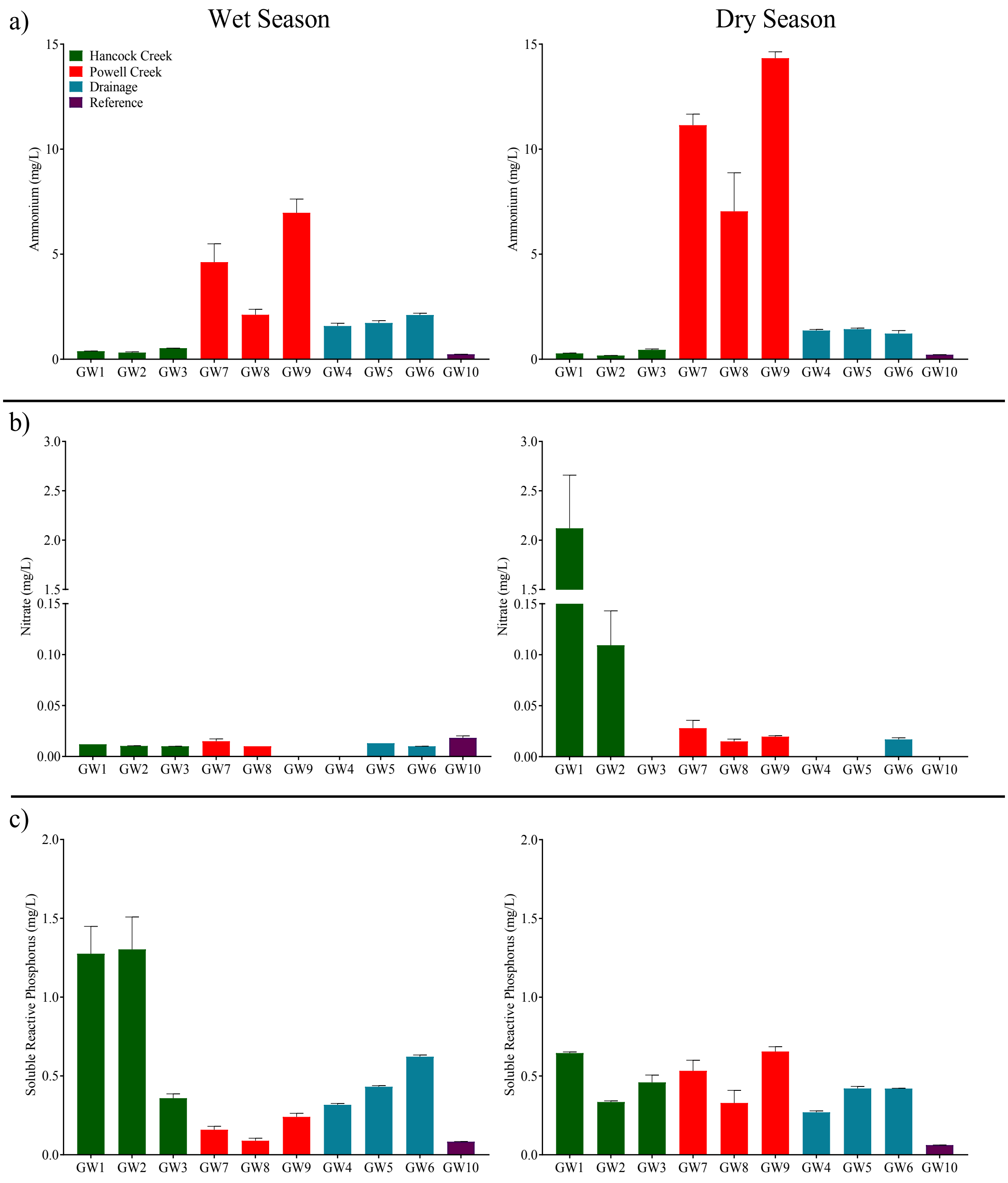

Figure 22. Groundwater dissolved reactive nutrient concentrations (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, a central drainage feature, and a reference area), and season (wet 2017 and dry 2018), including a) ammonium (NH_4), b) nitrate + nitrite (NO_3), and c) soluble reactive phosphorus (SRP).

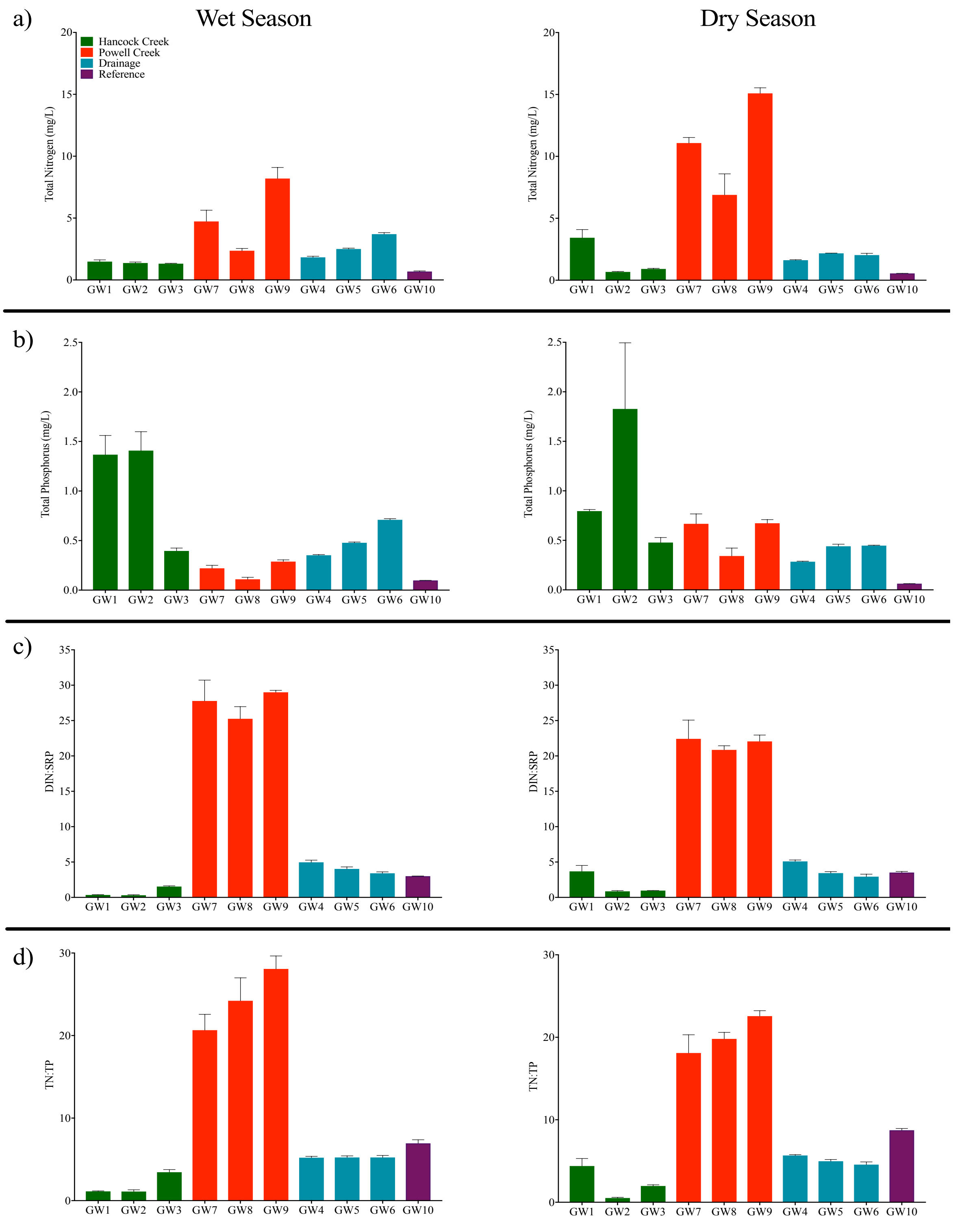

Figure 23. Groundwater dissolved total nutrient concentrations and ratios (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, a central drainage feature, and a reference area), and season (wet 2017 and dry 2018), including a) total nitrogen b) total phosphorus, c) dissolved inorganic nitrogen to soluble reactive phosphorus (DIN:SRP) and b) total nitrogen to total phosphorus (TN:TP).

In Powell Creek, overall NH_4 concentrations were the highest observed among the groundwater sites, ranging from 4.58 ± 1.15 to 10.65 ± 1.16 mg/L (**Appendix 5**). NO_3 concentrations were relatively low overall, fluctuating from 0.01 ± < 0.01 to 0.02 ± < 0.01 mg/L, while SRP concentrations spanned from 0.21 ± 0.05 to 0.45 ± 0.07 mg/L (**Appendix 3**). The highest overall TN concentrations were observed at Powell Creek, ranging between 4.62 ± 1.06 to 11.64 ± 1.14 mg/L (**Appendix 5**). TP concentrations varied from 0.23 ± 0.05 to 0.48 ± 0.06 mg/L (**Appendix 5**). The highest groundwater DIN:SRP and TN:TP ratios of the study were observed at Powell Creek (**Appendix 5**). DIN:SRP varied from 23.05 ± 1.09 to 25.53 ± 1.14, and TN:TP ranged from 19.38 ± 1.45 to 25.32 ± 1.16, indicating weak P-limitation (**Appendix 5**).

Seasonal differences were observed within Powell Creek. Dry season NH_4, NO_3, and SRP were higher than those recorded during the wet season, and NH_4 concentrations were highest during both seasons in relation to the other watersheds (**Fig. 22**). Additionally, TN and TP concentrations were higher during the dry season, and TN concentrations were the highest observed during both seasons compared to the other basins (**Fig. 23a,b**). DIN:SRP and TN:TP ratios were much higher than those observed in other basins during both the wet and dry seasons. Further, DIN:SRP and TN:TP were higher during the wet season relative to the dry season, indicting higher N-availability during the wet season (**Fig. 23c,d**).

Within the central drainage feature, overall NH_4 concentrations were moderately high compared to the other basins, ranging from 1.47 ± 0.08 to 1.66 ± 0.16 mg/L (**Appendix 5**). NO_3 concentrations were relatively low, fluctuating from BDL to 0.01 ± < 0.01 mg/L, while SRP concentrations were somewhat high, ranging from 0.29 ± 0.01 to 0.52 ± 0.03 mg/L (**Appendix 5**). Overall TN concentrations spanned from 1.73 ± 0.05 to 2.87 ± 0.27 mg/L and TP concentrations ranged from 0.32 ± 0.01 to 0.58 ± 0.04 mg/L (**Appendix 5**). DIN:SRP and TN:TP ratios were low, with DIN:SRP fluctuating from 3.17 ± 0.20 to 5.02 ± 0.17 and TN:TP ranging from 4.90 ± 0.22 to 5.44 ± 0.11 (**Appendix 5**).

Seasonal trends were often observed within the central drainage feature. NH_4 and SRP were consistently higher during the wet season, while NO_3 concentrations were relatively low and did not vary greatly between seasons (**Fig. 22**). TN and TP concentrations were also higher during the wet season compared to the dry season (**Fig. 23a,b**). With the exception of GW4, DIN:SRP and TN:TP ratios were higher during the wet season, but still indicated N-limitation (**Fig. 23c,d**).

The lowest overall NH_4 (0.23 ± 0.01 mg/L) and SRP (0.07 ± < 0.01 mg/L) concentrations were observed at the reference site, and NO_3 concentrations were also relatively low (0.02 ± < 0.01 mg/L; **Appendix 5**). Similarly, overall TN (0.62 ± 0.03 mg/L) and TP (0.08 ± 0.01 mg/L) concentrations were lowest at the reference site (**Appendix 5**). DIN:SRP (3.26

± 0.10) and TN:TP (7.84 ± 0.35) ratios at the reference site indicated N-limitation (**Appendix 5**).

Seasonal differences were not as distinct at the reference site. NH_4, NO_3, and SRP concentrations were only slightly higher during the wet season relative to the dry season (**Fig. 22**). TN and TP were higher during the wet season (**Fig. 23a,b**). DIN:SRP and TN:TP ratios were slightly higher during the dry season (**Fig. 23c,d**). For seasonal averages of nutrient concentrations in groundwater samples see **Appendix 6**.

3.3.4 Bacterial Prevalence

In Hancock Creek, overall bacterial concentrations were some of the lowest observed during the study (**Appendix 7**). Enterococci only ranged from 1 to 2 MPN/100 mL and *E. coli* was not detectable at any sites during the study (**Appendix 7**). During the wet season, enterococci was only detected at GW2, while during the dry season detections were observed at GW1 and GW3 (**Appendix 10**).

Powell Creek had the highest overall enterococci levels, and moderately high *E. coli* levels relative to the other basins (**Appendix 7**). Enterococci concentrations spanned from 3 to 811 ± 697 MPN/100 mL, while *E. coli* concentrations ranged from BDL to 45 MPN/100 mL (**Appendix 7**). Overall, GW9 was the only site with concentrations above the TPTV threshold for enterococci, while no sites exceeded the *E. coli* TPTV threshold. Enterococci concentrations were highly variable, ranging from BDL to 1,217 ± 1,204 MPN/100 mL during the wet season and from 1 to 49 ± 12 MPN/100 mL during the dry season (**Appendix 10**). The highest wet season detection of enterococci occurred at GW9 and was the only time concentrations exceeded the TPTV threshold. *E. coli* was only detected once during the wet season at GW9 at a concentration below the TPTV threshold and was not detected at any sites during the dry season (**Appendix 10**).

Overall enterococci concentrations in the central drainage feature were moderately high, but lower than the TPTV threshold, ranging from 2 ± < 1 to 49 MPN/100 mL (**Appendix 7**). Additionally, the highest *E. coli* concentrations were observed within the drainage feature and varied from BDL to 294 ± 202 MPN/100 mL, which were still below the TPTV threshold (**Appendix 7**). Wet season enterococci concentrations were lower than dry season concentrations, ranging from BDL to 3 MPN/100 mL during the wet season and from 2 ± 1 to 49 MPN/100 mL during the dry season (**Appendix 10**). *E. coli* was only detected during the dry season (**Appendix 10**).

The reference site had relatively low overall enterococci concentrations (4 ± 1 MPN/100 mL), while *E. coli* was not detected at the reference site during the study (**Appendix 7**). Wet season enterococci concentrations (5 ± 1 MPN/100 mL) were higher than dry season concentrations (3 MPN/100 mL) and were well below the TPTV threshold (**Appendix 10**).

3.3.5 Microbial Source Tracking: Chemical Tracers

The chemical tracers mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any groundwater sites during the study. Hancock Creek had the highest overall sucralose concentrations among the groundwater sites, ranging from 2.278 ± 0.543 to 41.675 ± 11.887 μg/L, which are all "significant" levels (**Appendix 11**). Acetaminophen, 2,4-D, and bentazon were not detected within Hancock Creek during the study (**Appendix 11**). Overall carbamazepine concentrations were low, but generally higher than other basins, spanning from 0.001 ± < 0.001 to 0.008 ± 0.002 μg/L (**Appendix 11**). Primidone concentrations were also low, but higher than other basins, ranging from BDL to 0.019 ± 0.002 μg/L (**Appendix 11**). GW1 consistently had the highest sucralose concentrations in both the wet and dry seasons, with higher concentrations in the dry season, while at GW2 and GW3 sucralose concentrations were higher during the wet season (**Fig. 24a**; **Appendix 12**). During both seasons sucralose concentrations were always "significant." Carbamazepine concentrations were similar between seasons (**Fig. 24b**; **Appendix 12**). Primidone was only detected at GW1 and GW2 during both the wet and dry season, and concentrations were higher at GW1 during the dry season while concentrations were higher at GW2 during the wet season (**Fig. 24c**; **Appendix 12**). Diuron, fluridone, imazapyr, and imidacloprid were not detected during the single sampling event they were measured (**Appendix 12**).

In Powell Creek, overall sucralose concentrations were relatively low, ranging from 0.299 ± 0.135 to 3.128 ± 2.336 μg/L (**Appendix 11**). Sucralose concentrations at GW7 and GW9 were "moderate," while concentrations at GW8 were "significant." The majority of the remaining chemical tracers were rarely detected within the Powell Creek watershed. Acetaminophen was detected once during the wet season at GW7, while carbamazepine and 2,4-D were not detected during the study (**Appendix 12**). Primidone and bentazon were only detected at GW9, with overall averages of 0.024 ± 0.009 μg/L and 0.023 ± 0.008 μg/L, respectively (**Appendix 11**). Sucralose was detected more consistently and in much higher concentrations during the dry season, ranging from 0.530 ± 0.028 to 4.685 ± 2.698 μg/L versus a range of 0.013 to 0.069 ± 0.022 μg/L during the wet season (**Fig. 24a**; **Appendix 12**). Dry season sucralose concentrations at GW7 and GW9 were "moderate" and GW8 concentrations were "significant"; while wet season concentrations were generally lower. At GW9, both primidone and bentazon were higher during the dry season (**Fig. 24c**; **Appendix 12**). Diuron, fluridone, imazapyr, and imidacloprid were not detected (**Appendix 12**).

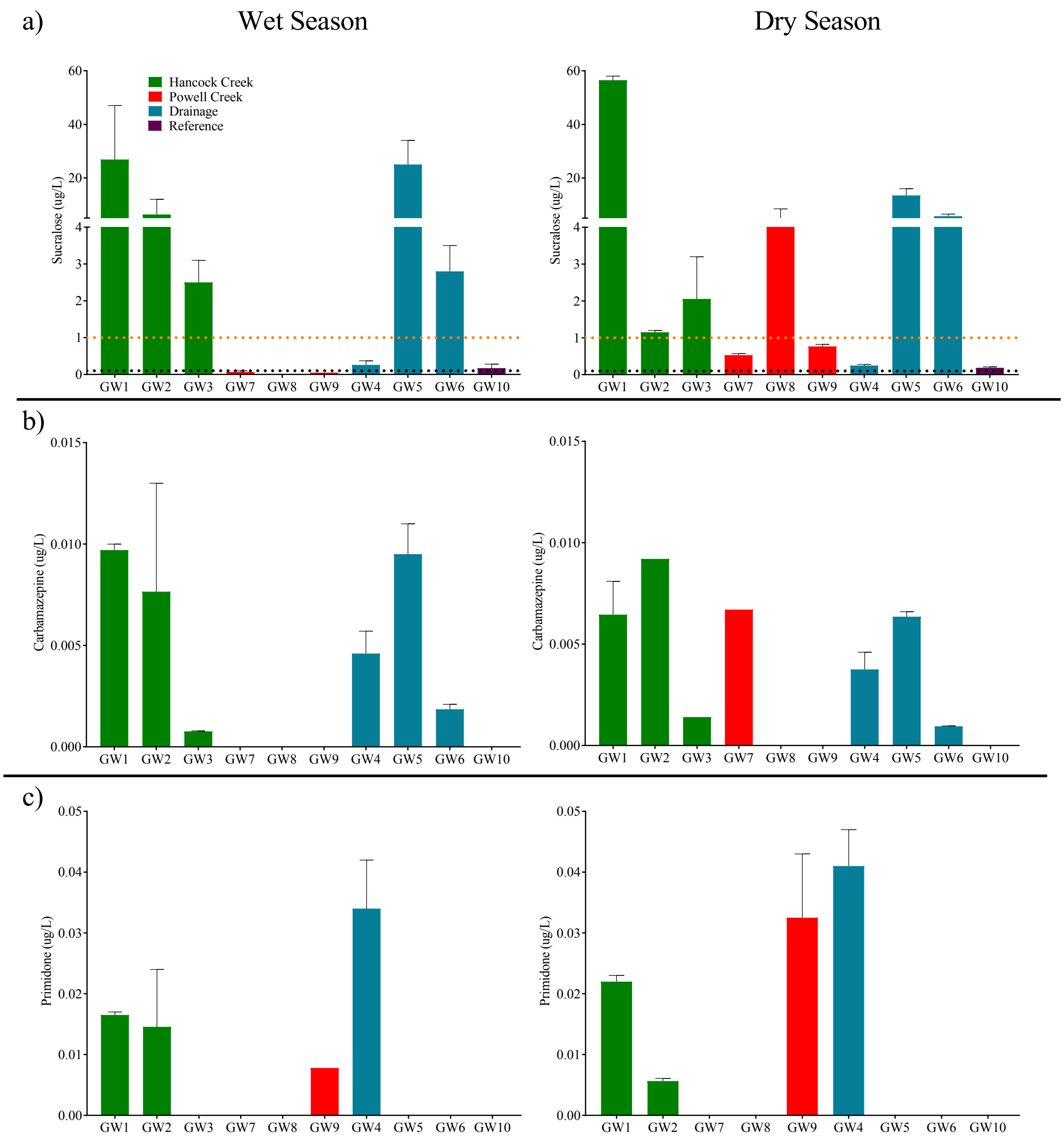

Figure 24. Groundwater human chemical tracers concentrations (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, a central drainage feature, and a reference area), and season (wet 2017 and dry 2018), including a) the artificial sweetener sucralose, with a black dotted line indicating "moderate" and an orange dotted line indicating "significant" concentrations, and the anticonvulsant pharmaceuticals, b) carbamazepine and c) primidone.

Within the central drainage feature, overall sucralose concentrations ranged from relatively low (0.253 ± 0.047 µg/L) to much higher (19.250 ± 5.056 µg/L; **Appendix 11**). At GW4 sucralose concentrations were "moderate," while concentrations at GW5 and GW6 were "significant," Both acetaminophen and 2,4-D were not detected in the central drainage feature during the study (**Appendix 11**). Carbamazepine concentrations were low, but high compared to other basins, ranging from 0.001 ± < 0.001 to 0.008 ± 0.001 µg/L (**Appendix 11**). Primidone and bentazon were only detected at GW4, with averages of 0.038 ± 0.005 µg/L and 0.002 ± < 0.001 µg/L, respectively (**Appendix 11**). With the exception of GW6, sucralose concentrations were higher during the wet season, ranging from 0.260 ± 0.078 to 25.000 ± 6.364 µg/L versus 0.245 ± 0.025 to 13.500 ± 1.768 µg/L during the dry season (**Fig. 24a**; **Appendix 12**). During both seasons sucralose concentrations were "moderate" at GW4 and "significant" at GW5 and GW6. Carbamazepine was slightly higher during the wet season relative to the dry season (**Fig. 24b**; **Appendix 12**). At GW4, primidone concentrations were higher during the dry season (**Fig. 24c**), while bentazon was only detected during the wet season (**Appendix 12**). Diuron, fluridone, imazapyr, and imidacloprid were not detected (**Appendix 12**).

Overall sucralose concentrations at the reference site were lower than the other groundwater sites at an average of 0.178 ± 0.046 µg/L, which is "moderate" (**Appendix 11**). Acetaminophen, carbamazepine, primidone, and 2,4-D were not detected at the reference site during the study (**Appendix 11**). Bentazon concentrations averaged 0.002 ± < 0.001 µg/L (**Appendix 11**). Sucralose concentrations were slightly higher during the dry season at the reference site (**Fig. 24a**), while bentazon concentrations were relatively similar between seasons (**Appendix 12**). Both wet and dry season sucralose concentrations were "moderate." During the single sampling event when they were collected, diuron, imazapyr, and imidacloprid were undetectable, but fluridone was present at a concentration of 0.002 µg/L (**Appendix 12**).

3.3.6 Aqueous Stable Isotope Analysis

In Hancock Creek, overall $\delta^{15}N\text{-}NH_4$ and $\delta^{15}N\text{-}NO_3$ signatures were relatively heavy and typically within the range for wastewater (> +3 ‰; **Appendix 15**). $\delta^{15}N\text{-}NH_4$ values spanned from +6.48 ± 0.56 to +9.25 ± 1.22 ‰, while $\delta^{15}N\text{-}NO_3$ values ranged from +0.49 ± 0.93 to +13.85 ± 7.16 ‰ (**Appendix 15**). Wet season concentrations of $\delta^{15}N\text{-}NH_4$ were heavier than dry season concentrations (**Fig. 25a**). $\delta^{15}N\text{-}NO_3$ values were much heavier during the dry season and were generally heavier than values observed in other basins during that season (**Fig. 25b**).

In Powell Creek, overall $\delta^{15}N\text{-}NH_4$ values varied from +2.20 ± 0.62 to +3.76 ± 0.29 ‰, while $\delta^{15}N\text{-}NO_3$ values spanned from -2.33 ± 1.65 to +2.45 ± 0.58 ‰ (**Appendix 15**). During both the wet and dry seasons $\delta^{15}N\text{-}NH_4$ signatures were relatively light in comparison to the other basins, and values were heavier during the wet season compared to the dry season (**Fig. 25a**). $\delta^{15}N\text{-}NO_3$ signatures were lightest in Powell Creek compared to the other basins during both seasons, and dry season values were heavier than those observed during the wet season (**Fig. 25b**).

In the central drainage feature, overall δ^{15}N-NH_4 signatures were moderately heavy, ranging from +3.71 ± 0.34 to +9.63 ± 0.26 ‰, while overall δ^{15}N-NO_3 values ranged from +2.05 ± 0.60 to +2.81 ± 0.65 ‰ (**Appendix 15**). δ^{15}N-NH_4 values were similar between the wet and dry seasons, while δ^{15}N-NO_3 values were heavier during the dry season relative to the wet season (**Fig. 25**).

At the reference site, overall δ^{15}N-NH_4 values were +3.41 ± 1.85 ‰, and overall δ^{15}N-NO_3 values were +3.65 ± 1.39 ‰ (**Appendix 15**). Both δ^{15}N-NH_4 and δ^{15}N-NO_3 values were heavier during the wet season compared to the dry season (**Fig. 25**).

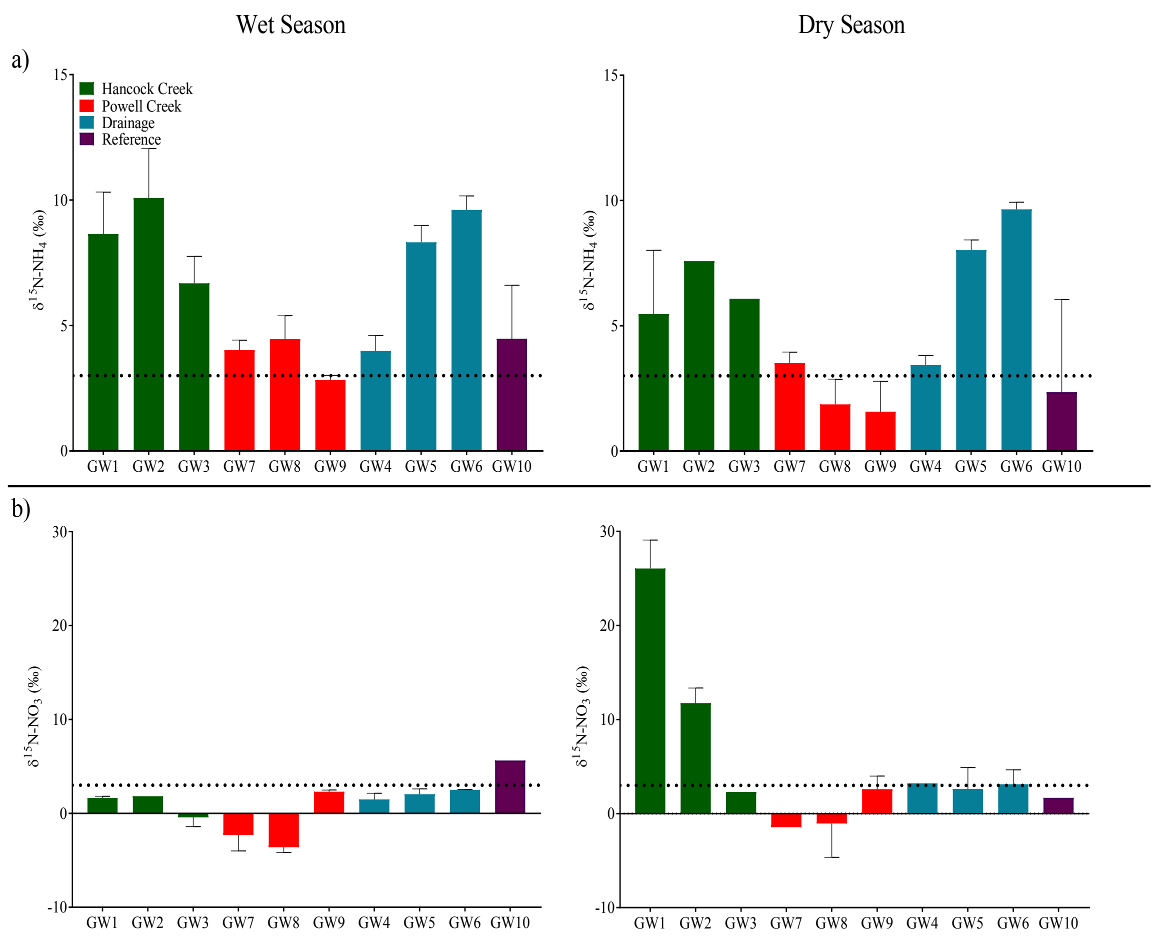

Figure 25. Groundwater aqueous stable nitrogen isotope values (average ± standard error) observed in North Fort Myers, FL by site, drainage basin (Hancock Creek, Powell Creek, a central drainage feature, and a reference area), and season (wet 2017 and dry 2018), including a) δ^{15}N-NH_4 and b) δ^{15}N-NO_3, with a dotted line to represent the average value for unprocessed wastewater (+ 3 ‰), such as septic tank effluent.

4. Discussion

The targeted sampling program was insightful as to contributing sources of bacteria and nutrients in the North Fort Myers watershed. In all three basins of the watershed, there were multiple lines of evidence that indicated leachate from septic systems was adversely affecting water quality. The evidence includes elevated DIN concentrations, particularly NH_4, high bacteria levels, the presence of human source tracers, including sucralose, carbamazepine, primidone, and the molecular marker HF183, and stable nitrogen isotopes with values within the range of wastewater. High groundwater levels were also consistently observed in the study area, suggesting that septic systems likely do not have the separation required by FAC Rule 62E-6. Further, there was evidence that surficial runoff from stormwater was adversely affecting water quality due to the presence of the chemical tracers 2,4-D, bentazon, diuron, fluridone, imazapyr, and imidacloprid. There were stable isotope values within the range of fertilizers, suggesting these may be another source of nutrients to North Fort Myers as well. Details specific to each section of the watershed are discussed below.

4.1 Hancock Creek

In Hancock Creek, both enterococci and *E. coli* surface water concentrations exceeded FDEP standards, especially during the dry season. Additionally, the avian bacteria markers, GFD and Gull2, were highest in Hancock Creek and were most elevated at downstream sites, suggesting a significant avian influence. This is corroborated by the strong guano smell frequently observed at sites SW9 and 16-3GR. The human marker HF183 was often detected in relatively low levels, and the ubiquitous presence of carbamazepine and “moderate” levels of sucralose help to link these bacteria to domestic wastewater as well. Except for the most upstream site at Yellow Fever Creek, POM stable nitrogen isotope signatures were consistently within the range of wastewater, indicating that phytoplankton are incorporating nitrogen from wastewater sources. Evidence of the influence of surface runoff was also apparent at Hancock Creek. While NH_4 and NO_3 levels were relatively low, SRP concentrations were generally high at Hancock Creek. Further, several herbicide chemical tracers were consistently detected in surface water samples. These combined data all reflect the influence of surficial runoff in Hancock Creek. The lowest DIN:SRP and TN:TP ratios were observed at Hancock Creek sites, in both surface and groundwater, indicating that this watershed is the most N-limited in the study area. In POM samples, C:N generally exceeded 6.6, especially during the wet season. C:P was below 106 and N:P was lower than 16, further supporting that this watershed was N-limited.

In groundwater at Hancock Creek, sucralose concentrations were especially high. For example, at GW1 these concentrations approached levels previously measured in WWTP effluent (up to 40 µg/L; Silvanima et al., 2018) and sewage effluent (67 µg/L; Lapointe et al., 2016). This indicates a strong link between wastewater and surface water quality. The average depth to water table was relatively low (2.7 ft) in the Hancock Creek groundwater wells, with lower depth to water during the wet season relative to the dry season. Therefore, septic systems in this area may not have adequate separation from the drainfield to the high water table without additional mounding. This is further supported by the aqueous stable nitrogen isotope signatures observed in groundwater samples (> +3 ‰), which were within the range of wastewater.

4.2 Powell Creek

Powell Creek bacterial concentrations often exceeded FDEP standards and were much higher during the dry season. Molecular markers showed consistent presence of the general avian marker GFD during both the wet and dry seasons, indicating that bird populations may influence water quality in the Powell Creek watershed. Detections of the human marker HF183 were found at each site during both seasons and were among the highest observed relative to the other basins. Surface water concentrations of sucralose, carbamazepine, and primidone were typically higher than other sites in the study area, with sucralose concentrations always exceeding "moderate" and sometimes exceeding "significant" classifications. Acetaminophen, a sensitive tracer indicative of untreated wastewater, was also detected at downstream sites during both seasons. The pervasive presence of HF183, coupled with the human waste chemical tracers, strongly indicate the presence of human wastewater in surface water of the Powell Creek watershed. Furthermore, POM stable nitrogen isotope signatures were within the range for wastewater, especially during the dry season. Lighter stable nitrogen isotope values in the wet season likely reflect the influence of rainfall and runoff containing fertilizers, both of which have depleted isotopic signatures (Lapointe et al., 2006; Lapointe and Bedford, 2007). Several herbicide chemical tracers were found in Powell Creek surface water samples, while concentrations in groundwater were largely undetectable, indicating that runoff is also affecting surface water quality. Surface water DIN:SRP and TN:TP ratios were elevated in comparison to the other basins in North Fort Myers, though still relatively low. C:N ratios in POM samples exceeded 6.6 during the wet season, also indicating N-limitation.

Groundwater concentrations of sucralose at Powell Creek were often lower than those observed in surface water, suggesting other groundwater conduits or an upstream wastewater source is contributing to surface water quality. However, aqueous stable nitrogen isotope signatures were within the range of wastewater at Powell Creek, especially during the wet season. Groundwater NH_4 concentrations were also much higher than the other basins, further indicating the presence of wastewater. N:P ratios in groundwater were greater than those observed in the other basins, reflecting high N content from wastewater, even in relation to the high natural P concentrations in this watershed. Water tables in the Powell Creek watershed were the highest in the study, with an average depth of 2.4 ft. The highest water tables were observed during the wet season. 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. It is noteworthy that 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. Despite the low occupancy rates, however these groundwater wells still showed the influence of wastewater.

4.3 Central Drainage Feature

The central drainage feature had bacteria levels that greatly exceeded FDEP standards. Based on the molecular marker analysis, the source of these bacteria was both avian (GFD) and human (HF183), as both markers were consistently present at the central drainage site. Sucralose was present in "moderate" concentrations in surface water, and carbamazepine was also consistently detected. Acetaminophen was also present during one of the wet season events, further supporting that wastewater is influential in this area. Dry season POM stable nitrogen isotope signatures were within the range for wastewater. The lighter signatures observed during the wet season are likely due to rainfall and fertilizer input from surficial runoff depleting the isotopic signatures (Lapointe et al., 2006; Lapointe and Bedford, 2007). Other evidence of surficial runoff was also observed in the central drainage feature. Multiple herbicides were present in surface water samples, but were rarely detected in groundwater samples. Surface water herbicide concentrations were typically greater during the wet season when runoff is expected to be highest. N:P ratios were typically low, reflecting the high P-availability. C:N in POM samples exceeded 6.6 during both seasons, indicating this watershed is N-limited.

Sucralose concentrations were typically much higher in groundwater at the central drainage feature than in nearby surface water, indicating a link between groundwater and surface water quality. Water tables were also high in the central drainage feature, with an average depth of 2.5 ft and higher levels during the wet season. Aqueous stable nitrogen isotopes in this watershed were within the range of wastewater during both seasons, indicating the influence of septic systems in the groundwater. Furthermore, NH_4 concentrations in groundwater were much higher than those observed in surface water in the central drainage feature, suggesting wastewater influence on groundwater is likely a contributing source to surface water quality degradation in this basin.

4.4 Groundwater Reference Site

The groundwater reference site had the lowest water table, with an overall average depth of 4.4 ft. At this site, MST parameters were generally among the lowest responses observed during the study. Sucralose concentrations were relatively low compared to other groundwater sites, and carbamazepine, primidone, and acetaminophen were all BDL. The herbicides bentazon and fluridone were the only other chemical tracers present at the reference site. Aqueous stable nitrogen isotopes were relatively light, but still often within the range for wastewater, and reactive nutrient concentrations were also typically much lower than other groundwater sites. N:P ratios were moderately low in comparison to the other groundwater basins. This site is the most undeveloped groundwater site in the study, and the relatively low responses observed indicate a reduced impact compared to residential land uses on the other watersheds.

4.5 Implications of Study

A conservative estimate of N-loading from septic systems to the Caloosahatchee River can be easily calculated. 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 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.

The results of this study corroborate the findings of previous studies in impaired coastal waters of Florida. Sucralose concentrations and aqueous stable nitrogen isotopes in the study area were similar to concentrations observed in Charlotte, Martin, and St. Lucie Counties (Lapointe et al., 2016; Lapointe et al., 2017; Lapointe et al., 2018). It is noteworthy that several times during this study groundwater sucralose concentrations approached those of WWTP effluent (up to 40 µg/L; Silvanima et al., 2018) or raw wastewater (67 µg/L; Lapointe et al., 2016) reflecting the severity of impairment. TN:TP ratios similar to sewage (10-19) were observed at North Shore Park and in Powell Creek groundwater samples. Additionally, N:P ratios in phytoplankton were lower during the wet season. At Powell Creek and the central drainage feature $\delta^{15}N$ values were also lower in the wet season. This indicates dilution due to higher rainfall and runoff, as well as more P-loading, similar to previous findings in Lee County by Lapointe et al. (2006) and Lapointe and Bedford (2007).

This study has many implications regarding the downstream effects of localized nutrient enrichment, such the HAB events which occurred in Lee County in 2017-2018. In the estuarine and coastal environment off of Fort Myers, *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, the Caloosahatchee River can be seeded with *M. aeruginosa* from Lake Okeechobee discharges. These blooms can experience exponential growth of one doubling per day or more when they encounter high levels of dissolved reactive nitrogen, especially ammonium (Lapointe et al., 2017), such as what is found in the North Fort Myers watershed. The $\delta^{15}N$ value of *M. aeruginosa* (+6.93 ‰) are similar to the mean value of POM for the surface water sites (+4.54 ‰) and mean groundwater ammonium $\delta^{15}N$ (+5.82 ‰) in the study area. Furthermore, the 2018 $\delta^{15}N$ of *K. brevis* (+3.85 ‰) is 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 (+7.83 ‰; Yentsch et al., 2008). Therefore, blooms are expected to be exacerbated during periods of heavy runoff to the estuarine and coastal environment which has a naturally high background P. Reduced salinity is also more favorable for red tide blooms (Odum, 1953; Slobodkin, 1953; Brand and Compton, 2007), so during heavy rain events or times of high discharge volumes from Lake Okeechobee these blooms are more likely to occur. These combined data further support the linkage between localized nutrient enrichment and HABs in Lee County.

The results of this study indicate that septic systems are not protective of water quality in North Fort Myers, which has also been found for other geographic areas (Verhougstraete et al., 2015). 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 persistent pollution are eliminated in Hancock and Powell Creek, as well as the central drainage feature, these pollutant loads will be greatly reduced. Also, implementing structures to better manage stormwater runoff will further enhance water quality. Most importantly, the susceptibility of these systems to localized HABs may be reduced by balancing the ecological stoichiometry of the watershed to achieve a nitrogen to phosphorus ratio where these microalgae will not thrive.

5. 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. 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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7. 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.

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: MMBR, 76(4), 685-706.

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.

Green, H.C., and Field, K.G. 2012. Sensitive detection of sample interference in environmental qPCR. Water Research, 46(10), 3251-3260.

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., 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., & 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.

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.

Lv, M., Sun, Q., Hu, A., Hou, L., Li, J., Cai, X., and Yu, C.P. 2014. Pharmaceuticals and personal care products in a mesoscale subtropical watershed and their application as sewage markers. Journal of Hazardous Materials, 280, 696-705.

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.

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.

Redfield, A.C. 1958. The biological control of chemical factors in the environment. American Scientist, 46(3), 230A-221.

Savoye, N., Aminot, A., Treguer, P., Fontugne, M., Naulet, N., & Kérouel, R. 2003. Dynamics of particulate organic matter δ15N and δ13C 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.

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.

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.

Verhougstraete, M. P., Martin, S. L., Kendall, A. D., Hyndman, D. W., & 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.

W. Dexter Bender & 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.

Yang, Y., He, Z., Lin, Y., Phlips, E.J., Yang, J., Chen, G., Stoffella, P.J., and Powell, C.A. 2008. Temporal and spatial variations of nutrients in the Ten Mile Creek of South Florida, USA and effects on phytoplankton biomass. Journal of Environmental Monitoring, 10(4), 508-516.

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.

8. Appendix

Appendix 1. Detailed site information by water type and drainage basin, including **s**tudy site names, site abbreviations, addresses, location descriptions, and GPS coordinates.

Water Type	Drainage Basin	Site Name	Site Abbreviation	Address / Site Description	Lattitude	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	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
	Reference	NOFOPZ-10	GW10	1397 Orchid Rd.	26.6726	-81.9176

Appendix 2. Seasonal averages (± standard error, except where only one measurement was made) of environmental parameters by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area), site, and season (wet 2017 and dry 2018); BDL = below detection level.

Water Type	Drainage Basin	Site	*Wet Season 2017*							
			Count	**pH**	**Salinity**	**Temperature (°C)**	**% Dissolved Oxygen**	**Conductivity (µS)**	**Biochemical Oxygen Demand (mg/L)**	**Color (CU)**
Surface Water	Hancock Creek	SW9	2	7.16±0.10	0.25±0.05	24.75±2.25	25.15±1.25	565±83	0.50	50±7
		SW8	2	7.30±0.11	0.35±0.05	25.60±1.90	45.60±12.10	718±105	0.65±0.05	77±4
		SW7	2	7.31±0.19	0.35±0.05	26.15±2.25	52.10±10.80	697±110	1.00±<0.01	94±12
		16-3 GR	2	7.24±0.19	0.35±0.05	25.95±2.05	50.70±14.20	650±114	1.00±0.10	99±3
		SW6	2	7.46±0.30	0.20±<0.01	26.30±2.40	73.60±16.10	357±8	1.50±<0.01	169±4
	Powell Creek	SW4	2	7.10±0.03	0.20±<0.01	25.05±1.55	66.35±3.45	410±89	0.70±<0.01	76±13
		SW1	2	7.05±0.11	0.20±<0.01	24.85±1.55	61.70±2.80	413±92	0.55±0.25	75±13
		SW2	2	7.05±0.10	0.25±0.05	24.65±1.85	22.70±18.10	481±92	0.75±0.25	80±12
		SW3	2	7.07±0.11	0.25±0.05	24.75±1.75	46.40±1.30	481±99	0.95±0.05	80±9
	Central Drainage	SW5	2	7.00±0.10	0.30±<0.01	24.30±2.30	24.55±13.15	553±13	1.20±0.60	153±6
Groundwater	Hancock Creek	GW1	2	6.62±0.08	0.45±<0.01	26.19±0.73	8.55±6.05	910±10	BDL	154±16
		GW2	2	6.68±0.09	0.43±0.07	27.44±0.91	19.10±16.60	867±131	BDL	141±3
		GW3	2	6.48±0.01	0.370	27.02±0.94	2.35±0.05	382±306	BDL	111±7
	Powell Creek	GW7	2	6.73±0.06	0.39±0.03	25.92±1.05	12.50±7.90	801±57	2.25±0.75	21±3
		GW8	2	6.91±0.07	0.70±0.03	26.80±0.88	42.25±21.95	1,411±57	2.75±1.35	117±78
		GW9	2	6.80±0.04	1.19±0.02	25.30±0.69	2.15±0.35	2,310±48	2.05±1.25	151±27
	Central Drainage	GW4	2	6.83±0.02	0.66±<0.01	28.13±0.94	2.45±0.05	1,324±5	BDL	382±314
		GW5	2	6.35±0.01	0.39±0.02	28.24±1.01	1.45±0.35	805±34	1.40±0.10	182±38
		GW6	2	6.68±0.01	0.58±0.02	27.63±0.91	1.00±0.50	1,173±38	0.300	243±166
	Reference	GW10	2	6.83±0.03	0.35±0.02	24.88±0.71	2.75±0.45	716±43	0.700	97±24

Water Type	Drainage Basin	Site	*Dry Season 2018*							
			Count	**pH**	**Salinity**	**Temperature (°C)**	**% Dissolved Oxygen**	**Conductivity (µS)**	**Biochemical Oxygen Demand (mg/L)**	**Color (CU)**
Surface Water	Hancock Creek	SW9	2	7.60±0.14	2.20±1.80	21.70±1.50	45.95±0.45	3,996±3,259	1.05±0.25	33±1
		SW8	2	7.55±0.06	8.05±1.35	22.60±2.80	59.00±4.30	12,518±3,469	2.10±1.20	66±1
		SW7	2	7.62±0.08	8.95±1.35	22.40±2.60	61.90±3.70	15,328±2,107	1.45±0.55	62±2
		16-3 GR	2	7.60±0.06	9.35±1.85	22.50±2.60	73.70±9.80	15,964±2,840	1.35±0.35	58±3
		SW6	2	7.79±0.10	11.25±1.95	22.20±2.20	93.15±4.05	18,855±2,999	0.80±0.20	49±6
	Powell Creek	SW4	2	7.28±0.10	0.65±0.35	21.40±2.30	52.80±6.10	1,329±703	0.90±0.30	34±6
		SW1	2	7.35±0.02	1.05±0.75	21.45±2.55	48.95±5.15	2,036±1,407	1.15±0.35	39±2
		SW2	2	7.44±0.03	4.85±2.75	21.00±3.10	54.35±4.35	8,642±4,604	1.45±0.25	53±0
		SW3	2	7.61±0.06	7.15±2.75	21.20±3.00	73.25±11.25	12,305±4,475	1.90±0.50	52±2
	Central Drainage	SW5	2	7.47±0.03	9.60±2.10	21.40±3.10	57.00±11.00	16,354±3,258	2.70±1.70	61±13
Groundwater	Hancock Creek	GW1	2	6.57±0.01	0.71±0.02	22.90±0.18	34.05±7.85	1,411±33	0.400	113±5
		GW2	2	6.53±0.02	1.88±<0.01	23.07±0.05	29.05±0.55	3,563±12	BDL	72±29
		GW3	2	6.51±0.02	1.23±0.25	22.91±0.37	9.65±3.25	2,386±466	0.300	73±1
	Powell Creek	GW7	2	6.79±0.02	0.51±0.03	22.25±0.19	20.70±2.70	1,016±69	3.60±0.70	165±105
		GW8	2	6.79±0.13	0.97±0.18	22.69±0.33	20.00±2.30	1,896±333	2.70±<0.01	107±50
		GW9	2	6.63±0.04	1.25±0.10	22.08±0.09	8.70±3.20	2,425±194	2.75±0.45	125±33
	Central Drainage	GW4	2	6.69±<0.01	0.64±<0.01	23.99±0.21	6.05±0.95	1,275±12	BDL	38±3
		GW5	2	6.22±0.03	0.35±0.03	23.73±0.24	4.80±0.30	707±55	0.500	150±9
		GW6	2	6.57±0.03	0.50±<0.01	23.99±0.08	4.25±0.15	1,008±14	1.80±0.20	76±37
	Reference	GW10	2	7.06±0.02	0.27±<0.01	23.03±0.36	4.25±0.05	541±11	0.300	45±2

Appendix 3. Monthly averages (± standard error, except where only one measurement was made) of dissolved nutrient concentrations, biochemical oxygen demand (BOD), and color concentration data by site for Hancock Creek surface water sites; BDL = below detection level.

Drainage Basin	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 Nitrogen (mg/L)	Total Phosphorus (mg/L)	DIN:SRP	TN:TP	Count	Biochemical Oxygen Demand (mg/L)	Color (CU)
Hancock Creek	SW9	43009	3	0.03±<0.01	0.06±<0.01	0.09±<0.01	0.13±<0.01	0.40±0.01	0.46±0.01	0.17±<0.01	0.67±<0.01	2.74±0.08	1	0.50	57
		43040	3	0.05±<0.01	0.07±<0.01	0.12±<0.01	0.14±<0.01	0.34±0.01	0.41±0.01	0.18±<0.01	0.84±0.02	2.24±0.05	1	BDL	43
		43070	1	0.02	0.06	0.07	0.10	0.42	0.48	0.13	0.76	3.69	1	BDL	26
		43101	1	0.04	0.04	0.08	0.14	0.33	0.37	0.16	0.54	2.31	1	BDL	27
		43132	3	BDL	0.01±<0.01	0.01±<0.01	0.20±<0.01	0.32±0.02	0.33±0.02	0.23±<0.01	0.06±<0.01	1.44±0.09	1	1.30	31
		43160	3	0.04±<0.01	0.01	0.04±0.01	0.24±<0.01	0.45±0.02	0.45±0.02	0.26±0.01	0.18±0.03	1.75±0.12	1	0.80	34
		43191	1	0.03	BDL	0.03	0.39	0.70	0.70	0.46	0.08	1.52	1	1.50	67
		43221	1	0.05	0.06	0.10	0.41	0.99	1.00	0.42	0.25	2.38	1	0.60	79
	SW8	43009	3	0.02	0.07±<0.01	0.08±0.01	0.16±<0.01	0.63±0.10	0.70±0.10	0.22±<0.01	0.47±0.04	3.14±0.46	1	0.70	81
		43040	3	0.03±<0.01	0.08±<0.01	0.11±<0.01	0.13±<0.01	0.51±0.08	0.60±0.09	0.17±<0.01	0.88±0.01	3.45±0.52	1	0.60	73
		43070	1	BDL	BDL	BDL	0.15	0.95	0.95	0.22	<0.01	4.32	1	1.40	60
		43101	1	BDL	BDL	BDL	0.15	0.81	0.81	0.24	<0.01	3.38	1	1.00	56
		43132	3	BDL	BDL	0.00±<0.01	0.15±<0.01	0.97±0.13	0.97±0.13	0.25±<0.01	<0.01±<0.01	3.93±0.57	1	3.30	68
		43160	3	0.07±<0.01	0.02±<0.01	0.09±<0.01	0.18±<0.01	0.77±0.05	0.79±0.05	0.20±<0.01	0.49±0.01	3.93±0.24	1	0.90	65
		43191	1	0.07	0.01	0.08	0.19	1.00	1.00	0.22	0.42	4.55	1	0.90	72
		43221	1	0.02	0.01	0.03	0.20	1.40	1.40	0.22	0.14	6.36	1	1.10	76
	SW7	43009	3	BDL	0.07±<0.01	0.07±<0.01	0.15±<0.01	0.68±0.05	0.75±0.05	0.21±0.01	0.44±0.01	3.54±0.36	1	1.00	105
		43040	3	0.01	0.01	0.02	0.10±<0.01	0.58±0.02	0.58±0.02	0.15±0.01	0.08±0.08	3.99±0.25	1	1.00	82
		43070	1	0.01	0.05	0.07	0.11	0.84	0.89	0.20	0.57	4.45	1	0.80	80
		43101	1	BDL	0.02	0.02	0.09	1.20	1.20	0.15	0.25	8.00	1	1.40	65
		43132	3	BDL	BDL	0.00±<0.01	0.09±<0.01	0.75±0.04	0.75±0.04	0.18±0.02	0.00±<0.01	4.30±0.21	1	2.00	64
		43160	3	0.06±<0.01	0.01±<0.01	0.07±<0.01	0.13±<0.01	0.83±0.02	0.85±0.01	0.17±0.01	0.59±0.01	5.09±0.21	1	0.90	60
		43191	1	0.03	BDL	0.03	0.12	1.00	1.00	0.17	0.28	5.88	1	1.40	55
		43221	1	BDL	BDL	0.00	0.15	1.00	1.00	0.20	0.00	5.00	1	1.20	57
	16-3GR	43009	4	0.03±<0.01	0.07±<0.01	0.10±<0.01	0.15±<0.01	0.74±0.08	0.80±0.07	0.21±0.01	0.67±0.01	3.81±0.29	2	1.10±<0.01	106±5
		43040	4	0.03±<0.01	0.03±0.01	0.05±<0.01	0.08±<0.01	0.69±0.07	0.72±0.06	0.15±0.01	0.66±0.02	4.73±0.37	2	1.25±0.35	99±3
		43070	2	0.02±<0.01	0.16±0.01	0.17±0.01	0.09±0.01	0.81±0.03	0.97±0.03	0.13±0.01	2.00±0.30	7.49±0.85	2	1.45±0.45	87±3
		43101	2	0.01	0.07	0.08	0.06±0.01	0.98±0.02	1.00±<0.01	0.14±<0.01	0.64±0.64	7.14±<0.01	2	1.85±0.65	65±3
		43132	4	0.02	BDL	0.00±<0.01	0.08±0.01	0.82±0.06	0.82±0.06	0.17±0.01	0.05±0.05	4.86±0.46	2	1.35±0.35	58±3
		43160	4	0.04±0.01	0.01±<0.01	0.05±0.01	0.10±0.01	0.87±0.02	0.88±0.02	0.14±0.01	0.53±0.09	6.56±0.33	2	1.45±0.45	52±3
		43191	2	0.02	BDL	0.01±0.01	0.11±0.01	0.93±0.02	0.93±0.02	0.18±0.03	0.10±0.10	5.41±0.66	2	1.35±0.15	51±2
		43221	1	BDL	BDL	<0.01	0.14	0.91	0.91	0.19	<0.01	4.79	1	1.70	50
	SW6	43009	3	0.02±<0.01	0.22±<0.01	0.24±<0.01	0.09±<0.01	0.93±0.03	1.13±0.03	0.14±0.01	2.56±0.03	8.34±0.55	1	1.50	172
		43040	3	0.03±<0.01	0.25±<0.01	0.28±<0.01	0.07±<0.01	0.81±0.05	1.05±0.05	0.11±<0.01	3.78±0.06	9.58±0.42	1	1.50	165
		43070	1	0.03	0.27	0.30	0.09	0.85	1.10	0.14	3.46	7.86	1	0.60	105
		43101	1	0.02	0.28	0.30	0.07	0.96	1.20	0.09	4.56	13.04	1	0.60	75
		43132	3	BDL	BDL	<0.01±<0.01	0.02±<0.01	0.71±0.02	0.71±0.02	0.05±<0.01	0.00±<0.01	15.43±0.68	1	1.00	55
		43160	3	0.03±<0.01	BDL	0.03±<0.01	0.05±<0.01	0.79±0.01	0.79±0.01	0.09±0.01	0.52±0.02	8.51±0.89	1	0.60	44
		43191	1	BDL	BDL	<0.01	0.07	0.93	0.93	0.11	<0.01	8.45	1	1.90	47
		43221	1	BDL	BDL	<0.01	0.09	1.10	1.10	0.26	<0.01	4.23	1	1.00	40

Appendix 4. Monthly averages (± standard error, except where only one measurement was made) of dissolved nutrient concentrations, biochemical oxygen demand (BOD), and color concentration data by site for Powell Creek and the central drainage basin surface water sites; BDL = below detection level.

Drainage Basin	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 Nitrogen (mg/L)	Total Phosphorus (mg/L)	DIN:SRP	TN:TP	Count	Biochemical Oxygen Demand (mg/L)	Color (CU)
Powell Creek	SW4	Oct-17	3	0.07±0.00	0.11±0.00	0.18±0.00	0.07±0.00	0.77±0.05	0.88±0.05	0.13±0.00	2.58±0.02	6.94±0.27	1	0.70	89
		Nov-17	3	0.04±0.00	0.08±0.00	0.12±0.00	0.08±0.00	0.70±0.03	0.78±0.03	0.12±0.01	1.52±0.04	6.62±0.76	1	0.70	63
		Dec-17	1	0.03	0.08	0.11	0.12	0.64	0.72	0.15	0.97	4.80	1	0.40	43
		Jan-18	1	0.06	0.10	0.16	0.19	0.65	0.75	0.22	0.88	3.41	1	BDL	36
		Feb-18	3	0.05±0.00	0.08±0.00	0.12±0.00	0.18±0.00	0.21±0.02	0.29±0.02	0.22±0.00	0.68±0.00	1.31±0.07	1	0.60	40
		Mar-18	3	0.03±0.00	0.09±0.00	0.12±0.00	0.23±0.00	0.49±0.02	0.59±0.02	0.32±0.00	0.54±0.02	1.86±0.06	1	1.20	29
		Apr-18	1	BDL	0.02	0.02	0.42	0.58	0.60	0.57	0.05	1.05	1	1.80	40
		May-18	1	0.12	0.03	0.15	0.44	0.64	0.67	0.46	0.33	1.46	1	0.50	57
	SW1	Oct-17	3	0.07±0.00	0.11±0.00	0.18±0.00	0.07±0.00	0.97±0.12	1.07±0.12	0.13±0.00	2.47±0.04	8.23±0.91	1	0.30	88
		Nov-17	3	0.04±0.00	0.08±0.00	0.12±0.00	0.08±0.00	0.56±0.02	0.64±0.01	0.11±0.00	1.52±0.03	5.85±0.13	1	0.80	63
		Dec-17	1	0.03	0.09	0.12	0.11	0.57	0.66	0.14	1.08	4.71	1	0.60	44
		Jan-18	1	0.04	0.10	0.14	0.17	0.38	0.48	0.22	0.80	2.18	1	BDL	35
		Feb-18	3	0.04±0.01	0.07±0.00	0.10±0.01	0.19±0.00	0.23±0.03	0.30±0.03	0.24±0.01	0.55±0.05	1.23±0.10	1	0.80	41
		Mar-18	3	0.02	0.04±0.00	0.05±0.01	0.23±0.00	0.52±0.02	0.56±0.02	0.30±0.01	0.21±0.02	1.87±0.02	1	1.50	37
		Apr-18	1	0.06	0.01	0.07	0.44	0.72	0.73	0.52	0.17	1.40	1	1.40	59
		May-18	1	0.14	0.06	0.20	0.36	0.87	0.93	0.37	0.56	2.51	1	0.60	68
	SW2	Oct-17	3	0.08±0.00	0.13±0.00	0.21±0.00	0.07±0.00	0.75±0.05	0.88±0.05	0.12±0.01	2.90±0.02	7.37±0.72	1	0.50	91
		Nov-17	3	0.07±0.00	0.10±0.00	0.16±0.00	0.07±0.00	0.66±0.05	0.76±0.05	0.11±0.00	2.37±0.00	6.91±0.42	1	1.00	68
		Dec-17	1	0.05	0.10	0.14	0.08	0.66	0.76	0.14	1.80	5.43	1	0.70	48
		Jan-18	1	0.11	0.14	0.26	0.11	0.66	0.80	0.18	2.29	4.44	1	0.40	49
		Feb-18	3	BDL	BDL	0.00±0.00	0.15±0.00	0.37±0.03	0.37±0.03	0.21±0.01	0.00±0.00	1.75±0.14	1	1.70	53
		Mar-18	3	0.07±0.00	0.03±0.00	0.10±0.00	0.16±0.00	0.83±0.03	0.86±0.03	0.22±0.01	0.61±0.02	3.90±0.14	1	1.20	52
		Apr-18	1	0.07	0.02	0.08	0.28	1.00	1.00	0.35	0.29	2.86	1	1.40	71
		May-18	1	0.10	0.04	0.14	0.27	0.89	0.93	0.29	0.52	3.21	1	0.90	78
	SW3	Oct-17	3	0.06±0.00	0.13±0.00	0.19±0.00	0.07±0.00	0.73±0.01	0.86±0.01	0.12±0.00	2.83±0.02	7.01±0.26	1	1.00	90
		Nov-17	3	0.05±0.00	0.10±0.00	0.15±0.00	0.06±0.00	0.68±0.06	0.78±0.06	0.11±0.01	2.33±0.06	6.88±0.20	1	0.90	71
		Dec-17	1	0.03	0.16	0.19	0.07	0.85	1.00	0.12	2.54	8.33	1	0.60	72
		Jan-18	1	0.08	0.18	0.26	0.10	0.77	0.95	0.14	2.69	6.79	1	0.40	63
		Feb-18	3	0.01	BDL	0.00±0.00	0.10±0.00	0.41±0.04	0.41±0.04	0.15±0.00	0.00±0.00	2.69±0.21	1	2.40	54
		Mar-18	3	0.04±0.00	0.02±0.00	0.06±0.00	0.10±0.00	0.84±0.01	0.86±0.01	0.13±0.00	0.57±0.01	6.48±0.16	1	1.40	50
		Apr-18	1	0.03	BDL	0.03	0.14	0.90	0.90	0.17	0.20	5.29	1	1.40	62
		May-18	1	0.03	0.01	0.04	0.18	0.83	0.84	0.25	0.22	3.36	1	1.10	75
Central Drainage	SW5	Oct-17	3	0.29±0.00	0.09±0.00	0.38±0.00	0.18±0.00	1.23±0.09	1.33±0.09	0.24±0.00	2.15±0.01	5.47±0.29	1	0.60	158
		Nov-17	3	0.20±0.00	0.19±0.00	0.38±0.00	0.10±0.00	1.03±0.09	1.23±0.09	0.23±0.00	3.76±0.02	5.77±0.26	1	1.80	147
		Dec-17	1	0.07	0.22	0.29	0.09	0.99	1.20	0.14	3.24	8.57	1	0.70	97
		Jan-18	1	0.09	0.13	0.22	0.06	0.92	1.10	0.10	3.73	11.00	1	0.60	77
		Feb-18	3	0.02±0.00	0.01	0.02±0.01	0.06±0.00	0.82±0.14	0.82±0.14	0.17±0.00	0.35±0.08	4.75±0.88	1	4.40	73
		Mar-18	3	0.04±0.00	0.02±0.00	0.06±0.00	0.06±0.00	0.97±0.07	0.98±0.06	0.10±0.01	1.03±0.01	9.87±0.37	1	1.00	48
		Apr-18	1	0.05	0.01	0.06	0.10	0.81	0.82	0.18	0.61	4.56	1	1.30	51
		May-18	1	0.11	0.15	0.26	0.19	0.98	1.10	0.22	1.37	5.00	1	0.90	59

Appendix 5. Overall average (± standard error) dissolved nutrient concentrations by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area), and site; BDL = below detection level.

Water Type	Drainage Basin	Site	Count	Ammonium (mg/L)	Nitrate + Nitrite (mg/L)	Dissolved Inorganic Nitrogen (mg/L)	Soluble Reactive Phosphorus (mg/L)	Total Kjeldahl Nitrogen (mg/L)	Total Nitrogen (mg/L)	Total Phosphorus (mg/L)	DIN:SRP	TN:TP
Surface Water	Hancock Creek	SW9	12	0.04±<0.01	0.04±0.01	0.06±0.01	0.18±0.01	0.38±0.02	0.41±0.02	0.21±0.01	0.44±0.10	2.04±0.15
		SW8	12	0.04±0.01	0.06±0.01	0.07±0.01	0.16±0.01	0.72±0.07	0.76±0.06	0.21±0.01	0.46±0.09	3.61±0.22
		SW7	12	0.05±0.01	0.04±0.01	0.04±0.01	0.12±0.01	0.71±0.03	0.73±0.03	0.18±0.01	0.28±0.08	4.23±0.20
		16-3GR	12	0.03±<0.01	0.04±0.01	0.05±0.01	0.10±0.01	0.78±0.04	0.81±0.03	0.17±0.01	0.49±0.08	4.93±0.34
		SW6	12	0.03±<0.01	0.23±0.01	0.14±0.04	0.06±0.01	0.81±0.03	0.92±0.05	0.10±0.01	1.71±0.46	10.47±0.92
	Powell Creek	SW4	12	0.05±<0.01	0.09±<0.01	0.14±0.01	0.14±0.02	0.54±0.07	0.64±0.07	0.20±0.02	1.33±0.24	4.18±0.80
		SW1	12	0.05±0.01	0.08±0.01	0.11±0.01	0.14±0.02	0.57±0.08	0.64±0.09	0.20±0.02	1.19±0.27	4.29±0.89
		SW2	12	0.07±<0.01	0.08±0.01	0.12±0.02	0.11±0.01	0.65±0.06	0.72±0.06	0.17±0.02	1.47±0.36	4.98±0.72
		SW3	12	0.05±<0.01	0.08±0.01	0.10±0.02	0.08±0.01	0.67±0.05	0.73±0.06	0.13±<0.01	1.43±0.36	5.77±0.55
	Central Drainage	SW5	12	0.14±0.03	0.09±0.02	0.21±0.05	0.10±0.01	1.01±0.06	1.09±0.07	0.18±0.02	1.82±0.39	6.53±0.67
Groundwater	Hancock Creek	GW1	12	0.33±0.02	1.42±0.43	1.39±0.40	0.96±0.13	1.39±0.09	2.46±0.43	1.08±0.13	2.01±0.64	2.76±0.66
		GW2	12	0.25±0.03	0.08±0.02	0.30±0.02	0.82±0.18	0.96±0.13	1.02±0.11	1.62±0.34	0.58±0.10	0.81±0.14
		GW3	12	0.49±0.03	0.01±<0.01	0.49±0.03	0.41±0.03	1.12±0.07	1.12±0.07	0.44±0.03	1.25±0.10	2.71±0.28
	Powell Creek	GW7	12	7.88±1.10	0.02±<0.01	7.89±1.10	0.35±0.07	8.08±1.11	7.90±1.07	0.44±0.08	25.09±2.06	19.38±1.45
		GW8	12	4.58±1.15	0.01±<0.01	4.58±1.15	0.21±0.05	4.77±1.13	4.62±1.06	0.23±0.05	23.05±1.09	22.01±1.53
		GW9	12	10.65±1.16	0.02±<0.01	10.66±1.16	0.45±0.07	11.64±1.14	11.64±1.14	0.48±0.06	25.53±1.14	25.32±1.16
	Central Drainage	GW4	12	1.47±0.08	BDL	1.47±0.08	0.29±0.01	1.73±0.05	1.73±0.05	0.32±0.01	5.02±0.17	5.44±0.11
		GW5	12	1.58±0.07	0.01±<0.01	1.58±0.07	0.43±0.01	2.33±0.06	2.33±0.06	0.46±0.01	3.73±0.19	5.11±0.14
		GW6	12	1.66±0.16	0.01±<0.01	1.67±0.16	0.52±0.03	2.87±0.27	2.87±0.27	0.58±0.04	3.17±0.20	4.90±0.22
	Reference	GW10	12	0.23±0.01	0.02±<0.01	0.23±0.01	0.07±<0.01	0.61±0.03	0.62±0.03	0.08±0.01	3.26±0.10	7.84±0.35

Appendix 6. Seasonal averages (± standard error, except where only one detection occurred) for dissolved nutrient concentrations by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area), site, and season (wet 2017 and dry 2018); BDL = below detection level.

			Wet Season 2017									
Water Type	**Drainage Basin**	**Site**	**Count**	**Ammonium (mg/L)**	**Nitrate + Nitrite (mg/L)**	**Dissolved Inorganic Nitrogen (mg/L)**	**Soluble Reactive Phosphorus (mg/L)**	**Total Kjeldahl Nitrogen (mg/L)**	**Total Nitrogen (mg/L)**	**Total Phosphorus (mg/L)**	**DIN:SRP**	**TN:TP**
Surface Water	Hancock Creek	SW9	6	0.04±0.01	0.06±<0.01	0.10±0.01	0.14±<0.01	0.37±0.01	0.43±0.01	0.18±<0.01	0.75±0.04	2.49±0.12
		SW8	6	0.02±<0.01	0.08±<0.01	0.09±0.01	0.14±0.01	0.57±0.06	0.65±0.06	0.20±0.01	0.67±0.09	3.29±0.32
		SW7	6	0.014	0.05±0.01	0.04±0.01	0.12±0.01	0.63±0.03	0.67±0.05	0.18±0.02	0.26±0.09	3.77±0.22
		16-3GR	6	0.03±<0.01	0.05±0.01	0.08±0.01	0.12±0.01	0.69±0.04	0.75±0.04	0.18±0.01	0.66±0.01	4.21±0.38
		SW6	6	0.03±<0.01	0.23±0.01	0.26±0.01	0.08±<0.01	0.87±0.04	1.09±0.03	0.12±0.01	3.17±0.27	8.96±0.42
	Powell Creek	SW4	6	0.06±0.01	0.10±0.01	0.15±0.01	0.08±<0.01	0.74±0.03	0.83±0.04	0.12±<0.01	2.05±0.24	6.78±0.37
		SW1	6	0.05±0.01	0.10±0.01	0.15±0.01	0.08±<0.01	0.76±0.11	0.86±0.11	0.12±<0.01	1.99±0.21	7.04±0.67
		SW2	6	0.07±<0.01	0.11±0.01	0.18±0.01	0.07±<0.01	0.71±0.04	0.82±0.04	0.12±<0.01	2.63±0.12	7.14±0.39
		SW3	6	0.06±<0.01	0.12±0.01	0.17±0.01	0.07±<0.01	0.71±0.03	0.82±0.03	0.12±<0.01	2.58±0.12	6.95±0.15
	Central Drainage	SW5	6	0.24±0.02	0.14±0.02	0.38±<0.01	0.14±0.02	1.13±0.07	1.28±0.06	0.24±<0.01	2.95±0.36	5.59±0.18
Groundwater	Hancock Creek	GW1	6	0.38±0.01	0.01±<0.01	0.39±0.01	1.28±0.17	1.48±0.14	1.48±0.14	1.37±0.19	0.34±0.05	1.13±0.06
		GW2	6	0.32±0.04	0.01±<0.01	0.32±0.04	1.30±0.21	1.37±0.08	1.37±0.08	1.41±0.19	0.31±0.08	1.11±0.20
		GW3	6	0.53±<0.01	0.01±<0.01	0.53±<0.01	0.36±0.03	1.32±0.03	1.32±0.03	0.40±0.03	1.53±0.11	3.45±0.32
	Powell Creek	GW7	6	4.62±0.87	0.02±<0.01	4.63±0.87	0.16±0.02	4.73±0.90	4.73±0.90	0.22±0.03	27.77±2.96	20.66±1.92
		GW8	6	2.12±0.26	0.01±<0.01	2.12±0.26	0.09±0.02	2.36±0.18	2.37±0.18	0.11±0.02	25.25±1.72	24.22±2.78
		GW9	6	6.97±0.65	BDL	6.97±0.65	0.24±0.02	8.20±0.89	8.20±0.89	0.29±0.02	29.00±0.27	28.07±1.57
	Central Drainage	GW4	6	1.58±0.14	BDL	1.58±0.14	0.32±0.01	1.83±0.08	1.83±0.08	0.35±0.01	4.96±0.30	5.21±0.16
		GW5	6	1.73±0.10	0.01±<0.01	1.73±0.10	0.43±0.01	2.50±0.07	2.50±0.07	0.48±0.01	4.03±0.28	5.24±0.19
		GW6	6	2.11±0.09	0.01±<0.01	2.11±0.09	0.62±0.01	3.70±0.12	3.70±0.12	0.71±0.01	3.41±0.20	5.23±0.25
	Reference	GW10	6	0.24±<0.01	0.02±<0.01	0.25±<0.01	0.08±<0.01	0.67±0.04	0.68±0.05	0.10±<0.01	3.00±0.03	6.94±0.43

			Dry Season 2018									
Water Type	**Drainage Basin**	**Site**	**Count**	**Ammonium (mg/L)**	**Nitrate + Nitrite (mg/L)**	**Dissolved Inorganic Nitrogen (mg/L)**	**Soluble Reactive Phosphorus (mg/L)**	**Total Kjeldahl Nitrogen (mg/L)**	**Total Nitrogen (mg/L)**	**Total Phosphorus (mg/L)**	**DIN:SRP**	**TN:TP**
Surface Water	Hancock Creek	SW9	6	0.04±<0.01	0.01±<0.01	0.03±0.01	0.22±0.01	0.38±0.03	0.39±0.03	0.24±0.01	0.12±0.03	1.60±0.10
		SW8	6	0.07±<0.01	0.02±<0.01	0.04±0.02	0.17±0.01	0.87±0.08	0.88±0.08	0.22±0.01	0.25±0.11	3.93±0.28
		SW7	6	0.06±<0.01	0.01±<0.01	0.04±0.02	0.11±0.01	0.79±0.03	0.80±0.03	0.17±0.01	0.30±0.13	4.70±0.22
		16-3GR	6	0.05±<0.01	0.01±<0.01	0.03±0.01	0.09±0.01	0.87±0.03	0.88±0.03	0.16±0.01	0.31±0.14	5.66±0.39
		SW6	6	0.03±<0.01	BDL	0.01±0.01	0.04±0.01	0.75±0.02	0.75±0.02	0.07±0.01	0.26±0.12	11.97±1.63
	Powell Creek	SW4	6	0.04±<0.01	0.09±<0.01	0.12±<0.01	0.20±0.01	0.35±0.06	0.44±0.07	0.27±0.02	0.61±0.03	1.59±0.13
		SW1	6	0.03±0.01	0.05±0.01	0.08±0.01	0.21±0.01	0.38±0.07	0.43±0.06	0.27±0.01	0.38±0.08	1.55±0.15
		SW2	6	0.07±<0.01	0.03±<0.01	0.05±0.02	0.15±<0.01	0.60±0.10	0.61±0.11	0.22±<0.01	0.30±0.14	2.82±0.49
		SW3	6	0.04±0.01	0.02±<0.01	0.03±0.01	0.10±<0.01	0.63±0.10	0.64±0.10	0.14±<0.01	0.29±0.13	4.59±0.86
	Central Drainage	SW5	6	0.03±0.01	0.02±<0.01	0.04±0.01	0.06±<0.01	0.90±0.08	0.90±0.08	0.14±0.02	0.69±0.16	7.31±1.22
Groundwater	Hancock Creek	GW1	6	0.28±0.02	2.12±0.54	2.40±0.56	0.65±0.01	1.30±0.12	3.43±0.66	0.80±0.02	3.68±0.84	4.39±0.91
		GW2	6	0.18±0.01	0.11±0.03	0.29±0.03	0.34±0.01	0.56±0.07	0.67±0.04	1.83±0.67	0.86±0.10	0.51±0.10
		GW3	6	0.45±0.05	BDL	0.45±0.05	0.46±0.05	0.91±0.04	0.91±0.04	0.48±0.05	0.97±0.01	1.98±0.14
	Powell Creek	GW7	6	11.14±0.52	0.03±0.01	11.16±0.52	0.53±0.07	11.42±0.39	11.07±0.45	0.67±0.10	22.41±2.65	18.10±2.20
		GW8	6	7.04±1.83	0.02±<0.01	7.05±1.83	0.33±0.08	7.17±1.82	6.88±1.70	0.34±0.08	20.85±0.58	19.81±0.78
		GW9	6	14.33±0.30	0.02±<0.01	14.34±0.30	0.66±0.03	15.08±0.45	15.08±0.45	0.67±0.04	22.06±0.90	22.56±0.66
	Central Drainage	GW4	6	1.37±0.05	BDL	1.37±0.05	0.27±0.01	1.62±0.04	1.62±0.04	0.29±<0.01	5.09±0.20	5.67±0.11
		GW5	6	1.43±0.05	BDL	1.43±0.05	0.42±0.01	2.17±0.02	2.17±0.02	0.44±0.02	3.44±0.20	4.97±0.21
		GW6	6	1.22±0.14	0.02±<0.01	1.23±0.15	0.42±<0.01	2.03±0.14	2.03±0.14	0.45±<0.01	2.93±0.35	4.56±0.34
	Reference	GW10	6	0.22±0.01	BDL	0.22±0.01	0.06±<0.01	0.55±0.01	0.55±0.01	0.06±<0.01	3.52±0.14	8.73±0.21

Appendix 7. Overall average (± standard error, except where only one detection occurred) bacterial concentrations and molecular markers by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area), and site; BDL = below detection level, NA = not analyzed.

Water Type	Drainage Basin	Site	Count	Enterococci (MPN/100mL)	*Escherischia coli* (MPN/100mL)	BacR-qPCR (TSC/100mL)	GFD-purified-qPCR (TSC/100mL)	GULL2-qPCR (TSC/100mL)	HF183-qPCR (GEU/100mL)
Surface Water	Hancock Creek	SW9	4	743±568	266±52	130	157±70	1,200	34±14
		SW8	4	119±70	296±114	BDL	164±72	620	36
		SW7	4	259±102	393±104	160	1,433±833	1,580±776	29±7
		16-3GR	4	1,269±423	693±85	150	7,025±444	14,775±2,443	113±73
		SW6	4	509±492	137±49	710	503±381	31,400±25,227	BDL
	Powell Creek	SW4	4	1,254±673	1,300±647	BDL	243±180	BDL	478±405
		SW1	4	1,273±662	1,312±640	BDL	453±358	240	117±59
		SW2	4	583±115	667±108	BDL	89±48	510	166±90
		SW3	4	344±76	549±198	BDL	126±89	1,000	493±443
	Central Drainage	SW5	4	1,199±265	1,724±1,060	330	67±29	BDL	653±279
Groundwater	Hancock Creek	GW1	4	1	BDL	NA	NA	NA	NA
		GW2	4	1	BDL	NA	NA	NA	NA
		GW3	4	2	BDL	NA	NA	NA	NA
	Powell Creek	GW7	4	31±11	BDL	NA	NA	NA	NA
		GW8	4	3	BDL	NA	NA	NA	NA
		GW9	4	811±697	45	NA	NA	NA	NA
	Central Drainage	GW4	4	2±1	1	NA	NA	NA	NA
		GW5	4	49	294±202	NA	NA	NA	NA
		GW6	4	2±<1	BDL	NA	NA	NA	NA
	Reference	GW10	4	4±1	BDL	NA	NA	NA	NA

Appendix 8. Monthly averages (± standard error, except where only one measurement was made) of bacteria concentration data by site for Hancock Creek surface water sites.

Drainage Basin	Site	Month	Count	Enterococci (MPN/100mL)	*Escherischia coli* (MPN/100mL)
Hancock Creek	SW9	Oct-17	1	45	291
		Nov-17	1	461	365
		Dec-17	1	727	649
		Jan-18	1	238	548
		Feb-18	1	2420	291
		Mar-18	1	44	118
		Apr-18	1	77	613
		May-18	1	1414	299
	SW8	Oct-17	1	55	146
		Nov-17	1	32	59
		Dec-17	1	687	199
		Jan-18	1	299	236
		Feb-18	1	328	517
		Mar-18	1	59	461
		Apr-18	1	387	517
		May-18	1	1986	866
	SW7	Oct-17	1	192	548
		Nov-17	1	68	144
		Dec-17	1	770	206
		Jan-18	1	345	345
		Feb-18	1	548	299
		Mar-18	1	228	579
		Apr-18	1	2420	2420
		May-18	1	2420	1203
	16-3GR	Oct-17	2	343±65	492±40
		Nov-17	2	735±132	850±191
		Dec-17	2	677±261	581±23
		Jan-18	2	1812±430	813±165
		Feb-18	2	2420±0	921±0
		Mar-18	2	881±297	669±39
		Apr-18	2	1595±583	752±46
		May-18	1	2420	1120
	SW6	Oct-17	1	7	56
		Nov-17	1	17	130
		Dec-17	1	55	70
		Jan-18	1	26	91
		Feb-18	1	1986	276
		Mar-18	1	25	84
		Apr-18	1	101	74
		May-18	1	344	649

Appendix 9. Monthly averages (± standard error, except where only measurement was made) of bacteria concentration data by site for Powell Creek and the central drainage basin surface water sites.

Drainage Basin	Site	Month	Count	Enterococci (MPN/100mL)	*Escherischia coli* (MPN/100mL)
Powell Creek	SW4	Oct-17	1	76	172
		Nov-17	1	98	186
		Dec-17	1	2420	2420
		Jan-18	1	2420	2420
		Feb-18	1	2420	2420
		Mar-18	1	2420	2420
		Apr-18	1	2420	2420
		May-18	1	2420	1300
	SW1	Oct-17	1	97	208
		Nov-17	1	155	201
		Dec-17	1	2420	2420
		Jan-18	1	2420	2420
		Feb-18	1	2420	2420
		Mar-18	1	2420	2420
		Apr-18	1	276	1203
		May-18	1	2420	921
	SW2	Oct-17	1	281	461
		Nov-17	1	548	517
		Dec-17	1	1300	1046
		Jan-18	1	2420	2420
		Feb-18	1	816	921
		Mar-18	1	687	770
		Apr-18	1	649	770
		May-18	1	2420	517
	SW3	Oct-17	1	488	1120
		Nov-17	1	133	261
		Dec-17	1	579	291
		Jan-18	1	921	921
		Feb-18	1	344	517
		Mar-18	1	411	299
		Apr-18	1	435	727
		May-18	1	1733	488
Central Drainage	SW5	Oct-17	1	921	272
		Nov-17	1	1986	517
		Dec-17	1	2967	1196
		Jan-18	1	2853	1890
		Feb-18	1	854	4840
		Mar-18	1	1036	1266
		Apr-18	1	2420	1733
		May-18	1	2420	1986

Appendix 10. Seasonal averages (± standard error, except where only one detection occurred) for bacterial concentrations and molecular markers by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area), site, and season (wet 2017 and dry 2018); BLD = below detection level, NA = not analyzed.

Water Type	Drainage Basin	Site	*Wet Season 2017*						
			Count	**Enterococci (MPN/100mL)**	***Escherichia coli* (MPN/100mL)**	**BacR-qPCR (TSC/100mL)**	**GFD-purified-qPCR (TSC/100mL)**	**GULL2-qPCR (TSC/100mL)**	**HF183-qPCR (GEU/100mL)**
Surface Water	Hancock Creek	SW9	2	253±208	328±37	BDL	90±41	BDL	34±20
		SW8	2	44±12	103±44	BDL	92±69	BDL	BDL
		SW7	2	130±62	346±202	160	1,985±1,365	2,285±1,615	18±4
		16-3GR	2	678±243	564±16	150	7,780±200	15,050±5,950	22
		SW6	2	12±5	93±37	710	935±705	5,050±2,050	BDL
	Powell Creek	SW4	2	87±11	179±7	BDL	41±25	BDL	46±26
		SW1	2	126±29	205±4	BDL	53	240	42±18
		SW2	2	415±134	489±28	BDL	54±22	BDL	104±86
		SW3	2	311±178	691±430	BDL	16±2	BDL	919±901
	Central Drainage	SW5	2	1,454±533	395±123	330	35±17	BDL	1,085±295
Groundwater	Hancock Creek	GW1	2	BDL	BDL	NA	NA	NA	NA
		GW2	2	1	BDL	NA	NA	NA	NA
		GW3	2	BDL	BDL	NA	NA	NA	NA
	Powell Creek	GW7	2	14±4	BDL	NA	NA	NA	NA
		GW8	2	BDL	BDL	NA	NA	NA	NA
		GW9	2	1,217±1,204	45	NA	NA	NA	NA
	Central Drainage	GW4	2	3	BDL	NA	NA	NA	NA
		GW5	2	BDL	BDL	NA	NA	NA	NA
		GW6	2	2	BDL	NA	NA	NA	NA
	Reference	GW10	2	5±1	BDL	NA	NA	NA	NA

Water Type	Drainage Basin	Site	*Dry Season 2018*						
			Count	**Enterococci (MPN/100mL)**	***Escherichia coli* (MPN/100mL)**	**BacR-qPCR (TSC/100mL)**	**GFD-purified-qPCR (TSC/100mL)**	**GULL2-qPCR (TSC/100mL)**	**HF183-qPCR (GEU/100mL)**
Surface Water	Hancock Creek	SW9	2	1,232±1,188	205±87	130	224±136	1,200	BDL
		SW8	2	194±135	489±28	BDL	310	620	36
		SW7	2	388±160	439±140	BDL	330	875±105	41±4
		16-3GR	2	1,860±560	823±99	BDL	6,270±50	14,500±500	158±122
		SW6	2	1,006±981	180±96	BDL	71±2	57,750±49,250	BDL
	Powell Creek	SW4	2	2,420±<1	2,420±<1	BDL	445±335	BDL	910±780
		SW1	2	2,420±<1	2,420±<1	BDL	653±628	BDL	192±99
		SW2	2	752±65	846±76	BDL	125±106	510	227±183
		SW3	2	378±34	408±109	BDL	237±154	1,000	67±34
	Central Drainage	SW5	2	945±91	3,053±1,787	BDL	130	BDL	220±80
Groundwater	Hancock Creek	GW1	2	1	BDL	NA	NA	NA	NA
		GW2	2	BDL	BDL	NA	NA	NA	NA
		GW3	2	2	BDL	NA	NA	NA	NA
	Powell Creek	GW7	2	49±12	BDL	NA	NA	NA	NA
		GW8	2	3	BDL	NA	NA	NA	NA
		GW9	2	1	BDL	NA	NA	NA	NA
	Central Drainage	GW4	2	2±1	1	NA	NA	NA	NA
		GW5	2	49	294±286	NA	NA	NA	NA
		GW6	2	2±1	BDL	NA	NA	NA	NA
	Reference	GW10	2	3	BDL	NA	NA	NA	NA

Appendix 11. Overall averages (± standard error, except where only one detection occurred) of chemical tracers by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area), and site; BDL = below detection level.

Water Type	Drainage Basin	Site	Count	2,4-D (µg/L)	Acetaminophen (µg/L)	Bentazon (µg/L)	Carbamazepine (µg/L)	Primidone (µg/L)	Sucralose (µg/L)	Count	Diuron (µg/L)	Fluridone (µg/L)	Imazapyr (µg/L)	Imidacloprid (µg/L)
Surface Water	Hancock Creek	SW9	4	BDL	BDL	0.002±<0.001	0.001±<0.001	BDL	0.215±0.017	1	BDL	0.012	0.028	BDL
		SW8	4	0.007±0.003	BDL	0.007±0.002	0.004±<0.001	0.008±0.001	0.790±0.183	1	0.002	0.095	0.036	0.004
		SW7	4	0.013±0.005	BDL	0.010±0.003	0.003±<0.001	0.006±0.001	0.678±0.159	1	0.003	0.048	0.031	0.005
		16-3GR	4	0.014±0.005	BDL	0.010±0.003	0.002±<0.001	0.005±<0.001	0.533±0.105	1	0.004	0.024	0.016	0.005
		SW6	4	0.030±0.012	BDL	0.007±0.002	0.001±<0.001	0.004	0.358±0.166	1	0.003	0.003	0.020	0.009
	Powell Creek	SW4	4	BDL	BDL	0.002±<0.001	0.015±0.001	0.009±0.001	0.958±0.282	1	BDL	0.006	0.038	0.004
		SW1	4	BDL	BDL	0.002±<0.001	0.013±0.001	0.010±0.003	0.968±0.283	1	BDL	0.005	0.034	0.003
		SW2	4	0.009±<0.001	0.021	0.004±0.001	0.010±0.002	0.006±0.001	0.830±0.213	1	BDL	0.003	0.034	0.005
		SW3	4	0.008±0.001	0.019±0.008	0.005±0.001	0.009±0.003	0.005±0.001	0.673±0.130	1	BDL	0.003	0.023	0.007
	Central Drainage	SW5	4	0.019±0.013	0.012±<0.001	0.009±0.003	0.002±<0.001	BDL	0.620±0.037	1	0.004	0.003	0.022	0.008
Groundwater	Hancock Creek	GW1	4	BDL	BDL	BDL	0.008±0.001	0.019±0.002	41.675±11.887	1	BDL	BDL	BDL	BDL
		GW2	4	BDL	BDL	BDL	0.008±0.002	0.010±0.005	3.755±2.750	1	BDL	BDL	BDL	BDL
		GW3	4	BDL	BDL	BDL	0.001±<0.001	BDL	2.278±0.543	1	BDL	BDL	BDL	BDL
	Powell Creek	GW7	4	BDL	0.023	BDL	BDL	BDL	0.299±0.135	1	BDL	BDL	BDL	BDL
		GW8	4	BDL	BDL	BDL	BDL	BDL	3.128±2.336	1	BDL	BDL	BDL	BDL
		GW9	4	BDL	BDL	0.023±0.008	BDL	0.024±0.009	0.526±0.209	1	BDL	BDL	BDL	BDL
	Central Drainage	GW4	4	BDL	BDL	0.002±<0.001	0.004±0.001	0.038±0.005	0.253±0.047	1	BDL	BDL	BDL	BDL
		GW5	4	BDL	BDL	BDL	0.008±0.001	BDL	19.250±5.056	1	BDL	BDL	BDL	BDL
		GW6	4	BDL	BDL	BDL	0.001±<0.001	BDL	4.275±0.949	1	BDL	BDL	BDL	BDL
	Reference	GW10	4	BDL	BDL	0.002±<0.001	BDL	BDL	0.178±0.046	1	BDL	0.002	BDL	BDL

Appendix 12. Seasonal averages (± standard error, except where only one detection occurred) of chemical tracers by water type (surface water and groundwater), drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference site), site, and season (wet 2017 and dry 2018); BDL = below detection level, NA = not analyzed.

Water Type	Drainage Basin	Site	*Wet Season 2017* Count	2,4-D (µg/L)	Acetaminophen (µg/L)	Bentazon (µg/L)	Carbamazepine (µg/L)	Primidone (µg/L)	Sucralose (µg/L)	Count	Diuron (µg/L)	Fluridone (µg/L)	Imazapyr (µg/L)	Imidacloprid (µg/L)
Surface Water	Hancock Creek	SW9	2	BDL	BDL	0.003±0.001	0.001±<0.001	BDL	0.220±0.028	0	NA	NA	NA	NA
		SW8	2	0.014	BDL	0.010±0.003	0.004±<0.001	0.006	0.760±0.311	0	NA	NA	NA	NA
		SW7	2	0.020±0.008	BDL	0.012±0.004	0.003±<0.001	BDL	0.605±0.265	0	NA	NA	NA	NA
		16-3GR	2	0.022±0.005	BDL	0.012±0.003	0.002±<0.001	BDL	0.470±0.163	0	NA	NA	NA	NA
		SW6	2	0.051±0.005	BDL	0.008±0.003	0.001	BDL	0.086±0.000	0	NA	NA	NA	NA
	Powell Creek	SW4	2	BDL	BDL	0.002±0.001	0.016±0.001	BDL	0.565±0.230	0	NA	NA	NA	NA
		SW1	2	BDL	BDL	0.002±0.001	0.015±0.001	BDL	0.585±0.251	0	NA	NA	NA	NA
		SW2	2	BDL	BDL	0.004±<0.001	0.014±0.001	BDL	0.640±0.247	0	NA	NA	NA	NA
		SW3	2	BDL	0.03	0.006±0.001	0.014±0.002	BDL	0.585±0.202	0	NA	NA	NA	NA
	Central Drainage	SW5	2	0.030±0.018	0.012±<0.001	0.013±0.004	0.002±0.001	BDL	0.640±0.042	0	NA	NA	NA	NA
Ground water	Hancock Creek	GW1	2	BDL	BDL	BDL	0.010±<0.001	0.017±<0.001	26.850±14.248	0	NA	NA	NA	NA
		GW2	2	BDL	BDL	BDL	0.008±0.005	0.015±0.007	6.360±3.988	0	NA	NA	NA	NA
		GW3	2	BDL	BDL	BDL	0.001±<0.001	BDL	2.500±0.424	0	NA	NA	NA	NA
	Powell Creek	GW7	2	BDL	0.023	BDL	BDL	BDL	0.069±0.022	0	NA	NA	NA	NA
		GW8	2	BDL	BDL	BDL	BDL	BDL	0.013	0	NA	NA	NA	NA
		GW9	2	BDL	BDL	0.011±0.008	BDL	0.008	0.049	0	NA	NA	NA	NA
	Central Drainage	GW4	2	BDL	BDL	0.002±0.001	0.005±0.001	0.034±0.006	0.260±0.078	0	NA	NA	NA	NA
		GW5	2	BDL	BDL	BDL	0.010±0.002	BDL	25.000±6.364	0	NA	NA	NA	NA
		GW6	2	BDL	BDL	BDL	0.002±<0.001	BDL	2.800±0.495	0	NA	NA	NA	NA
	Reference	GW10	2	BDL	BDL	0.002±0.001	BDL	BDL	0.171±0.077	0	NA	NA	NA	NA

Water Type	Location	Site	*Dry Season 2018* Count	2,4-D (µg/L)	Acetaminophen (µg/L)	Bentazon (µg/L)	Carbamazepine (µg/L)	Primidone (µg/L)	Sucralose (µg/L)	Count	Diuron (µg/L)	Fluridone (µg/L)	Imazapyr (µg/L)	Imidacloprid (µg/L)
Surface Water	Hancock Creek	SW9	2	BDL	BDL	0.002	<0.001±<0.001	BDL	0.210±0.000	1	BDL	0.012	0.028	BDL
		SW8	2	0.004±0.001	BDL	0.004±0.001	0.004±<0.001	0.009±0.001	0.820±0.057	1	0.002	0.095	0.036	0.004
		SW7	2	0.006±0.001	BDL	0.007±0.004	0.003±<0.001	0.006±0.001	0.750±0.021	1	0.003	0.048	0.031	0.005
		16-3GR	2	0.007±<0.001	BDL	0.009±0.005	0.002±<0.001	0.005±<0.001	0.595±0.053	1	0.004	0.024	0.016	0.005
		SW6	2	0.009±<0.001	BDL	0.007±0.003	0.002±<0.001	0.004	0.630±0.092	1	0.003	0.003	0.020	0.009
	Powell Creek	SW4	2	BDL	BDL	0.001±<0.001	0.013±<0.001	0.009±0.001	1.350±0.177	1	BDL	0.006	0.038	0.004
		SW1	2	BDL	BDL	0.002±0.001	0.010±0.001	0.010±0.003	1.350±0.177	1	BDL	0.005	0.034	0.003
		SW2	2	0.009±0.001	0.021	0.004±0.001	0.007±0.002	0.006±0.001	1.020±0.198	1	BDL	0.003	0.034	0.005
		SW3	2	0.008±0.002	0.009	0.004±<0.001	0.004±0.001	0.005±0.001	0.760±0.049	1	BDL	0.003	0.023	0.007
	Central Drainage	SW5	2	0.007±0.002	BDL	0.004±<0.001	0.002±<0.001	BDL	0.600±0.042	1	0.004	0.003	0.022	0.008
Ground water	Hancock Creek	GW1	2	BDL	BDL	BDL	0.006±0.002	0.022±0.001	56.500±1.061	1	BDL	BDL	BDL	BDL
		GW2	2	BDL	BDL	BDL	0.009±<0.001	0.006±<0.001	1.150±0.035	1	BDL	BDL	BDL	BDL
		GW3	2	BDL	BDL	BDL	0.001	BDL	2.055±0.810	1	BDL	BDL	BDL	BDL
	Powell Creek	GW7	2	BDL	BDL	BDL	0.007	BDL	0.530±0.028	1	BDL	BDL	BDL	BDL
		GW8	2	BDL	BDL	BDL	BDL	BDL	4.685±2.698	1	BDL	BDL	BDL	BDL
		GW9	2	BDL	BDL	0.034±0.010	BDL	0.033±0.007	0.765±0.039	1	BDL	BDL	BDL	BDL
	Central Drainage	GW4	2	BDL	BDL	BDL	0.004±0.001	0.041±0.004	0.245±0.025	1	BDL	BDL	BDL	BDL
		GW5	2	BDL	BDL	BDL	0.006±<0.001	BDL	13.500±1.768	1	BDL	BDL	BDL	BDL
		GW6	2	BDL	BDL	BDL	0.001±<0.001	BDL	5.750±0.530	1	BDL	BDL	BDL	BDL
	Reference	GW10	2	BDL	BDL	0.002	BDL	BDL	0.185±0.018	1	BDL	0.002	BDL	BDL

Appendix 13. Overall and seasonal averages (± standard error) of particulate organic matter, a proxy for phytoplankton, showing stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) by location (Hancock Creek, Powell Creek, a central drainage basin, *Microcystis aeruginosa* from Davis Ramp, and coastal red tide sites) and site; NS = not sampled.

Water Type	Drainage Basin	Site	*Overall Averages*						*Wet Season 2017*						*Dry Season 2018*					
			Count	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**C:N**	**C:P**	**N:P**	**Count**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**C:N**	**C:P**	**N:P**	**Count**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**C:N**	**C:P**	**N:P**
	Hancock Creek	SW9	12	-33.84±1.05	2.29±0.36	8.37±0.96	12.06±1.05	1.86±0.38	6	-30.44±0.47	2.34±0.64	11.23±0.30	10.71±1.71	0.95±0.15	6	-37.24±0.2	2.24±0.39	5.50±0.82	13.41±1.11	2.78±0.54
		SW8	12	-32.16±0.55	5.21±0.47	7.55±0.48	17.82±1.60	2.52±0.30	6	-31.98±0.25	5.00±0.93	8.54±0.23	13.86±1.21	1.62±0.13	6	-32.33±1.1	5.41±0.32	6.57±0.76	21.77±1.8	3.41±0.21
		SW7	12	-31.32±0.37	6.17±0.62	7.19±0.58	23.30±1.52	3.76±0.68	6	-32.22±0.38	7.03±1.06	8.16±0.16	21.67±1.94	2.65±0.21	6	-30.41±0.3	5.32±0.55	6.22±1.04	24.94±2.32	4.88±1.21
		16-3GR	12	-30.10±0.26	6.00±0.71	7.23±0.60	18.71±1.36	2.88±0.45	6	-30.80±0.13	6.17±1.46	8.37±0.27	18.30±0.81	2.20±0.12	6	-29.40±0.2	5.83±0.32	6.09±1.01	19.12±2.72	3.55±0.84
		SW6	12	-26.59±0.23	7.82±0.75	8.13±0.98	31.36±1.95	4.51±0.56	6	-26.92±0.42	8.27±1.15	10.96±0.70	34.18±2.68	3.13±0.18	6	-26.26±0.1	7.38±1.04	5.29±0.73	28.54±2.52	5.88±0.78
	Powell Creek	SW4	12	-32.98±1.02	4.07±0.68	7.81±0.79	18.51±3.39	2.44±0.35	6	-32.81±0.22	2.39±0.77	8.86±0.21	27.59±4.14	3.12±0.45	6	-33.16±2.1	5.75±0.55	6.76±1.51	9.42±0.71	1.77±0.39
		SW1	12	-33.46±0.59	4.65±0.92	7.22±0.79	17.40±1.63	2.64±0.25	6	-32.09±0.46	2.49±1.36	8.04±0.85	21.77±1.74	2.88±0.35	6	-34.82±0.7	6.80±0.11	6.41±1.33	13.02±1.01	2.40±0.36
		SW2	12	-32.66±0.55	3.13±1.26	7.33±0.65	16.70±1.12	2.45±0.21	6	-31.74±0.10	0.88±2.08	9.03±0.17	18.99±0.91	2.11±0.11	6	-33.58±1.0	5.38±0.81	5.63±0.82	14.40±1.61	2.78±0.37
		SW3	12	-30.97±0.40	2.79±0.63	7.60±0.61	20.18±1.56	2.82±0.24	6	-31.56±0.18	2.46±1.06	8.84±0.15	19.91±2.13	2.28±0.27	6	-30.38±0.7	3.12±0.75	6.36±1.01	20.45±2.4	3.36±0.24
Surface Water	Central Drainage	SW5	12	-28.68±0.92	3.27±0.96	7.33±0.72	26.71±1.51	4.46±0.82	6	-31.16±1.11	0.71±1.13	8.65±0.56	24.36±1.56	2.91±0.33	6	-26.21±0.2	5.83±0.37	6.00±1.12	29.05±2.31	6.02±1.37
	Blue-green Algae	Davis Ramp	3	-29.92±0.19	6.93±0.81	7.41±0.35	22.90±1.45	3.13±0.34	3	-29.92±0.19	6.93±0.81	7.41±0.35	22.90±1.45	3.13±0.34	0	NS	NS	NS	NS	NS
	Red Tide	Bonita	1	-9.36	3.83	6.12	50.80	8.32	0	NS	NS	NS	NS	NS	1	-9.36	3.83	6.12	50.80	8.32
		Lighthouse	1	-9.83	3.33	6.07	27.26	4.50	0	NS	NS	NS	NS	NS	1	-9.83	3.33	6.07	27.26	4.50
		Lovers Key	1	-12.17	4.23	6.11	30.85	5.06	0	NS	NS	NS	NS	NS	1	-12.17	4.23	6.11	30.85	5.06
		Lynn Hall	1	-19.29	4.72	2.91	20.18	6.94	0	NS	NS	NS	NS	NS	1	-19.29	4.72	2.91	20.18	6.94
		South Seas	1	-10.10	3.73	6.10	31.43	5.16	0	NS	NS	NS	NS	NS	1	-10.10	3.73	6.10	31.43	5.16
		Tarpon	1	-7.10	3.26	7.09	53.28	7.53	0	NS	NS	NS	NS	NS	1	-7.10	3.26	7.09	53.28	7.53

Appendix 14. Monthly depth to water table (single values or averages ± standard error when more than one measurement was made) by drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference site) and site.

Drainage Basin	Site	*October 2017*		*November 2017*		*December 2017*		*January 2018*		*February 2018*	
		Count	**Depth to Water Table**	**Count**	**Depth to Water Table**	**Count**	**Depth to Water Table**	**Count**	**Depth to Water Table**	**Count**	**Depth to Water Table**
Hancock Creek	GW1	1	1.3	3	3.60±0.69	3	4.66±0.08	4	4.58±0.50	5	3.03±0.07
	GW2	1	1.33	3	3.47±0.62	3	4.40±0.10	4	4.86±0.08	5	3.17±0.06
	GW3	1	0.8	3	2.99±0.72	3	4.09±0.23	4	3.21±0.59	5	2.02±0.06
Powell Creek	GW4	1	1.65	3	2.66±0.07	3	2.82±0.08	4	3.21±0.07	5	3.09±0.08
	GW5	1	1.3	3	2.56±0.18	3	2.47±0.10	4	2.98±0.05	5	2.68±0.11
	GW6	1	1.28	3	1.80±0.11	3	2.13±0.32	4	2.63±0.15	5	2.41±0.09
Central Drainage	GW7	1	0.61	3	1.81±0.10	4	2.10±0.06	4	2.50±0.08	5	2.40±0.11
	GW8	1	1.1	3	2.22±0.11	4	2.53±0.04	4	2.89±0.05	5	2.87±0.10
	GW9	1	2.45	3	2.55±0.08	3	2.73±0.12	4	3.19±0.08	5	2.95±0.22
Reference	GW10	1	3.83	3	4.22±0.01	3	4.36±0.02	4	4.47±0.01	5	4.49±0.02

Drainage Basin	Site	*March 2018*		*April 2018*		*May 2018*		*June 2018*		*July 2018*	
		Count	**Depth to Water Table**	**Count**	**Depth to Water Table**	**Count**	**Depth to Water Table**	**Count**	**Depth to Water Table**	**Count**	**Depth to Water Table**
Hancock Creek	GW1	4	3.17±0.05	5	3.38±0.06	4	2.24±0.76	4	1.85±0.16	4	1.85±0.16
	GW2	4	3.16±0.06	5	3.37±0.08	4	2.32±0.65	4	2.22±0.15	4	2.22±0.15
	GW3	4	1.95±0.05	5	2.10±0.09	3	1.72±0.33	4	1.53±0.23	4	1.53±0.23
Powell Creek	GW4	4	3.22±0.03	5	3.28±0.06	4	1.94±0.59	4	2.10±0.13	4	2.10±0.13
	GW5	4	2.78±0.02	5	2.88±0.07	4	1.65±0.57	4	1.97±0.08	4	1.97±0.08
	GW6	4	2.13±0.07	5	2.36±0.10	4	1.67±0.48	4	1.63±0.10	4	1.63±0.10
Central Drainage	GW7	4	2.75±0.06	5	2.89±0.08	2	2.52±0.20	4	1.47±0.08	4	1.47±0.08
	GW8	4	3.34±0.06	5	3.57±0.06	3	2.55±0.61	4	1.75±0.11	4	1.75±0.11
	GW9	4	3.24±0.05	5	3.34±0.08	4	1.95±0.66	4	2.26±0.09	4	2.26±0.09
Reference	GW10	4	4.73±0.05	5	5.14±0.07	4	4.40±0.65	4	3.82±0.08	4	3.82±0.08

Appendix 15. Overall averages (± standard error) of groundwater aqueous nitrogen isotope values of ammonium ($\delta^{15}N-NH_4$) and nitrate ($\delta^{15}N-NO_3$) drainage basin (Hancock Creek, Powell Creek, a central drainage basin, and a reference area) and site.

Water Type	Drainage Basin	Site	Count	$\delta^{15}N-NH_4$ (‰)	$\delta^{15}N-NO_3$ (‰)
Groundwater	Hancock Creek	GW1	4	7.06±1.55	13.85±7.16
		GW2	4	9.25±1.22	8.44±2.98
		GW3	4	6.48±0.56	0.49±0.93
	Powell Creek	GW7	4	3.76±0.29	-2.01±0.89
		GW8	4	3.16±0.94	-2.33±1.65
		GW9	4	2.20±0.62	2.45±0.58
	Central Drainage	GW4	4	3.71±0.34	2.05±0.60
		GW5	4	8.17±0.33	2.33±0.97
		GW6	4	9.63±0.26	2.81±0.65
	Reference	GW10	4	3.41±1.85	3.65±1.39

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