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Research Paper

Septic system–groundwater–surface water couplings in waterfront communities contribute to harmful algal blooms in Southwest Florida

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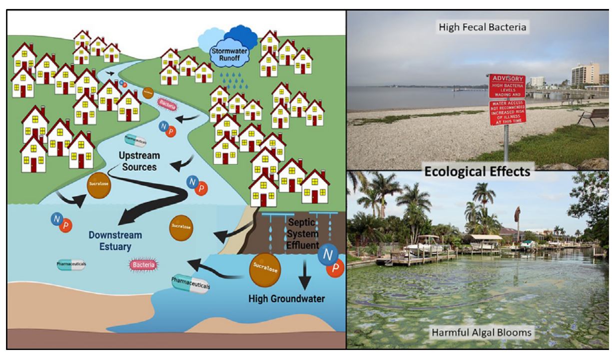
HIGHLIGHTS

- Groundwater is contaminated with nutrients and bacteria from septic systems.
- Septic systems do not function in high water tables.
- Surface waters are degraded due to human waste inputs.
- Sucralose was ubiquitous in groundwater and surface waters.
- Downstream harmful algal blooms may be mitigated through improved infrastructure.

GRAPHICAL ABSTRACT



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ABSTRACT

As human population growth has expanded in Southwest Florida, water quality has become degraded with an increased occurrence of harmful algal blooms (HABs). Red tide (*Karenia brevis*) originating offshore, intensifies in nearshore waters along Florida's Gulf Coast, and blue-green algae (*Microcystis* spp.) originating in Lake Okeechobee is discharged into the Caloosahatchee River. These HABs could be enhanced by anthropogenic nitrogen (N) and phosphorus (P) from adjacent watersheds. North Fort Myers is a heavily developed, low-lying city on the Caloosahatchee River Estuary serviced by septic systems with documented nutrient and bacterial pollution. To identify sources of pollution within North Fort Myers and determine connections with downstream HABs, this multiyear (2017–2020) study examined septic system- groundwater- surface water couplings through the analysis of water table depth, nutrients (N, P), fecal indicator bacteria (FIB), molecular markers (HF183, GFD, Gull2), chemical tracers (sucralose, pharmaceuticals, herbicides, pesticides), stable isotopes of groundwater (δ^{15}N-NH_4, δ^{15}N-NO_3) and particulate organic matter (POM; δ^{15}N, δ^{13}C), and POM elemental composition (C:N:P). POM samples were also collected during *K. brevis* and *Microcystis* spp. HAB events. Most (>80%) water table depth measurements were too shallow to support septic system functioning (<1.07 m). High concentrations of NH_4^+ and NO_x, up to 1094 μM and 482 μM respectively, were found in groundwater and surface water. δ^{15}N values of groundwater (+4.7‰) were similar to septic effluent (+4.9‰), POM (+4.7‰), and downstream HABs (+4.8 to 6.9‰), indicating a human waste N source. In surface water, FIB were elevated and HF183 was detected, while in groundwater and surface water sucralose, carbamazepine, primidone, and acetaminophen were detected. These data suggest that groundwater and surface water in North Fort Myers are coupled and contaminated by septic system effluent, which is negatively affecting water quality and contributing to the maintenance and intensification of downstream HABs.

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1. Introduction

Globally, coastal water quality degradation is an ongoing, evolving concern for urbanized watersheds with many ecological, economic, and public health implications (National Research Council, 2000). Excess nutrient inputs accelerate eutrophication, promote harmful algal blooms (HABs), and deplete dissolved oxygen (DO) in the water column, which can result in fish kills, habitat loss, and diminished water quality negatively impacting human health and the economy (Howarth et al., 2000). Many factors contribute to urban pollution, so there is high variability in water quality that is related to weather conditions, catchment rainfall, watershed characteristics, and drainage infrastructure (Walsh et al., 2005; Lapointe et al., 2012; Tran et al., 2015; Lapointe et al., 2017). These issues will be exacerbated by climate change, which is predicted to increase riverine nitrogen (N) loading by ~19% before the end of the century (Sinha et al., 2017).

Human waste contamination from onsite sewage treatment and disposal systems, commonly called "septic systems," can be a significant source of nutrient and microbial pollution to groundwater and surface water (Griffin et al., 1999; Lipp et al., 2001; Cahoon et al., 2006; Withers et al., 2011; Withers et al., 2014). As such, surface water contamination from septic system effluent has been observed in the coastal zone of all developed continents (Tuholske et al., 2021). In the United States, the state of Florida has ~2.67 million homes (~33%) serviced by septic systems for domestic waste management (Yang et al., 2016). Florida's coastal regions are particularly vulnerable to pollution from septic systems because of shallow water tables and porous soils that allow for rapid transport of contaminants to groundwater (Meeroff et al., 2008). Despite these poor conditions, Florida's coastal communities often contain high densities of septic systems (Flanagan et al., 2020) that can account for >50% of domestic waste disposal (Herren et al., 2021). As such, evidence of nutrient and bacterial pollution from septic system effluent contamination has been observed throughout the state (Lapointe et al., 1990; Lapointe and Krupa, 1995; Paul et al., 1995; Arnade, 1999; Corbett et al., 2000; Lipp et al., 2001; Bacchus and Barile, 2005; Lapointe and Bedford, 2007; Meeroff et al., 2008; Lapointe et al., 2015; Lapointe et al., 2017; Barile, 2018; Herren et al., 2021). Thus, source identification of nutrient and microbial pollution in Florida's coastal areas is necessary to determine the associated human health and environmental risks, as well as for the development of mitigation strategies.

Stable carbon (δ^{13}C) and N isotope (δ^{15}N) values are commonly used to identify nutrient pollution sources. For example, δ^{15}N values can help identify N sources, such as atmospheric deposition (<0‰), fertilizer (~−2 to +2‰), septic system effluent (+4.9‰) or processed human waste (>+3‰; Aravena et al., 1993; Costanzo et al., 2001; Kendall et al., 2007; Hinkle et al., 2008; Risk et al., 2009; Xue et al., 2009). Specifically, aqueous (dissolved) δ^{15}N from a water sample in the form of ammonium (δ^{15}N-NH_4^+) and nitrate (δ^{15}N-NO_3^-) can be used to distinguish between dissolved inorganic N (DIN) sources. Additionally, the δ^{15}N values of primary producers are often used to discriminate between natural and anthropogenic N sources (Costanzo et al., 2001; Cole et al., 2004; Lapointe et al., 2015). Finally, δ^{13}C values of plants can help identify carbon (C) sources because terrestrial C is more depleted (having lower values) than marine C, which is more enriched (Peterson and Fry, 1987).

Fecal indicator bacteria (FIB) are often used to assess watershed contamination, but this approach presents some challenges, including difficulty in discriminating between sources (e.g., human waste or environmental) and short survival times (Scott et al., 2002; Tran et al., 2015). Further, FIB are found in the feces of many animals and therefore provide no reliable indication regarding the source of fecal pollution where many potential non-point sources of fecal contaminants exist (Tran et al., 2015). Employing a suite of microbial source tracking tools can address these uncertainties and help to identify pollutant sources. For example, specific molecular markers can determine the source of bacterial contamination. HF183 is a molecular marker within the 16S rRNA genes of the *Bacteroides* species that reside in the human colon as part of the normal microbiota. Therefore, detection of HF183 in environmental waters serves as an indicator of human fecal pollution (Ahmed et al., 2008; Tran et al., 2015). BacR is a molecular marker within the 16S rRNA genes of *Bacteroides* species that reside in the digestive tract of most ruminants, such as deer, alpaca, cattle, llama, and goats. Thus, detection of BacR in environmental samples indicates ruminant fecal pollution (Reischer et al., 2006). Similarly, the avian marker GFD is an unclassified *Helicobacter* sp. and indicates the presence of many bird species, including gulls, goose, chicken, pigeon, egret, crow, etc. (Ahmed et al., 2016). Gull2 is associated with the bacteria species *Catellicoccus marimammalium* that is found in the feces of seagulls and other seabirds (Ryu et al., 2012).

Various chemical tracers can also help identify sources of nutrient and bacterial pollution. For example, the artificial sweetener sucralose, as well as certain pharmaceuticals, including the over the counter pain reliever acetaminophen and the prescription anticonvulsants carbamazepine and primidone are useful indicators of human waste contamination (Oppenheimer et al., 2011; Silvanima et al., 2018). Further, herbicide and pesticide chemical tracers can identify other sources of contamination to a waterbody, such as surficial runoff (Papadakis et al., 2018; Silvanima et al., 2018), which can contain nutrient and bacterial sources including pet waste, leaf litter, and grass clippings (Yang and Lusk, 2018; Krimsky et al., 2021).

A combination of the tools described above may be used to identify the sources of nutrient and bacterial pollution in urban areas with water quality concerns. Lee County, FL, is bisected by the Caloosahatchee River and Estuary, which historically was low in nutrients (Odum et al., 1955). However, the area has become highly developed since the 1950s (Fig. S1) and surface water in the Caloosahatchee River Estuary is now nutrient laden (Lapointe and Bedford, 2007; Vargo et al., 2008). Thus, some segments of the Caloosahatchee River Estuary are classified as impaired under Section 303(d) of the United States Clean Water Act of 1972 for nutrients, fecal coliforms, DO, and chlorophyll, while HABs are a recurring issue (see Fig. S2). For example, "red tide" blooms of the dinoflagellate *Karenia brevis* have become increasingly abundant, especially in nearshore environments (Brand and Compton, 2007), and have long been linked to nutrient enrichment from riverine inputs and estuarine flux (Slobodkin, 1953; Odum et al., 1955; Doig and Martin, 1974; Vargo et al., 2008; Yentsch et al., 2008; Medina et al., 2020; Medina et al., 2022), particularly during high flow years (Lapointe and Bedford, 2007; Heil et al., 2014). Additionally, beginning in 2003 red drift macroalgal HABs developed off the Lee County coast (Fig. S2) and were associated with increasing nutrient contributions from human waste, as well as rainfall and agricultural fertilizers (Lapointe and Bedford, 2007). Finally, extreme rainfall associated with hurricanes facilitated blooms of the freshwater blue-green alga *Microcystis aeruginosa* in the Caloosahatchee River Estuary and residential canals (Fig. S2) in 2005 (Lapointe et al., 2006), 2017, and 2018 (Glibert, 2020). Aside from the ecological and human health effects, these HABs can negatively impact local economies through the mortality of commercial seafood and by inhibiting ecotourism activities (Anderson et al., 2000). Despite these water quality issues, the population in Lee County continues to grow rapidly with a 24.5% increase from 2010 to 2019 (Fig. S1).

Within Lee County, the city of North Fort Myers has experienced degraded water quality over the last 30 years, including persistent nutrient and fecal bacterial pollution (W. Dexter Bender and Associates Inc., 1995). Therefore, to address the critical public health and water quality issues of nutrient and bacterial contamination in North Fort Myers, a multi-year microbial source tracking study was conducted to determine the sources of these impairments and assess connections with downstream HABs. Based on previous research, we hypothesized that effluent from septic systems was an important source of nutrient and bacterial pollution in North Fort Myers and thus location would be a more important factor for water quality than temporal factors, such as project year or season.

2. Materials and methods

2.1. Study area

Lee County encompasses 3139 km^2 and is bisected by the Caloosahatchee River and Estuary, which terminates into the Gulf of Mexico. The hydrology of the region has been highly modified over the last century with the conversion of the natural river into the C-43 canal and the connection to Lake Okeechobee via three lock-and-dam structures (S-77, S-78, and S-79) that allow the United States Army Corps of Engineers to control river flow with lake water discharges (Barnes, 2005). The unconfined water table aquifer in Lee County has high hydraulic conductivity with water flows from the topographic high to the southwest (Scott and Missimer, 2001). Rainfall is seasonally variable in subtropical Southwest Florida with wetter conditions from May to October (wet season) and drier conditions from November to April (dry season) and an average annual rainfall of ~155 cm (Liu et al., 2009), though variability is observed. Lee County Ordinance #08-08 limits fertilizer nutrient content and application rates and bans the application of fertilizers containing N and/or P during the wet season from June 1 to September 30.

There are ~39,768 "known" and ~57,054 "likely" septic systems in Lee County (~96,822 total estimated), however for many parcels there are no data available on domestic waste disposal (Florida Department of Health, 2020). Further, due to elevated seasonal high water tables (Arnade, 1999; Meeroff et al., 2008), many septic systems in Florida may not meet the state regulatory requirements. Septic systems require a minimum cover of 6″ over the drainfield, a drainfield depth of ~1′ (may be less in some soil types), and 2' of separation from the bottom of the drainfield to the high water table (FAC Rule 62E-6). Therefore, at least 3.5′ (1.07 m) of separation is needed from the ground surface to the water table to meet the minimum requirements. In some parcels with high water levels, the drainfield has been raised above the natural soil surface to help meet these requirements by adding additional separation between the initial effluent discharge and the groundwater ("mounding").

The urban areas of North Fort Myers were developed along natural creeks that flow into the tidal Caloosahatchee River Estuary that were modified by the addition of "finger" and drainage canals. Thus, North Fort Myers has three major drainage basins: Hancock Creek, Powell Creek, and a central drainage feature (Fig. 1). The primary land-use in North Fort Myers is residential with a high abundance of waterfront homes. The study area is serviced by an estimated 2164 septic systems (Fig. 1) and there is no application of reuse water.

In September 2017, ten shallow groundwater wells were installed using a hand auger to a depth of ~2.1 m. The wells were constructed from 2-in. (50.8 mm) diameter PVC with 1.5 m well screen and were sand packed between the well bore and casing to 0.31 m above the well screen with a 0.31 m thick bentonite cap on top of the sand pack. The groundwater monitoring wells were installed in Lee County right of way or at private residences where the owner consented. Nine wells were installed in high density, low elevation residential areas serviced by septic systems amended with finger canals in the drainage basins of Hancock Creek (GW1-GW3; 136 septic systems/km^2), a central drainage feature (GW4-GW6; 236 septic systems/km^2), and Powell Creek (GW7-GW9; 279 septic systems/km^2; Table 1). Unfortunately, the intensity of development in North Fort Myers prevented the inclusion of a completely "natural" location, so one "reference" groundwater well (GW10) was installed in a less densely developed upland area within the Hancock Creek watershed with no canals serviced by both sewer and septic systems (Fig. 1). Four of the ten surface water sites were along Powell Creek (SW1-SW4), one was in the central drainage feature (SW5), and five were along Hancock Creek (SW6-SW10; Fig. 1). The sites were freshwater with greater estuarine influence near the Caloosahatchee River Estuary.

2.2. Rainfall

Rainfall data over the study period (January 2017 to May 2020) was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (https://www.ncdc.noaa.gov/data-access). Fort Myers station US1FLLE0037 was selected as the primary data source due to its proximity to the study area and high temporal coverage (92.6%). These data are supplemented with data from other nearby stations in Fort Myers (US1FLLE0055, 3.1%; USW00012835, 2.0%; US1FLLE0056, 0.1%; US1FLLE0039, 0.1%) and Cape Coral (US1FLLE0053, 2.2%) for days in which data at the primary station are missing. Sampling was conducted during wet (October 2017, November 2017, August 2019, and September 2019) and dry (February 2018, March 2018, February 2020, and March 2020) seasons that were determined based on rainfall and seasonal water table fluctuations.

2.3. Sample collection and analyses

Samples were collected four times during the wet season (October 17–18, 2017, November 14–15, 2017, August 27–28, 2019, and September 25–26, 2019) and four times during the dry season (February 13–14, 2018, March 13–14, 2018, February 10–11, 2020, and March 10–11, 2020). Groundwater and surface water sampling were conducted simultaneously by two teams: Florida Atlantic University-Harbor Branch Oceanographic Institute researchers and Lee County Environmental Laboratory (LCEL) staff. Additionally, approximately weekly throughout the study period and during sampling events, depth to water in the groundwater wells was measured with a clean 100 m Geotech Water Meter Reader. Environmental parameters of groundwater and surface water, including pH, salinity (ppt), conductivity (μS), temperature (°C), and DO (%, mg/L) were measured during sampling events using calibrated multiparameter probes.

For groundwater sampling, a peristaltic pump and clean Tygon tubing were used by LCEL staff to purge triple the well volume before collecting samples per Florida Department of Environmental Protection (FDEP) standard operating procedures (FS2200). After purging, triplicate water samples were collected into HDPE bottles to determine concentrations of ammonium (NH_4^+), nitrate + nitrite (NO_x), soluble reactive P (SRP), total N (TN), and total P (TP). Singular water samples were collected into high-density polyethylene (HDPE) bottles for determination of enterococci and *Escherichia coli* counts, 5-day biochemical oxygen demand (BOD) concentrations, and color. These samples were submerged in ice and delivered to LCEL, Fort Myers, FL for analyses. For detailed methods and method detection limits (MDLs), see the Supplemental Methods. Groundwater was also collected for determination of aqueous stable N isotope values of ammonium ($\delta^{15}N$-NH_4) and nitrate ($\delta^{15}N$-NO_3). These samples were collected into 1 L HDPE bottles and stored on ice until shipment to various labs for processing and stable isotope analyses (see Supplemental Methods).

Surface water samples were collected during outgoing tides and handled similarly to groundwater for determination of NH_4, NO_x, SRP, TN, TP, enterococci, *E. coli*, BOD, and color. Additionally, at surface water sites and during HAB events, particulate organic matter (POM) was collected as a proxy for phytoplankton (see Supplemental Methods). Additionally, macroalgae was collected at sites when present, rinsed briefly with DI water, dried, and ground into a homogenous powder. The POM filters and tissue were analyzed for $\delta^{13}C$ and $\delta^{15}N$, as well as elemental composition (%C, %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). In year 1, %P was analyzed at UGA following the methodology of Aspila et al. (1976) on a Technicon Autoanalyzer II with an IBM-compatible, Labtronics, Inc. DP500 software data collection system (D'Elia et al., 1997). In year 2, because UGA ceased to perform these services, %P samples were analyzed at the University of Missouri Soil and Plant Testing Laboratory by Inductively Coupled Plasma Atomic Emission Spectroscopy (Viso and Zachariadis, 2018). C:N:P data were compared to a modified Redfield ratio of 360:30:1 (Redfield, 1958) to characterize temporal and spatial variation in nutrient status. Additionally, surface water samples for determination of molecular markers were collected at each site into 500 mL HDPE bottles, stored on ice, and shipped overnight to the FDEP lab. At the FDEP lab, they were analyzed using qPCR

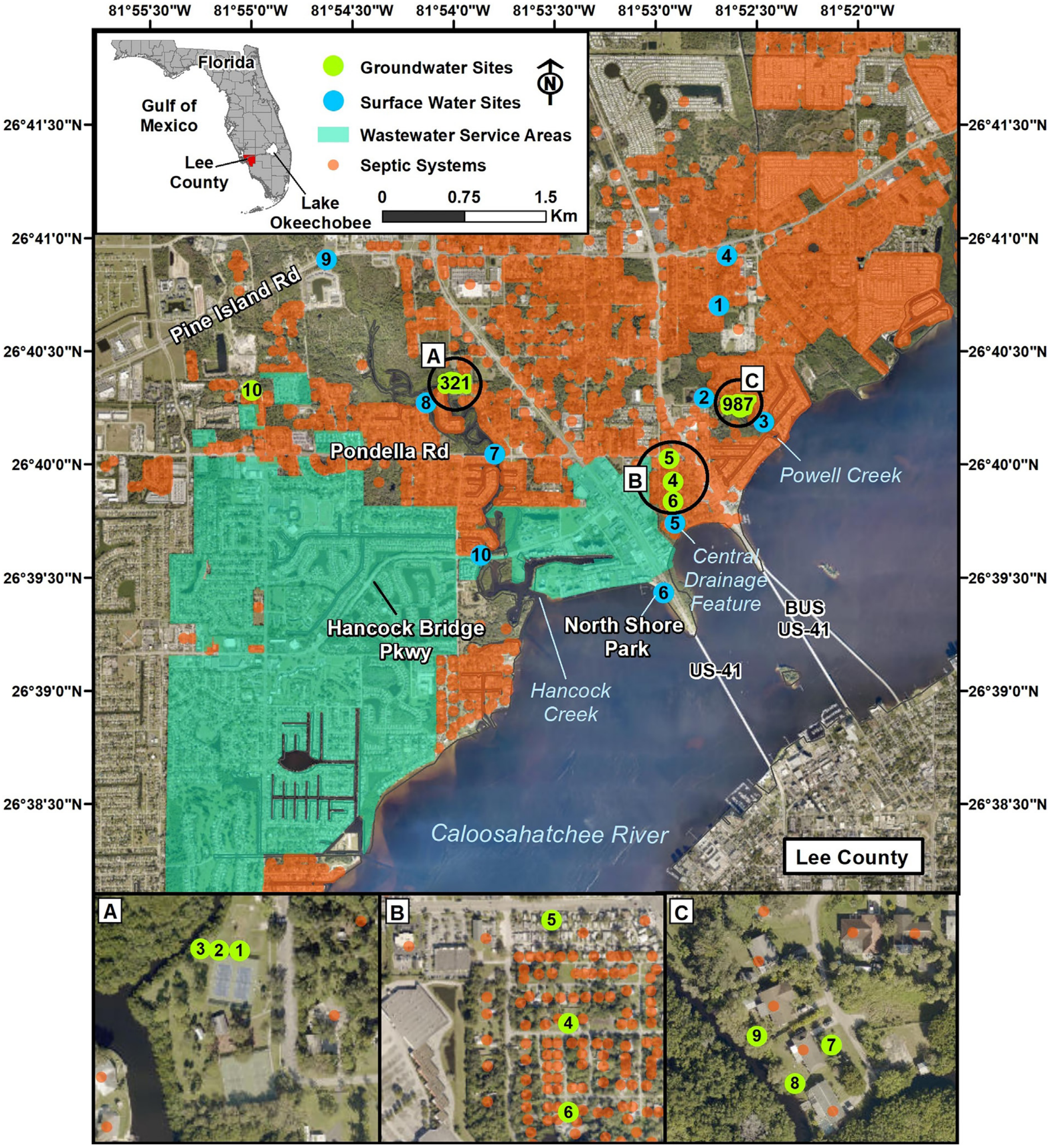

Fig. 1. Satellite imagery of the study area in North Fort Myers, FL, showing locations of groundwater wells (GW 1–10, green circles) in A) Hancock Creek drainage basin, B) the central drainage feature, and C) Powell Creek drainage basin, and surface water collection sites (SW 1–10, blue circles), as well as areas connected to centralized sewer for domestic waste disposal (teal shading) and parcels with septic systems (orange circles).

Table 1
Characteristics of watersheds within the North Fort Myers, FL study area, including total area (in km^2 and acre), septic system count, and number of septic systems per km^2 and acre. Estimated population using septic systems in each drainage basin is also shown using an average occupancy rate for the study area of 1.89 persons per unit based on Lee County census data.

Drainage basin	Area (km^2)	Area (acre)	Septic system count	Septic systems per km^2	Septic systems per acre	Estimated population using septic systems
Hancock Creek	5.22	1290	712	136	0.55	1346
Central drainage basin	2.54	628	599	236	0.95	1132
Powell Creek	2.63	650	735	279	1.13	1389
Overall	*10.39*	*2567*	*2046*	*197*	*0.80*	*3867*

to determine concentrations of the human marker HF183, the ruminant marker BacR, and the bird markers GFD and Gull2.

Finally, groundwater and surface water samples for analysis of chemical tracers were collected into amber glass bottles, stored on ice, and shipped overnight to the FDEP Laboratory, Tallahassee, FL. At the FDEP lab, samples were analyzed by high performance liquid chromatography, coupled with both thermospray-mass spectrometry and an ultraviolet detector (EPA method 8321B; see Supplemental Methods). Due to what was offered by the analytical lab, various chemicals were tested for during different sampling events. During all events, concentrations of the human waste tracers sucralose, carbamazepine, primidone, and acetaminophen were determined. The herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), bentazon, and triclopyr, as well as the psychoactive stimulant meta-Chlorophenylpiperazine (mCPP) were tested for in both seasons of year 1 (2017–2018). In the dry season of year 1 (2018), water samples were also analyzed to determine concentrations of an insecticide (imidacloprid), agricultural fungicide (pyraclostrobin), and other herbicides, including diuron, fenuron, fluridone, imazapyr, and linuron. Additional human waste tracers, ibuprofen, hydrocodone, and naproxen were also included in the suite of analytes during both seasons in year 2 (2019–2020).

2.4. Statistical analyses

For nutrient concentrations and bacterial counts, results flagged as below detection limits or less than the criterion of detection were substituted with a value equal to half the MDL for calculation of means, while original values reported by the lab were used in rank ordered, non-parametric analyses (Helsel, 2005). The following replacements were made for NH_4^+: one groundwater and 57 surface water samples and for NO_x: 148 groundwater and 61 surface water samples. For chemical tracers, any results below the MDL were considered as non-detects, per Silvanima et al. (2018) and replaced with zeros for data analyses. Estimated values and results flagged as between the MDL and the practical quantitation limit were included in data analyses (Helsel, 2005). To determine what factors were influential on water quality in the study area, parameters were compared between watersheds, project year (year 1, year 2), and season (dry, wet) with analysis of variance (ANOVA) if assumptions were met. The non-parametric Kruskal-Wallis test (groups of three or more, adjusted for ties) or Mann-Whitney *U* test (groups of two) were used if ANOVA assumptions were not met or if too many values (>15%) were below the MDL (USEPA, 2000; Helsel, 2005). Log transformation was attempted for all parameters having <15% non-detects before non-parametric statistics were employed. Significant main tests for ANOVA were followed by Tukey Honest Significant Difference test (HSD) and significant Kruskal-Wallis tests were followed by Dunn's test with a Bonferroni correction. Spearman's rank-order correlation was used to assess monotonic associations between groundwater and surface water variables. Only significant correlations with r > 0.30 are discussed in the Results and correlations between mathematically related variables (i.e., DIN and DIN:SRP) are not discussed. Analyses were conducted in SPSS 27, maps were made using ArcMap 10.8.1, and figures were created in GraphPad Prism 8. For all parameters and tests, differences were considered significant at $p < 0.05$ and data are presented as means with standard error (±S.E.) unless otherwise noted.

3. Results

3.1. Rainfall

All sampling events were conducted on days with little to no precipitation (Fig. 2). Mean annual rainfall in the study area ranged from a low of 1.24 m in 2018 to a high of 2.08 m in 2017. The most significant rainfall occurred from September 10–11, 2017, when Hurricane Irma passed through, contributing ~258 mm of precipitation over a two-day period. The cumulative rainfall ten days prior to the October 2017, November 2017, February 2018, and March 2018 sampling events was 18.54 mm, 5.33 mm, 22.86 mm, and 8.38 mm, respectively. The cumulative rainfall ten days prior to the August 2019, September 2019, February 2020, and March 2020 sampling events was 48.01 mm, 14.22 mm, 45.97 mm, and <0.01 mm, respectively.

3.2. Depth to water table

The depth to water table observed during the sampling events ranged from 0.19 to 2.08 m (Fig. 3) with an overall mean depth of 0.86 ± 0.04 m. During sampling events, depth to water table was variable by season (ANOVA, $F_{1,\,64} = 7.00$, $p = 0.01$) and watershed (ANOVA, $F_{3,\,64} = 15.5$, $p < 0.01$), but not project year (ANOVA, $F_{1,\,64} = 0.06$, $p = 0.80$) with no significant interactions (all $p > 0.05$). By season, depth to water table was significantly shallower in the wet season (0.75 ± 0.06 m) than the dry season (0.96 ± 0.05 m). By watershed, depth to water table was significantly deeper at the reference well (GW10 = 1.29 ± 0.03 m) and in Hancock Creek watershed (1.06 ± 0.09 m) than in the central drainage watershed (0.70 ± 0.05 m) or Powell Creek (0.67 ± 0.04 m; Fig. 3). In the central drainage watershed and Powell Creek, 100% of the depth to water measurements recorded during sampling events were too shallow to

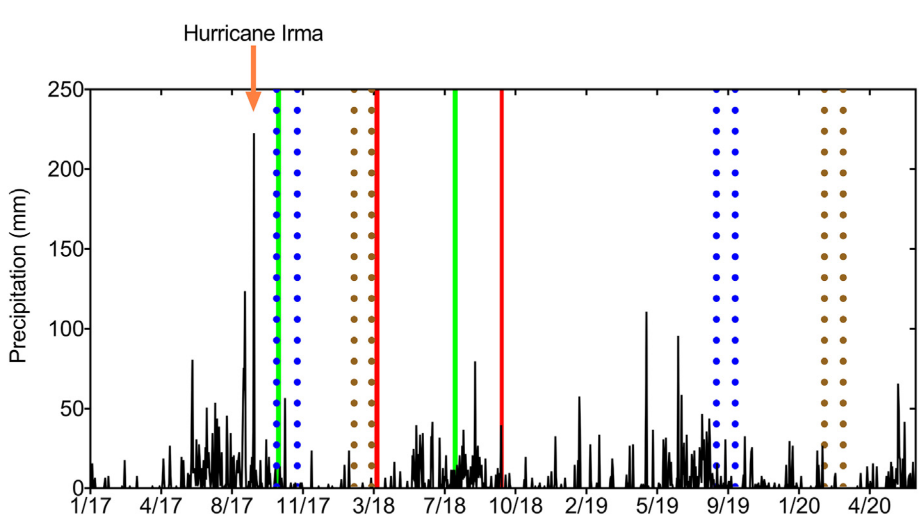

Fig. 2. Daily rainfall (mm) measured in North Fort Myers, FL from January 2017 through May 2020, showing sampling events conducted during the wet season (blue dotted lines), dry season (brown dotted lines), blue-green algae blooms (*Microcystis* spp.; green solid lines), and red tide blooms (*Karenia brevis*; red solid lines), as well as Hurricane Irma (orange arrow).

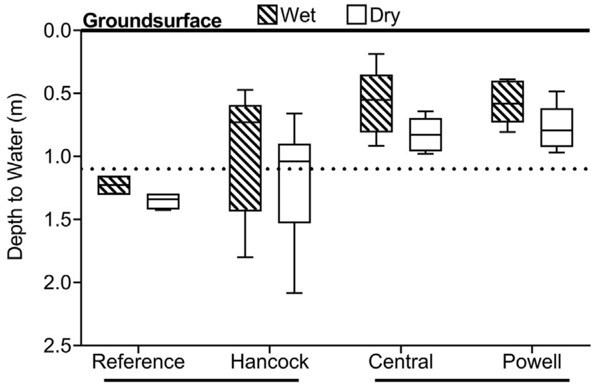

Fig. 3. Depth to water table measured in groundwater wells during sampling events by watershed (reference well, Hancock Creek, central drainage feature, and Powell Creek) and season (wet/dry); the dotted line indicates the approximate minimum separation required from the ground surface (at zero) to the water table required by FAC Rule 62E-6 (~1.07 m), values above this line indicate septic systems in this area may not be compliant with current requirements for new septic systems and do not have the separation needed to function properly. Lines below x-axis labels represent significant groupings.

support adequate treatment of effluent from a septic system without the addition of mounding (all <1.07 m). In Hancock Creek, 58% of depth to water measurements were too shallow and varied seasonally with 50% of dry season measurements <1.07 m and 67% of wet season measurements <1.07 m. In the reference well, 100% of depth to water measurements recorded during sampling events were >1.07 m (Fig. 3).

3.3. Nutrient concentrations

NH_4^+ concentrations were significantly higher in groundwater (153.6 ± 15 μM) than in surface water (2.82 ± 0.22 μM; Mann-Whitney *U* test, U = 1812, n = 478, p < 0.001). In groundwater, NH_4^+ concentrations ranged from below detection to 482 μM and were variable by watershed (Kruskal-Wallis test, H = 173, n = 238, df = 3, p < 0.001) and project year (Mann-Whitney *U* test, U = 5160, n = 238, p < 0.001), but not season (Mann-Whitney *U* test, U = 7786, n = 238, p = 0.184). Powell Creek groundwater had a significantly higher NH_4^+ concentration (395 ± 38 μM) than the other watersheds, while the central drainage watershed (97.0 ± 3.3 μM) was significantly higher than Hancock Creek watershed (20.8 ± 1.5 μM) and the reference well (17.4 ± 0.7 μM; Fig. 4). Groundwater NH_4^+ concentrations were significantly higher in year 1 (208 ± 26 μM) than in year 2 (98.3 ± 14 μM).

In surface water, NH_4^+ concentrations ranged from below detection to 21.1 μM and were variable by watershed (Kruskal-Wallis test, H = 36.5, n = 240, df = 2, p < 0.001) and season (Mann-Whitney *U* test, U = 9883, n = 240, p < 0.001), but not project year (Mann-Whitney U test, U = 6204, n = 240, p = 0.062). The central drainage feature (8.27 ± 1.6 μM) and Powell Creek (2.73 ± 0.14 μM) had significantly higher NH_4^+ concentrations than Hancock Creek (1.80 ± 0.13 μM; Fig. 4). Surface water NH_4^+ concentrations in the wet season were significantly higher (3.78 ± 0.04 μM) than the dry season (1.85 ± 0.13 μM).

NO_x concentrations were significantly higher in groundwater (23.7 ± 5.1 μM) than in surface water (3.97 ± 0.26 μM; Mann-Whitney *U* test, U = 40,185, n = 478, p < 0.001). In groundwater NO_x concentrations ranged from 0.36 to 482 μM and were variable by watershed (Kruskal-Wallis test, H = 51.9, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 6339, n = 238, p = 0.094) or project year (Mann-Whitney *U* test, U = 7623, n = 238, p = 0.221). Groundwater in the Hancock Creek watershed had significantly higher concentrations of NO_x (76.9 ± 15 μM) than the other watersheds, which were similar (all <0.90 μM; Fig. 4).

In surface water, NOx concentrations ranged from 0.36 to 19.6 μM and were variable by watershed (Kruskal-Wallis test, H = 27.5, n = 240, df = 2, p < 0.001) and season (Mann-Whitney *U* test, U = 11,104, n = 240, p < 0.001), but not project year (Mann-Whitney *U* test, U = 6203, n = 240, p = 0.061). Powell Creek had significantly higher surface water NO_x concentrations (4.60 ± 0.26 μM) than Hancock Creek (3.31 + 0.42 μM), while the central drainage feature had the highest average NO_x concentrations with high variability (4.74 ± 1.1 μM; Fig. 4). Wet season surface water NO_x concentrations were significantly higher (5.81 ± 0.42 μM) than in the dry season (2.13 ± 0.20 μM).

DIN concentrations were higher in groundwater (177 ± 15 μM) than surface water (6.79 ± 0.41 μM; Mann-Whitney *U* test, U = 983, n = 478, p < 0.001). Groundwater DIN concentrations were variable by watershed (Kruskal-Wallis test, H = 111, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 6830, n = 238, p = 0.637) or project year (Mann-Whitney *U* test, U = 6321, n = 238, p = 0.153). The groundwater DIN concentrations of all watersheds were significantly different from each other (Fig. 4). The highest groundwater DIN was observed in the Powell Creek watershed (396 ± 38 μM), followed by Hancock Creek watershed (97.7 ± 15 μM), the central drainage watershed (97.6 ± 3.3 μM), and the reference well (17. 9 ± 0.64 μM; Fig. 4).

Surface water DIN concentrations were variable by watershed (Kruskal-Wallis test, H = 30.9, n = 240, df = 2, p < 0.001), season (Mann-Whitney

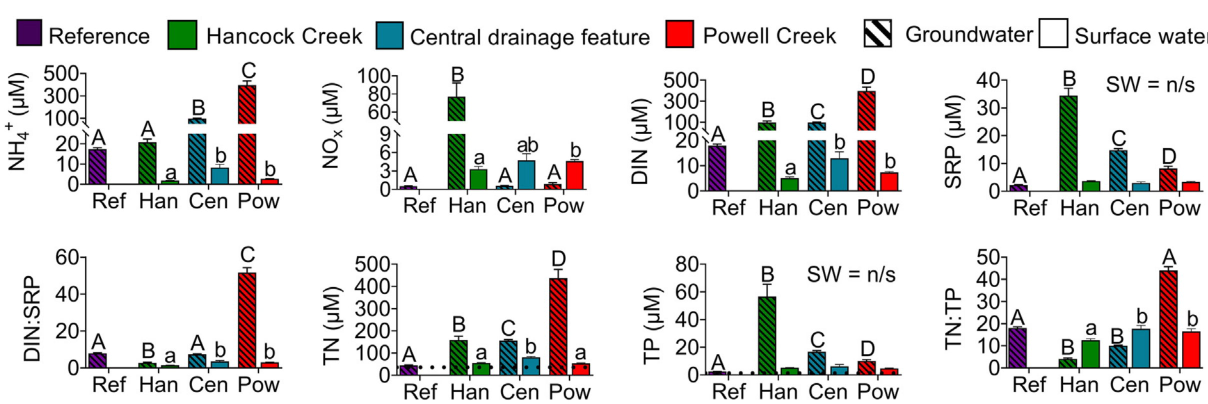

Fig. 4. Nutrient concentrations and molar ratios (mean ± SE) of groundwater (striped) and surface water (no pattern) in North Fort Myers, FL by watershed (reference, Hancock Creek, central drainage feature, and Powell Creek) including ammonium (NH_4^+), nitrate + nitrite (NO_x), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), the molar ratio of DIN:SRP, total nitrogen (TN) with a black dotted line indicating the Florida Department of Environmental Protection (FDEP) surface water target concentrations for the Lower Caloosahatchee Tidal Segments 1 and 2 (<35.7 μM), total phosphorus (TP) with a black dotted line indicating the FDEP surface water standard for the Lower Caloosahatchee (<1.29 μM), and the molar ratio of TN:TP. Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison.

U test, U = 10985, n = 240, $p < 0.001$), and project year (Mann-Whitney *U* test, U = 6053, n = 240, p = 0.033). Surface water DIN concentrations were significantly higher in the central drainage feature (13.0 ± 2.6 μM) and Powell Creek (7.33 ± 0.35 μM), than in Hancock Creek (5.11 ± 0.49 μM; Fig. 4). Surface water DIN concentrations were higher in the wet season (9.59 ± 0.68 μM) than in the dry season (3.99 ± 0.26 μM), while year 1 (7.63 ± 0.60 μM) was significantly higher than year 2 (5.94 ± 0.54 μM).

SRP concentrations were significantly higher in groundwater (17.5 ± 1.2 μM) than in surface water (3.47 ± 0.11 μM; Mann-Whitney *U* test, U = 9591, n = 478, $p < 0.001$). In groundwater, SRP concentrations ranged from 0.97 to 96.7 μM and were variable by watershed (Kruskal-Wallis test, H = 130, n = 238, df = 3, $p < 0.001$), but not season (Mann-Whitney *U* test, U = 7267, n = 238, p = 0.725) or project year (Mann-Whitney *U* test, U = 7746, n = 238, p = 0.210). SRP concentrations of groundwater by watershed were all significantly different (Fig. 4). The highest groundwater SRP concentration was in Hancock Creek watershed (34.4 ± 2.7 μM), followed by the central drainage watershed (14.8 ± 0.53 μM), Powell Creek watershed (8.20 ± 0.83 μM), and the reference well (2.27 ± 0.06 μM).

In surface water, SRP concentrations ranged from 0.45 to 7.65 μM and were variable by watershed (Kruskal-Wallis test, H = 6.80, n = 240, df = 2, p = 0.033), season (Mann-Whitney *U* test, U = 5033, n = 240, $p < 0.001$), and project year (Mann-Whitney *U* test, U = 5439, n = 240, $p < 0.001$). Multiple comparisons did not find significant differences in SRP concentrations by watershed (Dunn's test, all $p > 0.05$; Fig. 4), however the highest surface water SRP concentrations were in Hancock Creek (3.66 ± 0.14 μM), followed by Powell Creek (3.35 ± 0.19 μM), and the central drainage feature (3.00 ± 0.37 μM). Dry season surface water SRP concentrations were significantly higher (3.96 ± 0.16 μM) than in the wet season (2.99 ± 0.14 μM), while SRP concentrations were significantly higher in year 1 (3.84 ± 0.16 μM) than year 2 (3.10 ± 0.15 μM).

DIN:SRP were significantly higher in groundwater (19.0 ± 1.6) than in surface water (2.31 ± 0.13; Mann-Whitney *U* test, U = 10,000, n = 478, $p < 0.001$). Groundwater DIN:SRP ranged from 0.30 to 106 and were variable by watershed (Kruskal-Wallis test, H = 180, n = 238, df = 3, $p < 0.001$), but not project year (Mann-Whitney *U* test, U = 6,161, n = 238, p = 0.084) or season (Mann-Whitney *U* test, U = 6715, n = 238, p = 0.492). The highest groundwater DIN:SRP was in the Powell Creek watershed (51.6 ± 2.7), followed by the reference well (7.94 ± 0.30) and central drainage watershed (7.34 ± 0.37), which were similar, and then Hancock Creek watershed (2.80 ± 0.32; Fig. 4).

Surface water DIN:SRP were generally low (<5) for the North Fort Myers area (Fig. 4). DIN:SRP of surface water ranged from 0.17 to 8.57 and were variable by watershed (Kruskal-Wallis test, H = 45.5, n = 240, df = 2, $p < 0.001$) and season (Mann-Whitney *U* test, U = 12,343, n = 240, $p < 0.001$), but not project year (Mann-Whitney *U* test, U = 7293, n = 240, p = 0.863). Surface water DIN:SRP were significantly lower in Hancock Creek (1.48 ± 0.15), than in the central drainage basin (3.57 ± 0.49) or Powell Creek (3.04 ± 0.20; Fig. 4). Wet season surface water DIN:SRP water was higher (3.58 ± 0.20) than dry season (1.05 ± 0.07).

TN concentrations were significantly higher in groundwater (228 ± 16 μM) than in surface water (57.3 ± 1.2 μM; Mann-Whitney *U* test, U = 7822, n = 478, $p < 0.001$). In groundwater, TN concentrations ranged from 34.3 to 1143 μM and were variable by watershed (Kruskal-Wallis test, H = 98.8, n = 238, df = 3, $p < 0.001$), but not season (Mann-Whitney *U* test, U = 7304, n = 238, p = 0.673) or project year (Mann-Whitney *U* test, U = 6530, n = 238, p = 0.300). Groundwater TN concentrations of all watersheds were significantly different from each other (Fig. 4) with the highest in the Powell Creek watershed (437 ± 39 μM), followed by Hancock Creek watershed (159 ± 17 μM), the central drainage watershed (156 ± 4.5 μM), and the reference well (44.4 ± 1.6 μM).

Surface water TN often exceeded the FDEP target concentrations for the Lower Caloosahatchee Tidal Segments 1 and 2 of <0.5 mg/L (~35.7 μM) throughout the study area with a range of 17.9 to 114 μM. TN concentrations of surface water were variable by watershed (Kruskal-Wallis test, H = 34.0, n = 240, df = 2, $p < 0.001$), season (Mann-Whitney *U* test, U = 10,197, n = 240, $p < 0.001$), and project year (Mann-Whitney *U* test, U = 9155, n = 240, $p < 0.001$). By drainage basin, surface water TN concentrations were significantly higher in the central drainage feature (80.7 ± 3.7 μM) than in Hancock Creek (55.4 ± 1.5 μM) or Powell Creek (53.7 ± 1.8 μM; Fig. 4). Surface water TN concentrations were significantly higher in the wet season (64.3 ± 1.7 μM) than the dry season (50.3 ± 1.5 μM) and were significantly higher in year 2 (61.3 + 1.7 μM) than year 1 (53.3 ± 1.7 μM).

TP concentrations were significantly higher in groundwater (25.5 ± 3.0 μM) than in surface water (5.14 ± 0.19 μM; Mann-Whitney *U* test, U = 10,329, n = 478, $p < 0.001$). In groundwater, TP concentrations ranged from 1.65 to 549 μM and were variable by watershed (Kruskal-Wallis test, H = 145, n = 238, df = 3, $p < 0.001$), but not season (Mann-Whitney *U* test, U = 6693, n = 238, p = 0.466) or project year (Mann-Whitney *U* test, U = 1.09, n = 238, p = 0.297; Fig. 4). By watershed, groundwater TP concentrations were all significantly different (Fig. 4) with the highest groundwater TP concentration in the Hancock Creek watershed (56.7 ± 8.8 μM), followed by the central drainage watershed (16.8 ± 0.86 μM), Powell Creek watershed (10.1 ± 0.89 μM), and the reference well (2.49 ± 0.09 μM; Fig. 4).

Surface water TP concentrations exceeded the FDEP estuary-specific Numeric Interpretations of the Narrative Nutrient Criterion (F.S. Chapter 62-302) water quality standard for the Lower Caloosahatchee of <0.04 mg/L (~1.29 μM) throughout the study area with a range of 1.39 to 35.5 μM. TP concentrations were variable by season (Mann-Whitney *U* test, U = 4580, n = 240, $p < 0.001$) and project year (Mann-Whitney *U* test, U = 4766, n = 240, $p < 0.001$), but not by watershed (Kruskal-Wallis test, H = 5.28, n = 240, df = 2, p = 0.071). Surface water TP concentrations were highest in the central drainage feature (6.25 ± 1.3 μM), followed by Hancock Creek (5.21 ± 0.18 μM), and Powell Creek (4.79 ± 0.25 μM; Fig. 4). Surface water TP concentrations were significantly higher in the dry season (5.97 ± 0.33 μM) than the wet season (4.31 ± 0.16 μM) and were significantly higher in year 1 (5.59 ± 0.18 μM) than year 2 (4.70 ± 0.33 μM).

TN:TP of groundwater (19.0 ± 1.2) were not significantly different from surface water TN:TP (14.6 ± 0.64; Mann-Whitney *U* test, U = 28,834, n = 478, p = 0.856). In groundwater, TN:TP ranged from 0.37 to 76.0 μM and were variable by watershed (Kruskal-Wallis test, H = 197, n = 238, df = 3, $p < 0.001$) and project year (Mann-Whitney *U* test, U = 5952, n = 238, $p < 0.034$), but not season (Mann-Whitney *U* test, U = 7264, n = 238, p = 0.730). The highest TN:TP was observed in the Powell Creek watershed (44.0 ± 1.8) and the reference well (18.0 ± 0.6), which were significantly higher than the central drainage (10.0 ± 0.3) and Hancock Creek (4.11 ± 0.38) watersheds (Fig. 4). Year 1 groundwater TN:TP were significantly higher (21.3 ± 1.8) than year 2 (16.7 ± 1.6; Fig. 4).

TN:TP of surface water ranged from 2.21 to 50.1 and were variable by watershed (Kruskal-Wallis test, H = 9.21, n = 240, df = 2, p = 0.010), season (Mann-Whitney *U* test, U = 10,442, n = 240, $p < 0.001$), and project year (Mann-Whitney *U* test, U = 9676, n = 240, $p < 0.001$). Surface water TN:TP in Hancock Creek (12.6 ± 0.64) were significantly lower than either Powell Creek (16.4 ± 1.3) or the central drainage feature (17.7 ± 1.5; Fig. 4). Surface water TN:TP were significantly higher in the wet season (18.5 ± 1.0) than the dry season (10.8 ± 0.6), while year 2 (17.9 ± 1.1) was significantly higher than year 1 (11.3 ± 0.60).

3.4. Stable isotopes and C:N:P

$\delta^{15}N\text{-}NH_4^+$ values of groundwater in North Fort Myers were generally enriched (Fig. 5a) with a mean of +4.06 ± 0.4‰ and range of −5.72 to +12.1‰. Groundwater $\delta^{15}N\text{-}NH_4^+$ values were variable by project year (ANOVA, $F = _{1,\,62}$ 17.6, $p < 0.001$) and watershed (ANOVA, $F = _{3,\,62}$ 5.74, p = 0.002), but not season (ANOVA, $F = _{1,\,64}$ 3.00, p = 0.088). There was also a significant interaction between project year and watershed (ANOVA, $F = _{3,\,64}$ 2.97, p = 0.039), but no other interactions were

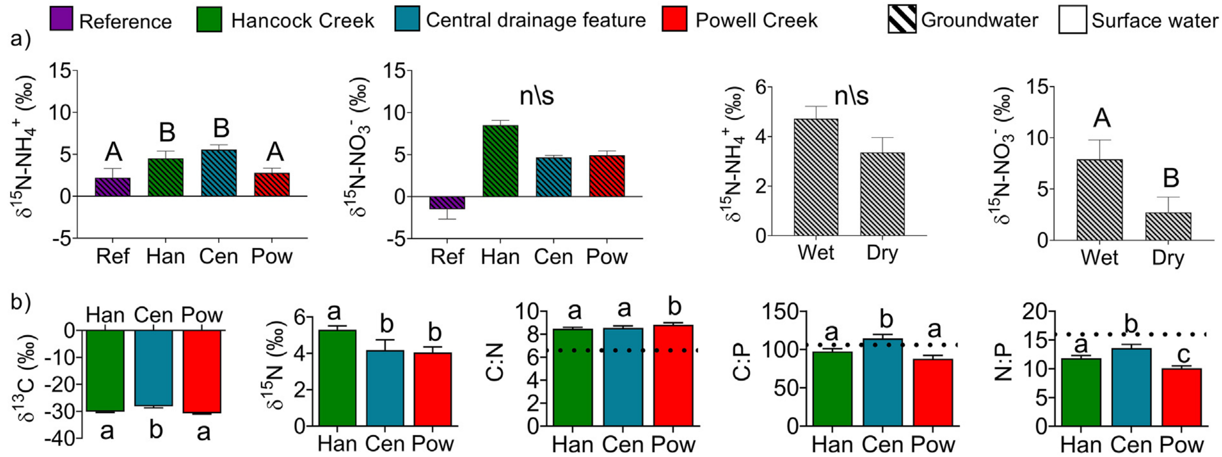

Fig. 5. Stable isotope values and elemental composition (mean ± SE) from North Fort Myers, FL including a) ammonium ($\delta^{15}N\text{-}NH_4^+$) and nitrate ($\delta^{15}N\text{-}NO_3^-$) isotopes of groundwater by watershed (reference well, Hancock Creek, central drainage watershed, and Powell Creek) and season (wet/dry), b) as well as carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes and molar C:N:P of particulate organic matter (POM), a proxy for phytoplankton, collected from surface water sites by watershed; 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). Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison.

significant (ANOVA, all $p > 0.050$). Multiple comparisons between project years did not reveal significant differences between year 1 (+5.57 ± 0.5‰) and year 2 (+2.62 ± 0.5‰; Tukey HSD pairwise comparison, $p > 0.05$). Multiple comparisons by watershed revealed that groundwater $\delta^{15}N\text{-}NH_4^+$ values were significantly higher in the central drainage watershed (+5.54 ± 0.6‰) and Hancock Creek watershed (+4.51 ± 0.9‰), than in Powell Creek watershed (+2.77 ± 0.6‰) or the reference well (+2.20 ± 1.1‰; Fig. 5a). In year 2, Powell Creek watershed had significantly higher $\delta^{15}N\text{-}NH_4^+$ values (+2.51 ± 1.1‰) than the reference well (+0.98 ± 1.1‰). Interestingly, in year 2 the central drainage watershed had the highest average groundwater $\delta^{15}N\text{-}NH_4^+$ values (+3.92 ± 0.6‰), but due to the high variability observed (range from −0.75 to 5.96‰), the difference was not significant. Similarly, in year 2 Hancock Creek had a large groundwater $\delta^{15}N\text{-}NH_4^+$ range (from −5.72 to +6.74‰), which lowered the watershed mean in that year (+1.99 ± 1.0‰). The reference well had the tightest range in year 2 (from −1.08 to +3.06‰).

Aqueous $\delta^{15}N\text{-}NO_3^-$ values of groundwater in North Fort Myers were variable with a mean of +5.37 ± 1.2‰ and range of −10.5 to +44.1‰. Groundwater $\delta^{15}N\text{-}NO_3^-$ values were variable by season (ANOVA, $F = {}_{1,\,58}\,6.16$, $p = 0.016$), but not by project year (ANOVA, $F = {}_{1,\,58}\,0.017$, $p = 0.896$) or watershed (ANOVA, $F = {}_{3,\,58}\,1.95$, $p = 0.131$). The interaction of project year and season was significant (ANOVA, $F = {}_{3,\,58}\,14.7$, $p < 0.001$). Wet season groundwater $\delta^{15}N\text{-}NO_3^-$ values (+7.89 ± 1.9‰) were significantly higher than dry season values (+2.71 ± 1.5‰; Fig. 5a). While watershed was not a significant factor, $\delta^{15}N\text{-}NO_3^-$ values of groundwater were highest in Hancock Creek watershed (+8.49 ± 2.7‰), followed by Powell Creek watershed (+4.90 ± 2.6‰), the central drainage watershed (+4.65 ± 1.2‰), and the reference well (−1.52 ± 2.8‰; Fig. 5a). The interaction of project year and season revealed that groundwater $\delta^{15}N\text{-}NO_3^-$ values from the year 2 wet season (+14.3 ± 2.9‰) were significantly higher than the year 1 wet season (+0.82 ± 0.6‰) and the year 2 dry season (+0.08 ± 1.8‰), while the year 1 dry season was not significantly different from any sampling events (+6.00 ± 2.2‰).

Surface water POM $\delta^{13}C$ values were generally depleted throughout the study area (Fig. 5b) with values ranging from −38.3 to −24.8‰ and an overall mean of −30.2 ± 0.19‰. POM $\delta^{13}C$ values were variable by project year (Mann-Whitney *U* test, $U = 10{,}355$, $n = 240$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 15.9$, $n = 240$, $p < 0.001$), but not season (Mann-Whitney *U* test, $U = 7.189$, $n = 240$, $p = 0.983$). Surface water POM $\delta^{13}C$ values were significantly higher in year 2 (−29.0 ± 0.19‰) than in year 1 (−31.3 ± 0.28‰). By watershed, the central drainage feature had significantly more enriched POM $\delta^{13}C$ values (−28.1 ± 0.56‰), than Hancock Creek (−30.1 ± 0.25‰) or Powell Creek (−30.7 ± 0.29‰).

Surface water POM $\delta^{15}N$ values were generally enriched (>+3‰) throughout the study area with values ranging from −8.45 to +11.9‰ and an overall mean of +4.69 ± 0.18‰. POM $\delta^{15}N$ values were variable by season (Mann-Whitney *U* test, $U = 5117$ $n = 239$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 10.3$, $n = 239$, $p = 0.006$), but not project year (Mann-Whitney *U* test, $U = 7306$, $n = 240$, $p = 0.756$). The dry season had significantly higher surface water POM $\delta^{15}N$ values (+5.28 ± 0.22‰) than the wet season (+4.10 ± 0.27‰). Surface water POM $\delta^{15}N$ values were significantly higher in Hancock Creek (+5.30 ± 0.22‰), than the central drainage feature (+4.18 ± 0.57‰) and Powell Creek (+4.05 ± 0.31‰; Fig. 5b).

Surface water POM C:N ratios ranged from 0.96 to 13.7 with an overall mean of 8.63 ± 0.09. POM C:N ratios were variable by project year (Mann-Whitney *U* test, $U = 5222$, $n = 240$, $p < 0.001$), season (Mann-Whitney *U* test, $U = 9250$, $n = 240$, $p < 0.001$), and watershed (Kruskal-Wallis test, $H = 8.21$, $n = 240$, $p = 0.016$). POM C:N ratios were significantly higher in year 1 (8.83 ± 0.11) than in year 2 (8.43 ± 0.15). Wet season POM C:N ratios (9.04 ± 0.14) were significantly higher than in the dry season (8.22 ± 0.11). Powell Creek POM C:N ratios (8.83 ± 0.17) were significantly higher than the central drainage feature (8.54 ± 0.19) and Hancock Creek (8.49 ± 0.12; Fig. 5b).

Surface water POM C:P ratios ranged from 21.9 to 221 with an overall mean of 95.5 ± 2.5. The POM C:P ratios were variable by project year (Mann-Whitney *U* test, $U = 10{,}371$, $n = 240$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 13.5$, $n = 240$, $p = 0.001$), but not season (Mann-Whitney *U* test, $U = 7690$, $n = 240$, $p = 0.362$). Surface water POM C:P ratios were significantly higher in year 2 (110 ± 0.38) than year 1 (81.1 ± 2.9). The central drainage feature had significantly higher POM C:P ratios (115 ± 5.0) than Hancock Creek (97.7 ± 3.6) and Powell Creek (88.0 ± 4.2; Fig. 5b).

Surface water POM N:P ratios ranged from 2.05 to 26.6 with an overall mean of 11.3 ± 0.31. POM N:P ratios were variable by project year (Mann-Whitney *U* test, $U = 10{,}758$, $n = 240$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 15.1$, $n = 240$, $p = 0.001$), but not season (Mann-Whitney *U* test, $U = 6983$, $n = 240$, $p = 0.687$). POM N:P ratios were significantly higher in year 2 (13.3 ± 0.44) than year 1 (9.36 ± 0.35). All watersheds had significantly different POM N:P ratios (Fig. 5c). The central drainage feature had the highest POM N:P ratio (13.6 ± 0.65), followed by Hancock Creek (11.8 ± 0.47), and Powell Creek (10.19 ± 0.44; Fig. 5b).

Green macroalgae in the order Ulotrichales were collected at SW6 (North Shore Park) on October 17, 2017 and March 14, 2018 (unidentifiable to genus). No other sites had macroalgae present and/or accessible during sampling events. In October 2017, the δ^{13}C and δ^{15}N values for macroalgae were − 18.3 ± 0.24‰ and + 7.74 ± 0.09‰, respectively. In March 2018, the δ^{13}C and δ^{15}N values were − 29.7 ± 0.03‰ and + 5.01 ± 0.06‰, respectively. Overall, the δ^{13}C and δ^{15}N values for green macroalgae collected from SW6 were − 24.0 ± 2.54‰ and + 6.38 ± 0.61‰, respectively.

Microcystis spp. POM samples collected from Davis Boat Ramp on October 19, 2017, had depleted δ^{13}C values similar to those from the study area (−29.9 ± 0.19‰) and enriched δ^{15}N values within the range of human waste (+6.93 ± 0.81‰; Fig. 6a, b). Further, *Microcystis* spp. POM samples collected during a bloom on July 19, 2018, at North Shore Park (SW6) also had depleted δ^{13}C values (−28.8 ± 0.02‰) and enriched δ^{15}N values (+8.79 ± 0.04‰; Fig. 6a, b). Another localized *Microcystis* spp. bloom ~12 km downstream from the study area in the Caloosahatchee River Estuary was sampled on July 19, 2018, from a residential finger canal near Normandy Court in Cape Coral, FL. This very high biomass HAB event (see Fig. S2b) had δ^{13}C values that were slightly higher than the study area (−26.5 ± 0.08‰) and enriched δ^{15}N values (+7.42 ± 0.04‰).

Karenia brevis bloom POM samples collected in coastal areas of Lee County during 2018 had more enriched δ^{13}C values (−15.9 ± 1.2‰) than the samples collected in the study area (−30.2 ± 0.19‰), while the δ^{15}N values were similar (+4.94 ± 0.24‰ vs. 4.69 ± 0.18%, respectively; Fig. 6a,c). The *K. brevis* POM samples collected from several beaches on March 14, 2018, had more enriched δ^{13}C values (−11.3 ± 1.7‰) and lower δ^{15}N values (+3.85 ± 0.23‰) than those collected from Lighthouse Beach Park, Sanibel, FL, on September 29, 2018, which had more depleted δ^{13}C values (−18.7 ± 0.57‰) and more enriched δ^{15}N values (+5.60 ± 0.11‰). On March 14, 2018, *K. brevis* POM samples from Lighthouse Beach had more enriched δ^{13}C values (−9.83‰) and more depleted δ^{15}N values (+3.33‰) than those collected on September 29, 2018, which had more depleted δ^{13}C values (−18.7 ± 0.57‰) and more enriched δ^{15}N values (+5.60 ± 0.11‰; Fig. 6a,c).

3.5. Bacterial abundance

Enterococci counts were significantly lower in groundwater (39.2 ± 30 MPN/100 mL) than in surface water (594 ± 88 MPN/100 mL; Mann-Whitney *U* test, U = 6163, n = 160, p < 0.001). Groundwater enterococci

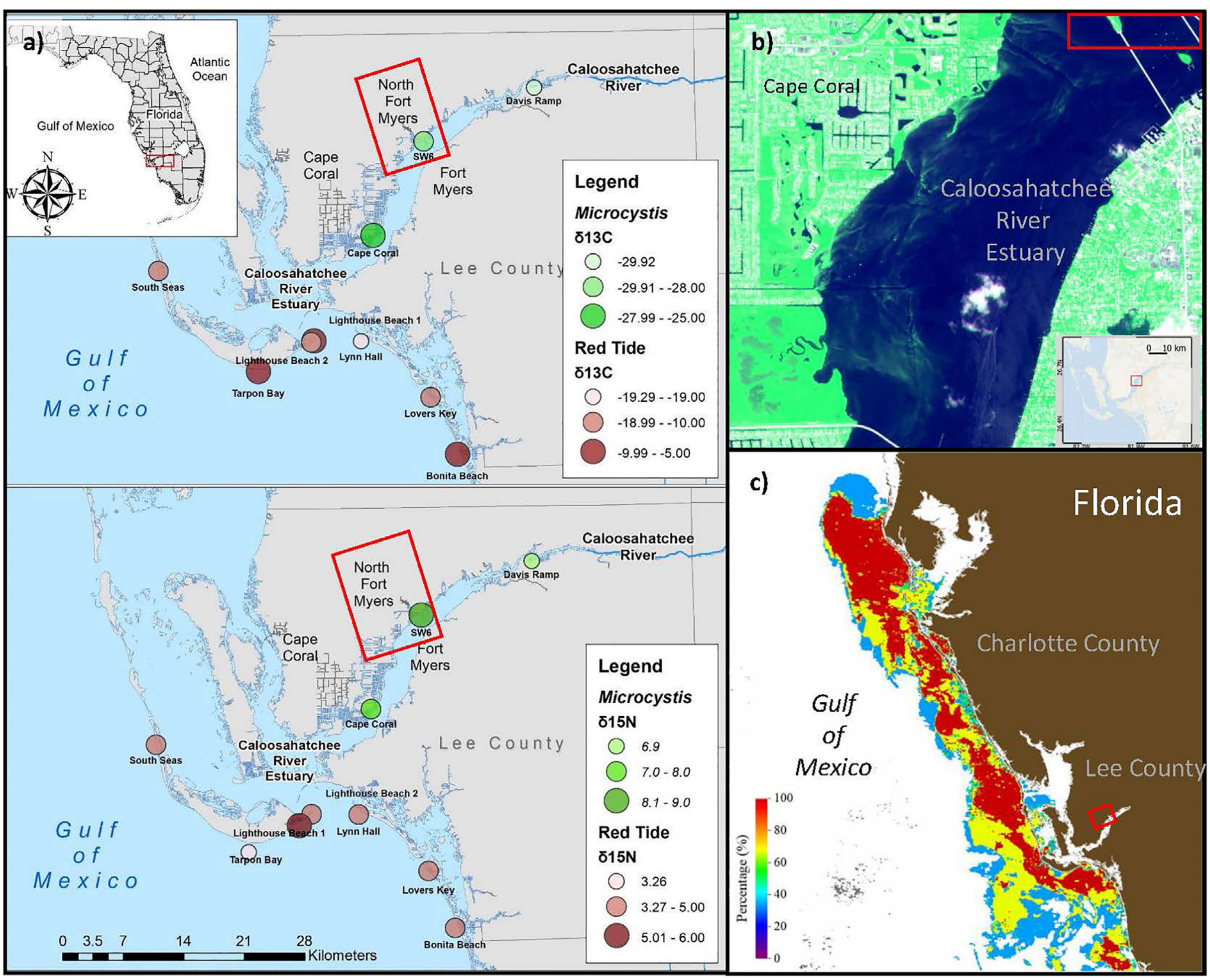

Fig. 6. Harmful algal bloom event sampling locations (dots) in Lee County, FL for blue-green algae, primarily *Microcystis* spp., (green) and red tide (*Karenia brevis*; red), a) showing average (n = 3; Davis Ramp, SW6, Cape Coral, Lighthouse Beach 2) or single sample values (Bonita Beach, Lovers Key, Lynn Hall, Lighthouse Beach 1, Tarpon Bay, and South Seas) by location for δ^{13}C and δ^{15}N. b) A false-color Red-Green-Blue image from the PlanetScope DOVE on July 11, 2018, showing surface cyanobacterial scums in the Caloosahatchee River Estuary. In this image, clouds appear white, land appears green, water appears blue, and cyanobacterial scums appear greenish over the water background. and c) Frequency of red tide observations between September 24–30, 2018, estimated from satellite and field observations. Here, “red tide” is defined as *K. brevis* concentrations >10^5 cells/L. Panels b and c courtesy of Chuanmin Hu. The approximate North Fort Myers study area is denoted in each panel by a red box.

counts ranged from <0.5 to >2420 MPN/100 mL and were not significantly different by watershed (Kruskal-Wallis test, H = 2.28, n = 80, df = 3, p = 0.516), season (Mann-Whitney *U* test, U = 0.03, n = 80, p = 0.862), or project year (Mann-Whitney *U* test, U = 0.27, n = 80, p = 0.603). Groundwater enterococci counts were highest in the Powell Creek watershed (114 ± 100 MPN/100 mL), followed by the Hancock Creek (11.0 ± 8.1 MPN/100 mL), central drainage (5.3 ± 2.3 MPN/100 mL), and reference (2.3 ± 0.69 MPN/100 mL) watersheds (Fig. 7a).

Surface water enterococci counts ranged from <5 to >2420 MPN/100 mL and were variable by watershed (Kruskal-Wallis test, H = 7.58, n = 80, df = 2, p = 0.023) and season (Mann-Whitney *U* test, U = 7.32, n = 80, p = 0.007), but not by project year (Mann-Whitney *U* test, U = 598, n = 80, p = 0.051). Pairwise comparisons revealed no significant differences in surface water enterococci counts between watersheds (Dunn's test, all p > 0.05). The highest surface water enterococci counts were observed in the central drainage feature (883 ± 278 MPN/100 mL), followed by Powell Creek (767 ± 162 MPN/100 mL), and Hancock Creek (397 ± 97 MPN/100 mL; Fig. 7a). Seasonal effects were observed in surface water enterococci counts with a higher count in the dry (907 ± 150 MPN/100 mL), than the wet (280 ± 59 MPN/100 mL) season (Fig. 7b).

Escherichia coli counts were significantly lower in groundwater (8.60 ± 7.2 MPN/100 mL) than in surface water (650 ± 91 MPN/100 mL; Mann-Whitney *U* test, U = 6345, n = 160, p < 0.001). Groundwater *E. coli* counts ranged from 0.5 to 579 MPN/100 mL and were not variable by watershed (Kruskal-Wallis test, H = 2.06, n = 80, df = 3, p = 0.560), season (Mann-Whitney *U* test, U = 1.00, n = 80, p = 0.317), or project year (Mann-Whitney *U* test, U = 0.07, n = 80, p = 0.786). Though not significantly different, the highest groundwater *E. coli* counts were observed in the central drainage watershed (25 ± 24 MPN/100 mL), followed by the Powell Creek (2.4 ± 1.9 MPN/100 mL) and Hancock Creek (0.75 ± 0.18 MPN/100 mL) watersheds, as well the reference well (0.5 ± 0 MPN/100 mL; Fig. 7a).

Surface water *E. coli* counts ranged from 38 to >4840 MPN/100 mL and were variable by watershed (Kruskal-Wallis test, H = 16.3, n = 80, df = 2, p < 0.001) and season (Mann-Whitney *U* test, U = 9.82, n = 80, p = 0.002), but not by project year (Mann-Whitney *U* test, U = 704, n = 80, p = 0.355). Powell Creek had significantly higher surface water *E. coli* counts (893 ± 147 MPN/100 mL) than Hancock Creek (323 ± 44 MPN/100 mL; Fig. 7a). The central drainage feature had the highest surface water *E. coli* counts (1310 ± 570 MPN/100 mL), but the variability in that watershed was also largest and the number of sites lowest (n = 1); therefore, differences between the central drainage watershed and the other watersheds were not statistically significant. Significantly higher surface water *E. coli* counts were observed in the dry season (969 ± 164 MPN/100 mL) than the wet season (331 ± 37 MPN/100 mL; Fig. 7b).

BOD concentrations were significantly higher in groundwater (1.19 ± 0.16 mg/L) than in surface water (1.02 ± 0.08 mg/L; Mann-Whitney *U* test, U = 3806, n = 160, p = 0.036). Groundwater BOD concentrations ranged from 0.15 to 5.2 mg/L and were variable by watershed (Kruskal-Wallis test, H = 40.0, n = 80, df = 3, p < 0.001), but not by season (Mann-Whitney *U* test, U = 1.61, n = 80, p = 0.204) or project year (Mann-Whitney *U* test, U = 0.57, n = 80, p = 0.451). Powell Creek watershed had the highest groundwater BOD concentration (2.77 ± 0.31 mg/L) and was significantly higher than the other watersheds, which all had similar BOD concentrations (reference well = 0.59 ± 0.19 mg/L, central drainage watershed = 0.57 ± 0.12 mg/L, and Hancock Creek watershed = 0.43 ± 0.14 mg/L; Fig. 7a).

Surface water BOD concentrations ranged from 0.15 to 4.4 mg/L and were variable by season (Mann-Whitney *U* test, U = 15.6, n = 80, p < 0.001), but not by watershed (Kruskal-Wallis test, H = 2.32, n = 80, df = 2, p = 0.314) or project year (Mann-Whitney *U* test, U = 609, n = 80, p = 0.065). The highest surface water BOD by watershed was at the central drainage feature (1.49 ± 0.43 mg/L), followed by Hancock Creek (0.98 ± 0.11 mg/L), and Powell Creek (0.96 ± 0.08 mg/L; Fig. 7a). Surface water BOD concentrations were significantly higher in the dry season (1.30 ± 0.12 mg/L) than the wet season (0.74 ± 0.07 mg/L; Fig. 7b).

Average FIB concentrations were relatively low in groundwater, though individual samples were occasionally high. For example, in Hancock Creek groundwater, an elevated enterococci concentration of 195 MPN/100 mL was observed on 08/27/2019, while a high BOD concentration of 3.20 mg/L was observed on 02/10/2020. In the central drainage basin *E. coli* concentrations up to 579 MPN/100 mL were observed with corresponding

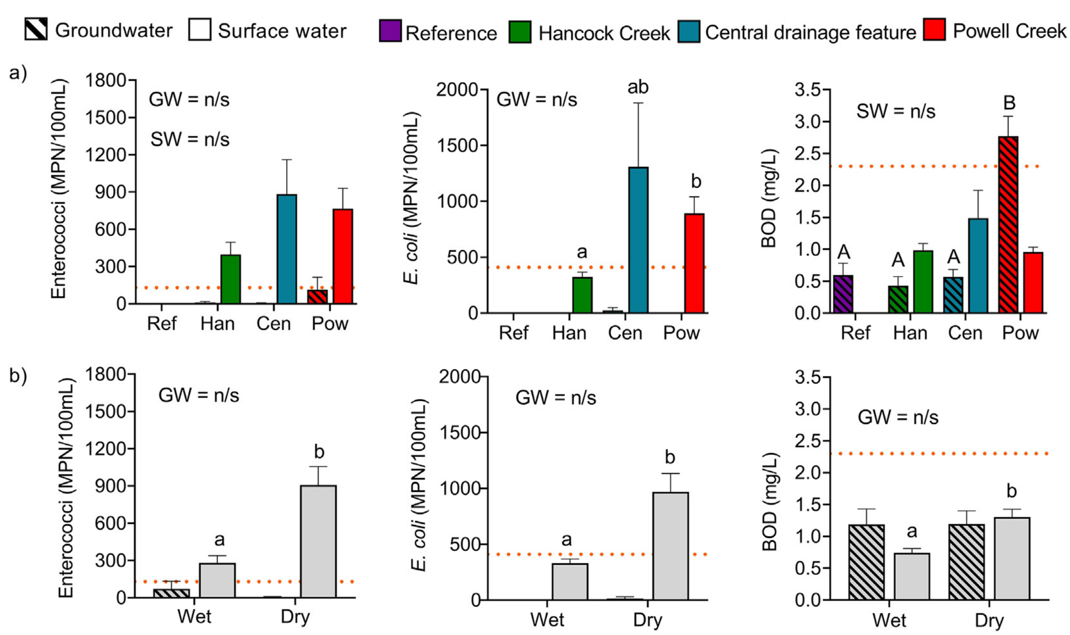

Fig. 7. Fecal indicator bacteria abundance, including enterococci and *Escherichia coli* (*E. coli*), as well as biochemical oxygen demand (BOD) concentrations of groundwater (striped) and surface water (no pattern) by a) watershed (reference well, Hancock Creek, central drainage feature, and Powell Creek), and b) season (wet/dry); dotted lines indicate Florida Department of Environmental Protection surface water criteria for enterococci (marine water ≥130 MPN/100 mL), BOD (2.4 mg/L), and *E. coli* (fresh water ≥410 MPN/100 mL). Some surface water standards do not apply to groundwater or the study area because it is freshwater and are shown as a point of reference only. Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison. All show mean count ± SE.

enterococci concentrations of 49 MPN/100 mL from the same date (02/13/2018), while elevated BOD concentrations (>1 mg/L) were observed in six of 24 groundwater samples. Finally, in Powell Creek basin a very high enterococci concentration of >2420 MPN/100 mL was observed on 10/18/2017, while elevated BOD concentrations (>1 mg/L) were consistently observed in 20 of 24 groundwater samples, with 16 of those being >2 mg/L. Thus, in Powell Creek groundwater, average BOD concentrations were particularly high (2.77 ± 0.31 mg/L).

3.6. Molecular markers

Concentrations of the human marker HF183 spanned from below detection to 1820 GEU/100 mL in surface water. HF183 concentrations were variable by watershed (Kruskal-Wallis test, H = 13.3, n = 80, df = 2, p = 0.001), but not by season (Mann-Whitney *U* test, U = 1.34, n = 80, p = 0.247) or project year (Mann-Whitney *U* test, U = 0.476, n = 80, p = 0.490). HF183 concentrations were significantly higher in the central drainage feature (406 ± 175 GEU/100 mL) than in Hancock Creek (31.3 ± 114 GEU/100 mL), but not Powell Creek (177 ± 76 GEU/100 mL; Fig. 8).

Avian markers were also detected in surface water. For example, concentrations of GFD fluctuated from below detection to 32,600 TSC/100 mL. GFD concentrations were variable by watershed (Kruskal-Wallis test, H = 8.58, n = 80, df = 2, p = 0.014) and season (Mann-Whitney *U* test, U = 7.32, n = 80, p = 0.007), but not by project year (Mann-Whitney *U* test, U = 0.755, n = 80, p = 0.385). Multiple comparisons by watershed did not find any significant differences in GFD concentrations (Dunn's test, all p > 0.05; Fig. 8). Significantly higher GFD concentrations were observed in the wet season (2472 ± 961 TSC/100 mL) than in the dry season (488 ± 223 TSC/100 mL). Gull2 was only detected in Hancock Creek and concentrations ranged from below detection to 107,000 TSC/100 mL (Fig. 8). Gull2 concentrations were not variable by season (Mann-Whitney *U* test, U < 0.001, n = 80, p = 0.992) or project year (Mann-Whitney *U* test, U = 2.81, n = 80, p = 0.094). The ruminant marker BacR was not detected during the study.

3.7. Chemical tracers

Though apparently higher, groundwater sucralose concentrations (5769 ± 1291 ng/L) were not significantly different from surface water concentrations (635.3 ± 37.6 ng/L; Mann-Whitney *U* test, U = 2897, n = 160, p = 0.300). In groundwater, sucralose was detected at least once in all wells with individual concentrations ranging from below detection to 58,000 ng/L. Concentrations of sucralose in groundwater were variable by watershed (Kruskal-Wallis test, H = 42.3, n = 80, df = 3, p < 0.001), but not by season (Mann-Whitney *U* test, U = 2.51, n = 80, p = 0.113) or project year (Mann-Whitney *U* test, U = 0.65, n = 80, p = 0.419). By watershed, groundwater sucralose concentrations in the reference well (195 ± 26 ng/L) and Powell Creek (580 ± 349 ng/L) were significantly lower than in the central drainage basin (5554 ± 1665 ng/L) or Hancock Creek (13,030 ± 3507 ng/L; Fig. 9).

Sucralose was ubiquitous in surface water of the study area with detections occurring at every site and individual concentrations ranging from 86 to 1600 ng/L. Sucralose varied by watershed (Kruskal-Wallis test, H = 6.81, n = 80, df = 2, p = 0.033), but not season (Mann-Whitney *U* test, U = 1.54, n = 80, p = 0.214) or project year (Mann-Whitney *U* test, U = 0.265, n = 80, p = 0.607). Multiple comparisons by watershed showed no significant differences in surface water sucralose concentrations (Dunn's test, all p > 0.05). Though no significant differences were found between watersheds, the highest surface water sucralose concentrations were observed in Powell Creek (749 ± 66 ng/L), followed by the central drainage feature (710 ± 74 ng/L) and Hancock Creek (529 ± 46 ng/L; Fig. 9).

Carbamazepine concentrations were significantly higher in groundwater (12.4 ± 4.9 ng/L) than in surface water (5.77 ± 0.7 ng/L; Mann-Whitney *U* test, U = 4070, n = 160, p = 0.003). Carbamazepine was not detected in the reference well, but was detected in groundwater of all other watersheds in low concentrations (Fig. 9) with individual values ranging from below detection to 310 ng/L. Groundwater carbamazepine concentrations were variable by watershed (Kruskal-Wallis test, H = 50.3, n = 80, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 0.450, n = 80, p = 0.501) or project year (Mann-Whitney *U* test, U = 0.020, n = 80, p = 0.892). The highest groundwater carbamazepine concentrations were found in the Hancock Creek watershed (36.8 ± 15 ng/L) and the central drainage watershed (4.13 ± 0.6 ng/L), which were significantly higher than Powell Creek (0.28 ± 0.28 ng/L) and the reference well (no detection; Fig. 9).

In surface water, carbamazepine was detected at least once at every site in low concentrations with an overall range from below detection to 28 ng/L (Fig. 9). Surface water carbamazepine concentrations varied by watershed (Kruskal-Wallis test, H = 52.9, n = 80, df = 2, p < 0.001), but not season (Mann-Whitney *U* test, U = 0.854, n = 80, p = 0.355) or project year (Mann-Whitney *U* test, U = 0.946, n = 80, p = 0.331). Powell Creek had significantly higher carbamazepine concentrations in surface water (11.9 ± 1.1 ng/L) than Hancock Creek (1.71 ± 0.18 ng/L) or the central drainage feature (1.44 ± 0.26 ng/L; Fig. 9).

Groundwater primidone concentrations (6.16 ± 1.3 ng/L) were not significantly different from those in surface water (3.04 ± 0.4 ng/L; Mann-Whitney *U* test, U = 3382, n = 160, p = 0.481). Primidone was not detected in the reference well but was detected in groundwater of all other watersheds with individual concentrations ranging from below detection to 47 ng/L. Concentrations of primidone in groundwater were not variable by project year (Mann-Whitney *U* test, U = 2.35, n = 80, p = 0.125), watershed (Kruskal-Wallis test, H = 4.28, n = 80, df = 3, p = 0.233), or season (Mann-Whitney *U* test, U = 0.003, n = 80, p = 0.953). All watersheds with detections had similar groundwater primidone concentrations (Powell Creek = 5.50 ± 2.2 ng/L, Hancock Creek = 5.27 ± 1.75 ng/L, and central drainage = 9.75 ± 3.3 ng/L; Fig. 9).

In surface water, primidone was detected at least once at every site in low levels with an overall range from below detection to 14 ng/L (Fig. 9). Surface water primidone concentrations varied by season (Mann-Whitney *U* test, U = 31.7, n = 80, p < 0.001), but not by watershed (Kruskal-Wallis test, H = 1.48, n = 80, df = 2, p = 0.478) or project year (Mann-Whitney *U* test, U = 0.328, n = 80, p = 0.567). Surface water primidone

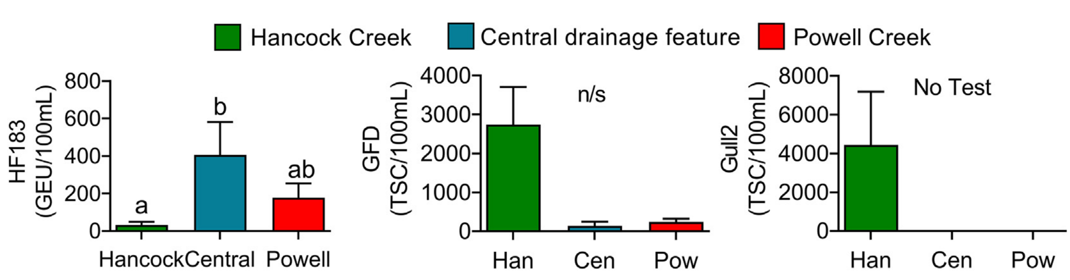

Fig. 8. Molecular markers concentrations in surface water (mean ± SE) of North Fort Myers, FL by watershed (Hancock Creek, a central drainage feature, and Powell Creek) including the human marker, HF183, and the avian markers GFD and Gull2. Significant differences are represented by lowercase letters, while "n/s" represents a non-significant statistical comparison.

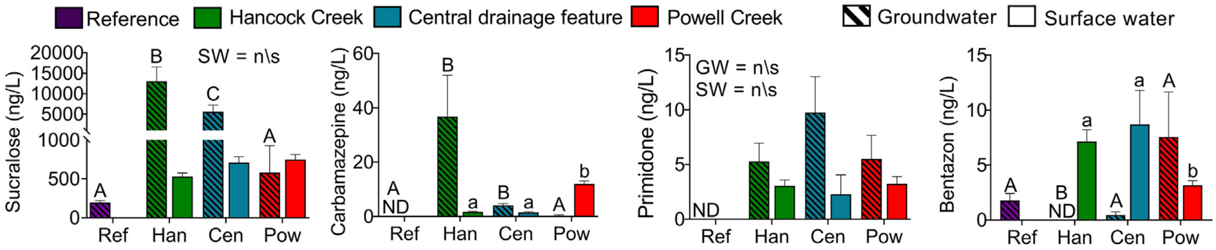

Fig. 9. Chemical tracer concentrations (mean ± SE) of groundwater (striped) and surface water (no pattern) observed in North Fort Myers, FL by watershed (reference well, Hancock Creek, central drainage basin, and Powell Creek), including the artificial sweetener sucralose, the anticonvulsant pharmaceuticals carbamazepine and primidone, and the herbicide bentazon. Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison.

concentrations were significantly higher in the dry (5.28 ± 0.58 ng/L) than the wet (0.80 ± 0.31 ng/L) season. Though no significant differences were found between watersheds, the highest surface water primidone concentrations were observed in Powell Creek (3.23 ± 0.66 ng/L), followed by Hancock Creek (3.04 ± 0.54 ng/L) and the central drainage feature (2.29 ± 1.8 ng/L; Fig. 9).

Acetaminophen was detected in groundwater and surface water in the central drainage and Powell Creek basins with no significant difference between groundwater (3.63 ± 3.0 ng/L) and surface water concentrations (1.92 ± 0.76 ng/L; Mann-Whitney *U* test, U = 3394, n = 80, p = 0.131). Acetaminophen was only detected three times in groundwater during the study with one detection in Powell Creek (GW7) on 11/15/217 (23 ng/L) and two detections from the same well (GW5) in the central drainage watershed on 08/27/2019 (27 ng/L) and 09/16/2019 (240 ng/L). Surface water acetaminophen concentrations ranged from below detection to 38 ng/L. Acetaminophen concentrations in surface water varied by watershed (Kruskal-Wallis test, H = 12.0, n = 80, df = 2, p = 0.002), but not season (Mann-Whitney *U* test, U = 0.533, n = 80, p = 0.465) or project year (Mann-Whitney *U* test, U = 0.506, n = 80, p = 0.477). Surface water acetaminophen concentrations were significantly higher in the central drainage feature (6.50 ± 3.6 ng/L) and Powell Creek (3.18 ± 0.16 ng/L), than in Hancock Creek (no detection).

Groundwater bentazon concentrations were significantly higher (2.59 ± 1.3 ng/L) than those in surface water (0.01 ± 0.001 ng/L; Mann-Whitney *U* test, U = 1185, n = 80, p < 0.001). Bentazon was detected in groundwater in three of the four watersheds in very low concentrations with a range from below detection to 44 ng/L. In groundwater, concentrations of bentazon were variable by watershed (Kruskal-Wallis test, H = 8.92. n = 40, df = 3, p = 0.030), but not season (Mann-Whitney *U* test, U = 216, n = 40, p = 0.678). Due to the high variability observed in Powell Creek (7.56 ± 4.1 ng/L) and the low concentration observed in the central drainage basin (0.47 ± 0.27 ng/L), only the reference well (1.78 ± 0.64 ng/L) and Hancock Creek (no detections) had significantly different bentazon concentrations from each other (Fig. 9).

Surface water bentazon concentrations ranged from below detection to 17 ng/L. In surface water, bentazon concentrations varied by watershed (Kruskal-Wallis test, H = 9.33, n = 40, df = 2, p = 0.009) and season (Mann-Whitney *U* test, U = 4.86, n = 40, p = 0.027). Surface water bentazon concentrations were significantly higher in the central drainage feature (8.7 ± 3.1 ng/L) and Hancock Creek (7.15 ± 1.1 ng/L) than in Powell Creek (3.16 ± 0.41 ng/L; Fig. 9). By season, bentazon concentrations were significantly higher in the wet season (7.23 ± 1.1 ng/L) than the dry season (4.19 ± 0.8 ng/L).

Diuron concentrations in surface water ranged from below detection to 4.1 ng/L and did not vary by watershed (Kruskal-Wallis test, H = 5.67, n = 10, df = 2, p = 0.059). Though no significant differences were found between watersheds, the highest surface water diuron concentrations were observed in the central drainage feature (one detection = 3.9 ng/L), followed by Hancock Creek (2.48 ± 0.70 ng/L), and Powell Creek (no detections).

Fluoridone was detected at all sites throughout the study area with concentrations ranging from 3 to 95 ng/L. Surface water fluoridone concentrations did not vary by watershed (Kruskal-Wallis test, H = 3.18, n = 101, df = 2, p = 0.203). Though no significant differences were found between watersheds, the highest surface water fluoridone concentrations were observed in Hancock Creek (36.4 ± 17 ng/L), followed by Powell Creek (4.13 ± 0.62 ng/L) and the central drainage feature (one detection = 3.30 ng/L).

Imazapyr was detected at all surface water sites throughout the study area with concentrations ranging from 16 to 38 ng/L. Surface water imazapyr concentrations did not vary by watershed (Kruskal-Wallis test, H = 2.48, n = 10, df = 2, p = 0.290) Though no significant differences were found between watersheds, the highest surface water imazapyr concentrations were observed in Powell Creek (32.3 ± 3.2 ng/L), followed by Hancock Creek (26.2 ± 3.6 ng/L), and the central drainage feature (one detection = 22 ng/L).

Imidacloprid was detected at all surface water sites throughout the study area, except for SW9, with concentrations ranging from below detection to 8.6 ng/L. Surface water imidacloprid concentrations did not vary by watershed (Kruskal-Wallis test, H = 1.50, n = 10, df = 2, p = 0.474). Though no significant differences were found between watersheds, the highest surface water imidacloprid concentrations were observed in the central drainage feature (one detection = 7.90 ng/L), followed by Powell Creek (4.87 ± 0.80 ng/L) and Hancock Creek (4.38 ± 1.4 ng/L).

Some chemical tracers were not widely detected in the North Fort Myers study area. The chemical tracers mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any groundwater wells during the study, while fluoridone was only detected at the reference well. The chemical tracers hydrocodone, ibuprofen, naproxen, mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any surface water sites during the study.

3.8. Correlations between variables

Fecal indicator bacteria had no strong (≥0.30) correlations with each other or human waste tracers (Fig. S11). In fact, enterococci and *E. coli* had weak (<0.30) negative correlations with some of the human waste tracers (Fig. S11). Further, groundwater BOD concentrations were negatively correlated with carbamazepine (Spearman r = −0.51, n = 74, p < 0.001), sucralose (Spearman r = −0.39, n = 74, p = 0.001), SRP (Spearman r = −0.35, n = 74, p = 0.002), TP (Spearman r = −0.37, n = 74, p = 0.001), and $\delta^{15}N$-NH_4^+ (Spearman r = −0.38, n = 72, p = 0.001), while positive correlations were observed between groundwater BOD concentrations and NH_4^+ (Spearman r = 0.51, n = 74, p < 0.001), DIN (Spearman r = 54, n = 74, p < 0.001), DIN:SRP (Spearman r = 70, n = 74, p < 0.001), TN (Spearman r = 48, n = 74, p < 0.001), and TN:TP (Spearman r = 67, n = 74, p < 0.001). Additionally, groundwater NH_4^+ concentrations were negatively correlated with carbamazepine (Spearman r = −0.45, n = 74, p < 0.001), sucralose (Spearman r = −0.37, n = 74, p = 0.001), NO_x (Spearman r = −0.32, n = 74, p = 0.005), and SRP (Spearman r

= −0.31, n = 74, p = 0.007). Groundwater NOx concentrations were positively correlated with carbamazepine (Spearman r = 0.42, n = 74, p < 0.001), SRP (Spearman r = 0.35, n = 74, p = 0.002), and $\delta^{15}N$-NH_4^+ (Spearman r = 0.32, n = 74, p = 0.002). Groundwater SRP and TP concentrations were also positively correlated with carbamazepine (Spearman r = 0.62, n = 74, p < 0.001; Spearman r = 0.68, n = 74, p < 0.001, respectively) and sucralose (Spearman r = 0.72, n = 74, p < 0.001; Spearman r = 0.67, n = 74, p < 0.001, respectively). Groundwater $\delta^{15}N$-NH_4^+ was positively correlated with sucralose (Spearman r = 0.30, n = 74, p = 0.010) and color (Spearman r = 0.33, n = 72, p = 0.010). The color of groundwater was also positively correlated with sucralose (Spearman r = 0.30, n = 74, p = 0.020). Groundwater pH was negatively correlated with sucralose (Spearman r = −0.65, n = 74, p < 0.001), carbamazepine (Spearman r = −0.46, n = 74, p < 0.001), and SRP (Spearman r = −0.45, n = 74, p < 0.001). Finally, groundwater salinity was positively correlated with primidone (Spearman r = 0.51, n = 74, p < 0.001), DIN (Spearman r = 0.36, n = 74, p = 0.002), and TN (Spearman r = 0.30, n = 74, p = 0.011).

Enterococci concentrations were positively correlated with *E. coli* (Spearman r = 0.78, n = 74, p < 0.001) and in surface water fecal indicator bacteria had strong positive correlations with human waste tracers (Fig. S12). Specifically, surface water enterococci concentrations were positively correlated with primidone (Spearman r = 0.32, n = 74, p = 0.005), sucralose (Spearman r = 0.306, n = 74, p = 0.008), and HF183 (Spearman r = 0.30, n = 74, p = 0.008). Positive correlations were also observed between surface water *E. coli* concentrations and carbamazepine (Spearman r = 0.37, n = 74, p = 0.001), primidone (Spearman r = 0.376, n = 74, p = 0.001), sucralose (Spearman r = 0.30, n = 74, p = 0.008), and HF183 (Spearman r = 0.32, n = 74, p = 0.009). Similarly, BOD was positively correlated with primidone (Spearman r = 0.40, n = 74, p < 0.000), as well as salinity (Spearman r = 0.38, n = 74, p = 0.001). HF183 concentrations in surface water were positively correlated with acetaminophen (Spearman r = 0.43, n = 74, p < 0.001), NH_4^+ (Spearman r = 0.43, n = 74, p < 0.001), DIN (Spearman r = 0.31, n = 74, p = 0.007), and TN (Spearman r = 0.35, n = 74, p = 0.002), but negatively correlated with pH (Spearman r = −0.36, n = 74, p = 0.002). Surface water NH_4^+ concentrations were positively correlated with acetaminophen (Spearman r = 0.30, n = 74, p = 0.009) and NO_x (Spearman r = 0.55, n = 74, p < 0.001), while surface water NO_x and DIN concentrations were positively correlated with carbamazepine concentrations (Spearman r = 0.42, n = 74, p < 0.001; Spearman r = 0.38, n = 74, p = 0.001, respectively). Surface water SRP concentrations were positively correlated with primidone (Spearman r = 0.35, n = 74, p = 0.002), but negatively correlated with TN (Spearman r = −0.55, n = 74, p < 0.001). Surface water pH was negatively correlated with reactive N, including NH_4^+ (Spearman r = −0.52, n = 74, p < 0.001), NO_x (Spearman r = −0.51, n = 74, p < 0.001), DIN (Spearman r = −0.57, n = 74, p < 0.001), and DIN:SRP (Spearman r = −0.47, n = 74, p < 0.001). Finally, surface water color was negatively correlated with primidone (Spearman r = −0.33, n = 74, p = 0.004), but positively correlated with DIN:SRP (Spearman r = 0.31, n = 74, p = 0.008) and TN (Spearman r = 0.30, n = 74, p = 0.010).

4. Discussion

This study demonstrated the nutrient and microbial couplings between septic systems, groundwater, surface water, and HABs in the highly modified Caloosahatchee River Estuary and downstream coastal waters. This multi-year study revealed that these human waste contaminant sources remain relatively consistent over time with location generally being the most influential factor for water quality. In each watershed there were multiple lines of evidence that indicated septic system effluent was adversely affecting water quality. Notably, shallow water tables demonstrated that these systems did not have the physical separation required for adequate treatment of effluent (>1 m separation from the ground surface to the seasonally high table). Evidence of human waste contamination observed in all three affected watersheds included high groundwater and surface water DIN concentrations, enriched $\delta^{15}N$ values of groundwater and POM that closely matched septic effluent (+4.9‰; Hinkle et al., 2008), and elevated surface water FIB with the presence of HF183, as well as detections of sucralose, carbamazepine, and primidone in groundwater and surface water. These findings demonstrate that septic systems are not protective of water quality in these low elevation watersheds, which has also been found in other urbanized waterfront residential areas, such as Michigan's Lower Peninsula (Verhougstraete et al., 2015), North Carolina (Humphrey et al., 2011; Cahoon et al., 2016), and Jepara, Indonesia (Adyasari et al., 2018). The presence of the chemical tracers 2,4-D, bentazon, diuron, fluoridone, imazapyr, and imidacloprid provided evidence that stormwater runoff was also adversely affecting surface water quality. Finally, detections of GFD and Gull2 indicated that avian fecal matter may also negatively affect water quality at some sites in North Fort Myers. Thus, these findings may be useful for understanding water quality and HAB drivers in other waterfront communities.

4.1. Nitrogen enrichment and harmful algal blooms

The high nutrient concentrations observed in North Fort Myers groundwater and surface water reflects enrichment from septic system effluent. Groundwater NH_4^+ concentrations were particularly high in the central drainage and Powell Creek basins, while NO_x concentrations were at background levels (Fig. 4). These drainage basins had the highest water tables (Fig. 3), which would likely suppress coupled nitrification-denitrification (Lapointe et al., 1990; Mallin, 2013). Conversely, in Hancock Creek where water tables were deeper, groundwater NO_x concentrations were highest and NH_4^+ concentrations were lower, indicating that some nitrification was occurring. $\delta^{15}N$-NO_3^- values were also highest in Hancock Creek groundwater (+8.49‰), further linking these high NO_x concentrations to a human waste source. Despite the variability between reactive N species, DIN, SRP, TN, and TP concentrations were all significantly higher in groundwater near high density septic systems than in the less densely developed, upland reference well. Surface water in the drainage basins with the shallower water tables also had higher reactive N concentrations and N:P. The various significant correlations of HF183, carbamazepine, and acetaminophen with surface water NH_4^+, NO_x, and TN concentrations help to connect this N loading to effluent from septic systems (Fig. S12). Conversely, surface water P concentrations were not variable between watersheds and may not be as affected by septic system effluent loading because some of these additional P inputs are likely adsorbed by sediments (Lapointe et al., 1990; Weiskel and Howes, 1992). The study region has P-rich phosphorus deposits in the Bone Valley Formation, which provide for natural elevation of P concentrations (Odum, 1953). Despite this, P was elevated above FDEP standards throughout the study area (Fig. 4), which may be reflective of anthropogenic inputs from human waste. These findings support previous work that identified local basin nutrient sources as more “severe” than Lake Okeechobee discharges and proposed that improving water in local basins would have the greatest effect on estuarine water quality (Doering and Chamberlain, 1999; Lapointe and Bedford, 2007).

As such, nutrient enrichment from the local North Fort Myers basin may support HAB events, such as those that occurred during this study in 2017–2018 following Hurricane Irma (Glibert, 2020). While this study is one of few to connect downstream HABs with upstream nutrient loading from septic systems, this has been observed in other locations, such as the St. Lucie Estuary, FL (Lapointe et al., 2017), Waquoit Bay, Massachusetts (Valiela et al., 1992) and Lake Huron, Ontario, Canada (Rakhimbekova et al., 2021). In many areas of Florida, due to the high background P (Odum, 1953), N has been identified as the element most capable of promoting algal growth and toxicity– especially for cyanobacteria (Kramer et al., 2018), which has been observed in other locations as well (Gobler et al., 2016). In the estuarine and coastal environments adjacent to Lee County, *M. aeruginosa* and *K. brevis* blooms thrive in low N:P conditions (Ketchum and Keen, 1948; Odum, 1953; Lapointe et al., 2006; Yentsch et al., 2008; Lapointe et al., 2012; Lapointe et al., 2017), but *K. brevis* can also be sustained at higher N:P (Odum et al., 1955). At SRP concentrations typical of coastal Southwest Florida, growth of *K. brevis* in lab cultures was a linear function of ammonia in sewage effluent (Doig and Martin, 1974). In addition to the exacerbating effects of excess N loading to HABs, reduced salinity has also been shown to favor *K. brevis* and *Microcystis* spp. blooms (Slobodkin, 1953; Brand and Compton, 2007; des Aulnois et al., 2019; Medina et al., 2020). During wet periods, such as following tropical storms or hurricanes, the downstream Caloosahatchee River Estuary can be seeded with *M. aeruginosa* from Lake Okeechobee freshwater discharges. Similarly, blooms of *M. aeruginosa* occurred in Southeast Florida's St. Lucie River Estuary in 2005, 2013, and 2016 that were attributed to algal "seeding" from Lake Okeechobee discharges combined with high biomass local basin blooms supported by urban nutrient loading (Lapointe et al., 2012; Phlips et al., 2012; Lapointe et al., 2017; Kramer et al., 2018). In 2016, the phytoplankton biomass in Lake Okeechobee was N-limited during *M. aeruginosa* bloom conditions (Kramer et al., 2018); thus after heavy rainfall and discharges these blooms experienced exponential growth in urbanized estuaries with low salinities and high levels of DIN, especially NH_4^+ (Lapointe et al., 2017; Kramer et al., 2018). This is consistent with this study, where cyanobacterial bloom samples collected from nearby Cape Coral residential canals (Fig. S2) had very high concentrations of NH_4^+ (2107 μM) and SRP (148 μM) with enriched POM $\delta^{15}N$ values (+7.41‰; Fig. 6). Comparable $\delta^{15}N$ values (+6.93‰) were observed in *M. aeruginosa* samples collected from the Caloosahatchee River Estuary in 2005 (Lapointe et al., 2006) demonstrating the consistency of this issue and nutrient source over time.

There were similarities observed between the study area and coastal HABs. For example, in North Fort Myers $\delta^{15}N$ values were enriched in cyanobacteria (+8.79‰) and green macroalgae (+6.38) samples collected at North Shore Park, as well as in groundwater aqueous NH_4^+ samples (+3.90‰), groundwater aqueous NO_3^- samples (+6.36‰), and surface water POM samples (+4.69‰). NH_4^+ is often the dominant inorganic N species in the study area (Lapointe and Bedford, 2007; Dixon et al., 2014; Heil et al., 2014), which could have implications for bloom development. This connection is supported by enriched $\delta^{15}N$ values observed in *K. brevis* samples (+4.94‰) collected from coastal areas of Lee County in 2018 that are similar to those of septic effluent (+4.9‰; Hinkle et al., 2008). Further, alongside urban development in the study area, NH_4^+ and *K. brevis* bloom concentrations have increased (Brand and Compton, 2007). Interestingly, many of the *K. brevis* blooms between 1998 and 2001 initiated offshore of Lee County before moving north (Vargo et al., 2008). Additionally, in this study, *K. brevis* $\delta^{13}C$ values from the most terrestrially influenced site, Lighthouse Beach, were more depleted during the wet season (September 2018) than in the dry season (March 2018) and at the other more coastal sites, indicative of a more freshwater C source. The $\delta^{15}N$ values were also the most enriched during this sampling event, further demonstrating the link between upstream freshwater sources and N enrichment. These values are similar to $\delta^{15}N$ values of POM collected during cruises between 1998 and 2001 at stations with >50,000 *K. brevis* cells/L^{-1} (~+4.4‰), which led the authors to conclude that a common seasonal N supply was available for *K. brevis* blooms (Havens et al., 2004). Enriched $\delta^{15}N$ values were also observed in red drift macroalgae (+4.86‰) and *K. brevis* samples (+7.83‰) collected from Lee County coastal waters in 2004 and 2005 following hurricanes Charley, Frances, and Jeanne (Lapointe et al., 2006; Lapointe and Bedford, 2007). Therefore, it is reasonable to expect that HABs in estuarine and coastal environments of Southwest Florida may be intensified following periods of heavy or prolonged rain events that reduce salinity and increase local basin N loading, including from less conspicuous sources like submarine groundwater discharge (Hu et al., 2006).

4.2. Bacterial abundance

The low groundwater FIB concentrations observed near septic systems was not unprecedented. Other studies in Florida (Herren et al., 2021), along Chesapeake Bay (Reay, 2004), and in Australia (Ahmed et al., 2005) have observed similar patterns where groundwater FIB are lower than in adjacent surface water. For example, Herren et al. (2021) found very low to non-detectable *E. coli*, fecal coliform, and enterococci concentrations in groundwater near septic systems and elevated concentrations in adjacent surface water that they attributed to the well distance from septic systems and the diffuse nature of the sub-surface septic plume. Additionally, bacteria can move quickly in saturated conditions and rapidly permeable soils (Cogger, 1988; Bicki and Brown, 1990; Harris, 1995), such as what was observed in North Fort Myers. These circumstances can lead to low FIB groundwater residence time due to preferential conduit flow (Andreo et al., 2006). Previous monitoring in North Fort Myers observed patchiness in "the power and magnitude" of groundwater bacteria concentrations and noted difficulty in definitively discriminating contributing sources (W. Dexter Bender and Associates Inc., 1995) These issues in the use of FIB as human waste tracer are well established and support the importance of using a multifaceted microbial source tracking approach to track human fecal contamination (Scott et al., 2002; Field and Samadpour, 2007; Tran et al., 2015; Kelly et al., 2018). In this study because wells were installed in parks or private residences, no septic system plume tracking was conducted prior to well installation and well locations were haphazardly selected, thus groundwater samples may not reflect the full magnitude of impacts to groundwater from septic systems. Despite these issues, even the relatively low concentrations of FIB observed in North Fort Myers groundwater are concerning as they exceed the United States Safe Drinking Water Act, which has been observed in other locations near septic systems (Hunter et al., 2021). The North Fort Myers study area has ~141 domestic wells that could be affected by this contamination.

Surface water FIB concentrations in the study area were higher than groundwater, often exceeding FDEP water quality standards. In warm environments, such as the study area, bacteria can be sourced from the gut of an animal, including humans, but become "naturalized" allowing them to persist and multiply in the environment if conditions are favorable (Devane et al., 2020). In North Fort Myers, the high background P may help to maintain these high surface water bacterial abundances (Mallin and Cahoon, 2020). Additionally, submarine groundwater discharge from septic systems can promote bacterial survival in surface water through delivery of nutrients and fresh water (Reay, 2004). In this study, the surface water sites near dense residential areas with high water tables tended to have higher microbial densities, which can be linked to human waste inputs because of the presence of chemical human waste tracers and HF183 (Figs. 5, 6, and S12). For example, the watersheds with greater densities of septic systems, Powell Creek and the central drainage feature (279 and 236 septic systems/km^2, respectively; Table 1) also had the highest enterococci and *E. coli* concentrations. In Hancock Creek, the densely residential site, SW10 had the highest enterococci and *E. coli* counts, while in Powell Creek, an upstream to downstream gradient was observed for both enterococci and *E. coli* counts, which may indicate dilution from exchange with the Caloosahatchee River Estuary. FIB in the central drainage feature was as high as the most upstream site in Powell Creek, which could indicate a higher initial FIB concentration due to the high density of septic systems in this drainage basin or a closer human waste source to the sampling location in this watershed.

Septic contamination of surface water with FIB is further supported by the high concentration of DIN (especially NH_4^+), sucralose, and HF183 observed at the central drainage feature (Figs. 4–6). The positive correlation between groundwater BOD and NH_4^+ (Fig. S11) provides additional evidence of non-functioning septic systems, particularly in Powell Creek where both were highest. While there was a negative correlation between surface water BOD and NH_4^+, surface water BOD was highest in the central drainage feature (1.49 ± 0.43 mg/L), which similarly had the highest NH_4^+ concentrations (8.27 ± 1.6 μM) further supporting this relationship. High NH_4^+ concentrations exert a BOD on receiving waters (referred to as nitrogenous BOD or NBOD) because DO is consumed as bacteria and other microbes oxidize NH_4^+ into NO_x.

Like other locations in Florida, tidal pumping likely increases nutrient and bacterial loading from septic systems during outgoing tides (Lapointe et al., 1990; Lipp et al., 2001; Buszka and Reeves, 2021). This connectivity between septic systems, groundwater, and surface water is supported by significant positive correlations between FIB and human waste tracers observed in surface water during outgoing tides. For example, enterococci and *E. coli* were positively correlated with carbamazepine, primidone, and sucralose, while acetaminophen was weakly correlated with both (Fig. S12). Further supporting this connection, downstream bacteria populations in other locations have been matched to septic tank source populations using biochemical fingerprinting (Ahmed et al., 2005).

4.3. Molecular markers

The detection of HF183 in surface water of all three watersheds (Fig. 6) demonstrated the ubiquitous presence of human fecal bacteria in North Fort Myers. Because there is no application of reuse water in the study area groundwater contaminated with human enteric bacteria from septic systems is the most likely source of HF183. Similar fecal pollution has been observed in the St. Lucie Estuary, another Florida ecosystem affected by high-density, waterfront septic systems at low elevation (Lapointe et al., 2012; Lapointe et al., 2017; Kelly et al., 2020), as well as in other locations, including North Carolina (Cahoon et al., 2006), Georgia (Sowah et al., 2014), Michigan's Lower Peninsula (Verhougstraete et al., 2015), and Puerto Rico (Jent et al., 2013). In future studies, HF183 should be sampled in both groundwater and adjacent surface water to better connect septic system bacterial contamination to surface water concentrations.

Bird molecular markers were also detected in all the surface water surveyed in North Fort Myers, but were particularly prevalent in the Hancock Creek watershed (Fig. 6). Seabird feces contain high amounts of enterococci and can be a significant source of bacteria to beaches (Grant et al., 2001). While elevated bacteria levels are concerning and seabird feces have the potential to transfer zoonotic agents to humans, such as bacteria and viruses (Epstein et al., 2006), the risk of seabird fecal contamination to humans is relatively unknown (Field and Samadpour, 2007). For example, in South Florida, transmission of salmonella from white ibis (*Eudocimus albus*) to humans in park environments has been suspected, but not confirmed (Hernandez et al., 2016). Further, bird feces also likely contribute nutrients to the study area. Thus the exclusion of gulls and other seabirds from beaches can significantly improve water quality (Goodwin et al., 2017), which suggests that discouragement of human food provisioning within beach areas might have similar effects and positive benefits for both humans and birds (Murray et al., 2021). As such, at North Shore Park where the Gull2 molecular marker was observed, FIB and nutrient concentrations might be reduced through a public education campaign, such as informational signs or social media campaigns, designed to inform park visitors of the water quality implications to feeding wild bird populations (Murray et al., 2021). Further, trash receptacles could be modified with heavy sealing lids that would lessen the availability of discarded food materials attracting birds to the area. Analyses of long-term databases on Florida beaches showed that while covered trash receptacles alone were not associated with a change in FIB, the discouragement of bird groupings was associated with a decrease in FIB (Kelly et al., 2018). These types of programs would likely be helpful in reducing bird FIB and nutrient contributions to waterfront parks in other locations.

4.4. Chemical tracers

There are some advantages to using chemical tracers over FIB and molecular markers, including source specificity, stability, and higher probability of detection (Lim et al., 2017). In this study chemical human waste tracers were useful for connecting nutrient and bacterial pollution to a source. For example, sucralose, carbamazepine, and primidone were present in groundwater and surface water in all three basins serviced primarily by septic systems (Fig. 9) confirming the ubiquitous influence of human waste throughout North Fort Myers. Similar widespread detection of sucralose in surface water has been observed in other regions with septic system influence (Watanabe et al., 2016; Spoelstra et al., 2020). Sucralose concentrations were similar to values observed in groundwaters in other areas of Florida, including Indian River (Herren et al., 2021), Martin, Charlotte, and St. Lucie Counties (Lapointe et al., 2016; Lapointe and Herren, 2016; Lapointe et al., 2017; Lapointe et al., 2020a), as well as North Carolina (Hunter et al., 2021), Vietnam and the Philippines (Watanabe et al., 2016). Acetaminophen was also detected in the central drainage and Powell Creek basins in both groundwater and surface water further demonstrating the presence of poorly treated human waste in these basins, which could be a result of the shallow water tables in these basins combined with higher densities of septic systems (Table 1). Despite the subtle differences between watersheds, the ubiquitous presence of these human waste tracers and their positive correlations with other indicators in surface water (Fig. S12) confirms that effluent from septic systems is contaminating both the groundwater and surface water of North Fort Myers.

In North Fort Myers, the presence of herbicides and pesticides provided evidence that stormwater runoff was also contributing to water quality degradation. Impervious surface area has been correlated with estuarine fecal coliform abundance, indicating that smart urban planning and stormwater mitigation techniques, including constructed wetlands and green areas, can be useful for improving urban water quality (Mallin et al., 2000). Thus, reducing N and P in stormwater from urban residential catchments has been identified as a primary opportunity to reduce nutrient loading (Yang and Toor, 2018). In Florida, turfgrass has been estimated to take up ~80 to 90% of applied fertilizers within the turf thatch with minimal amounts reaching groundwater and surface water, even in the wet season (Hochmuth et al., 2009; Shaddox and Unruh, 2018), so this is likely not a major nutrient source in the study area. However, a detailed study in Florida documented shifting patterns of $\delta^{15}N\text{-}NO_3^-$ throughout individual storm events, ranging from atmospheric deposition, inorganic fertilizers, soil, manure, and human waste (Jani et al., 2020), so multiple sources may contribute nutrients to stormwater runoff depending on conditions. In South Florida, climate change is expected to greatly exacerbate stormwater runoff issues in the next thirty years, which will demand drastic improvements of existing stormwater drainage infrastructure through retrofitting, rehabilitations, and new construction in order to minimize local basin pollutant loading (Huq and Abdul-Aziz, 2021). Therefore, working to minimize the amount of untreated stormwater runoff flowing into surface water represents another important component of improving water quality in North Fort Myers.

4.5. High groundwater

High groundwater levels were ubiquitous in the study area and may provide an accurate indicator for locations where septic systems may not provide adequate domestic waste treatment. This is because proper functioning of a septic system can only occur when a sufficient volume of unsaturated soil is present to absorb and treat the effluent (Bicki and Brown, 1990). Thus, in these areas, septic systems may not be an appropriate option for domestic waste management, particularly when they are located close to surface water important for recreation and fisheries. High groundwater tables were first documented in North Fort Myers almost 30 years ago (W. Dexter Bender and Associates Inc., 1995) and this study confirms that poor conditions for septic systems persist. Resource managers and civic leaders could use groundwater levels and proximity to surface water to help prioritize locations for septic to sewer conversions or other mitigation strategies, such as the use of Distributed Wastewater Systems that provide onsite advanced wastewater treatment (Lapointe and Brewton, 2021). High water table mapping in Florida coastal regions has recently been improved using fine resolution Lidar Digital Elevation Model data coupled with spatial interpolators like multiple linear regression and support vector machine techniques (Zhang et al., 2021) that could make these efforts more efficient.

This study supports the findings of previous research that septic systems contribute to the human waste contamination of groundwater and adjacent surface water. For example, shallow seasonal high water tables that interfere with the functioning of septic systems (Bicki and Brown, 1990) have also been observed throughout Florida in Jupiter and Tequesta (Lapointe and Krupa, 1995), St. George Island (Corbett et al., 2002), Dania Beach

(Meeroff et al., 2008), and Port St. Lucie (Lapointe et al., 2020a). Similar issues with shallow water tables interfering with the performance of septic systems have been observed in many other United States coastal communities, such as in North Carolina (Humphrey et al., 2011), Rhode Island (Cox et al., 2020), and Texas (Forbis-Stokes et al., 2016). Thus, there are far ranging implications of this work for other waterfront communities seeking to better manage human waste inputs and improve water quality.

4.6. Seasonality

While generally not as important as proximity to septic systems, some seasonal effects were also observed during this study, particularly for surface water. To begin with, water tables were shallower in the wet season compared to the dry season (Fig. 3 and Fig. S4). While groundwater concentrations of FIB and nutrients did not vary seasonally, there were seasonal differences observed in surface water. For example, in surface water higher concentrations of enterococci, *E. coli*, and BOD were observed in the dry season compared to the wet season (Fig. 4b). In the wet season, surface water concentrations of NH_4^+, NO_x, DIN, and TN were higher, while SRP and TP concentrations were higher in the dry season. Due to the relative variability observed in reactive nutrient concentrations by season, DIN: SRP was also higher in the wet season. These differences between seasons support a higher rate of N-loading from septic systems when water tables are elevated in the wet season, while elevated P in the dry season may support the higher surface water bacterial abundances observed during these sampling events (Mallin and Cahoon, 2020). Groundwater and surface water concentrations of sucralose and carbamazepine, as well as surface water concentrations of acetaminophen and HF183 were not variable by season, suggesting that septic systems are providing a year-round source of human waste in North Fort Myers. Interestingly, while groundwater primidone concentrations were not seasonally variable, surface water concentrations were higher in the dry season. This could be an effect of seasonal residency increasing loading of this specific tracers in the dry season or slower degradation due to cooler temperatures in the dry season. While concentrations of bentazon in groundwater were not seasonally variable, those in surface water were, suggesting an increase in surficial runoff during the wet season.

Increased surficial runoff may also contribute to the elevated N observed during the wet season. For example, rainfall can also carry animal (bird, dog, cat, etc.) waste and fertilizers into the adjacent surface water (Krimsky et al., 2021). This is supported by higher concentrations of the avian GFD marker in the wet season, though Gull 2 was not seasonally variable. Future studies could also seek to quantify the effect of pet waste on water quality by including molecular markers for dog and cat fecal bacteria. $\delta^{15}N$-NH_4^+ values were not seasonally variable, suggesting a constant NH_4^+ source in the groundwater, such as septic system effluent. However, $\delta^{15}N$-NO_3^- were significantly higher in the wet season (+7.89 ± 1.9‰) than the dry season (+2.71 ± 1.5‰), suggesting a more enriched NO_x source, such as human waste, is dominant in groundwater in the wet season (Fig. 7a). POM $\delta^{13}C$ values were not seasonally variable reflecting a consistent C source, however POM $\delta^{15}N$ values were significantly higher in the dry season (+5.28 ± 0.22‰) than the wet season (+4.10 ± 0.27‰; Fig. 7b), which could reflect more diversity in N sources from increased surficial runoff, as opposed to primarily septic system effluent. Lapointe and Bedford (2007) found a similar seasonal pattern for macroalgal $\delta^{15}N$ in Lee County coastal waters during 2004, with higher values in the dry season (+5.84‰) compared to the wet season (+3.89‰), which they attributed to greater inputs of isotopically depleted atmospheric and fertilizer N in the wet season. POM C:N were also higher in dry season than the wet season, reflecting the demonstrated higher N availability in the wet season.

4.7. Ecological impacts

There are many ecological effects of nutrient loading to the downstream estuary. Excess N loading represents a threat to seagrasses in Florida (Lapointe et al., 2020b) and other coastal areas globally (Hauxwell and Valiela, 2004; Orth et al., 2006; Short et al., 2014; Thomsen et al., 2020). As such, seagrasses are among the most threatened ecosystems on earth (Waycott et al., 2009) and it is important to consider threats to seagrass that stakeholders perceive as persistent (Unsworth et al., 2019). During sampling events, residents in the Powell Creek study area shared anecdotal accounts of large-scale seagrass losses where the creek terminates into the Caloosahatchee River Estuary that they attributed to poor water quality. This was likely tape grass (*Vallisneria americana*), as most of its coverage is located upstream of the Fort Myers bridges (Doering et al., 2002). Loss of seagrass habitats can potentially have cascading effects on ecologically and recreationally important fish populations, such as gag grouper (*Mycteroperca microlepis*) and spotted seatrout (*Cynoscion nebulosus*) that are dependent on this structure during juvenile life history stages (Barnes, 2005). Further, the vulnerable Florida manatee (*Trichechus manatus*) depends on seagrasses and adequate water quality to preserve their essential habitat (Barnes, 2005), while the critically endangered smalltooth sawfish (*Pristis pectinate*) uses the remaining natural shoreline along the Caloosahatchee River Estuary dominated by the red mangrove (*Rhizophora mangle*) as essential habitat (Poulakis et al., 2011). In fact, telemetry studies have defined the area in the Caloosahatchee River Estuary between the North Fort Myers bridges into which the study area drains as a *P. pectinate* "nursery hotspot" (Scharer et al., 2017). Accordingly, a juvenile sawfish (species undetermined) was observed and reported to Florida Fish and Wildlife Conservation Commission during the March 2020 sampling event of this study at North Shore Park.

4.8. Implications for management

Preserving and improving water quality in urbanized areas is an ongoing challenge for waterfront communities globally that will intensify as climate change progresses. This multi-year study indicates that septic systems are not protective of water quality in North Fort Myers, which has also been observed in other locations, including England (Withers et al., 2011, 2014) and Cape Cod, MA (Howes et al., 2004). Thus, in these locations there is a unique opportunity to significantly improve water quality by reducing dependence upon aging septic systems adjacent to surface water. These issues in North Fort Myers are caused by aging septic systems installed in high densities (~0.63/acre; Bicki and Brown, 1991) in areas with shallow water tables (Bicki and Brown, 1990). Additionally, the presence of canals in these residential areas with septic systems may increase the rate of pollutant transfer from groundwater to surface water via tidal pumping (Lapointe et al., 1990; Lipp et al., 2001; Buszka and Reeves, 2021). The amount of impervious ground cover, such as concrete or asphalt, in urban areas is another contributing factor to water quality issues (Chelsea Nagy et al., 2012; Flanagan et al., 2020). The combination of these conditions is likely to degrade water quality, which may continue to worsen with climate change. Municipalities should consider all these factors when planning sustainable development and infrastructure improvements, such as septic to sewer programs and stormwater treatment areas. For example, it would be beneficial for coastal areas with high densities of septic systems and canals to be prioritized for septic to sewer conversions (Buszka and Reeves, 2021) or other advanced wastewater treatment options, such as Distributed Wastewater Treatment Systems (Lapointe and Brewton, 2021). Finally, the susceptibility of these systems to localized HABs may be reduced by balancing the ecological stoichiometry of the watershed to achieve a nutrient load and N:P ratio where these microalgae will not thrive, leading to fewer HAB events. The scale of the red tide issue demands watershed-scale solutions and N management based on a holistic view, considering both oceanographic and anthropogenic processes (Medina et al., 2022).

5. Conclusions

Urban water quality is complex because it is affected by myriad environmental, economic, and political issues. This means that resource managers must be able to identify sources contributing to water quality decline and then prioritize mitigation and abatement strategies. Due to the nature of human waste inputs (i.e., reactive nutrients, pathogens, bacteria, pharmaceuticals, etc.), improved wastewater infrastructure and management, including advanced wastewater treatment (nutrient removal), in low elevation, high density waterfront communities serviced by septic systems should be prioritized as climate change will continue to exacerbate water quality issues and HABs. Further, stormwater improvements and citizen education campaigns on subjects such as feeding wild birds, removal of pet waste, and fertilizer use may be other methods to help minimize the effect of urbanization on water quality.

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CRediT authorship contribution statement

Rachel A. Brewton: conceptualization, data curation, methodology, data visualization, project administration, writing- original draft, writing- review and editing. **Lisa B. Kreiger**: conceptualization, data curation, methodology, writing- review and editing. **Kevin N. Tyre**: data curation, methodology, formal analyses, writing- review and editing. **Diana Baladi**: data curation, writing- review and editing. **Lynn E. Wilking**: formal analyses, writing- original draft. **Laura W. Herren**: conceptualization, data visualization, project administration, writing- review and editing. **Brian E. Lapointe**: conceptualization, methodology, writing- review and editing, project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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