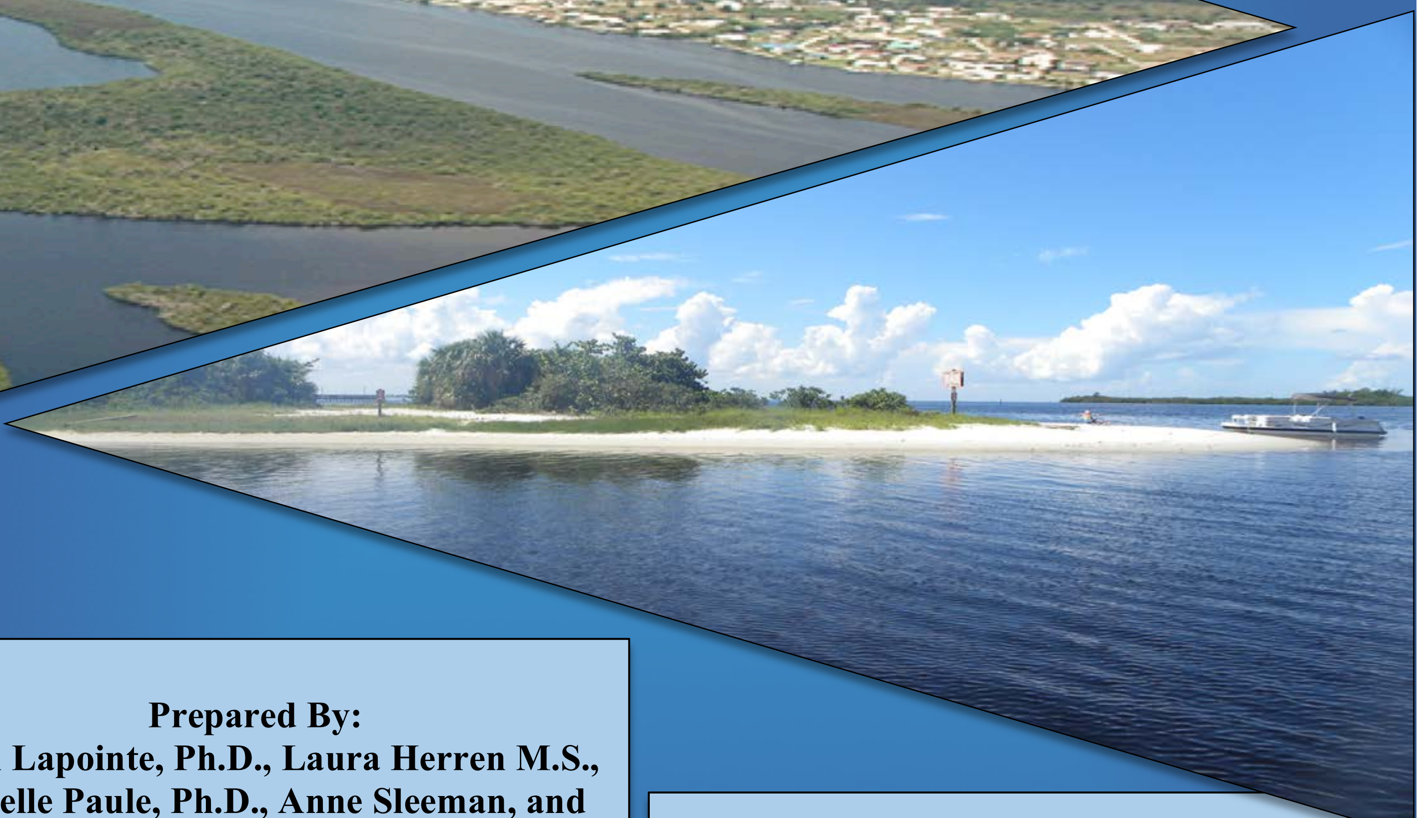
CHARLOTTE COUNTY
WATER QUALITY ASSESSMENT

PHASE I: DATA ANALYSIS AND RECOMMENDATIONS FOR LONG-TERM MONITORING



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Executive Summary

Human activities are increasing nutrient and microbial pollution to coastal waters worldwide, accelerating the problems of coastal eutrophication and harmful algal blooms. In Charlotte County, steady population growth combined with the use of septic systems has increased pollution to Charlotte Harbor, an estuary of national significance and a member of the National Estuary Program. Unfortunately, this important water body is listed as having high-level eutrophication and impaired water quality. This impacted ecosystem provides necessary seagrass habitat for many aquatic organisms and birds, including the critically endangered smalltooth sawfish (*Pristis pectinata*), among other important species.

Charlotte County supported Florida Atlantic University-Harbor Branch Oceanographic Institution to conduct this Phase I research project to analyze historical nutrient and water quality data in the Port Charlotte area. To better understand the relationship of septic systems and water quality in Charlotte County, we analyzed existing data from the area relating to nutrient and bacterial pollution of surface waters, groundwaters, and stormwater focusing primarily on state and county datasets. In addition, reconnaissance sampling for nutrients and tracers of human waste pollution was conducted to identify the relative importance of septic tank effluent compared to other sources of nutrient pollution, such as fertilizers. The usefulness of these data were considered in regards to the expense of future sampling to develop a cost-effective, but comprehensive approach for monitoring the changes in nutrient loading throughout the septic to centralized sewer conversion process.

Multiple lines of evidence suggest that septic systems are a primary source of nutrient loading from the Port Charlotte study area into Charlotte Harbor. Analysis of the historical data and the reconnaissance sampling both indicated that septic systems are contributing significantly to nutrient and bacterial pollution of surface waters and groundwaters. In historical data, increases in human population correlate strongly with rising concentrations of nitrogen, phosphorus, and chlorophyll *a*, illustrating how wastewater pollution from increasing human activities is impacting Charlotte Harbor water quality. Furthermore, high concentrations of total nitrogen, nitrate, ammonia, biochemical oxygen demand, and enteric bacteria were consistently found downgradient of septic systems. Additionally, the limited vertical separation between the ground surface and seasonally high water tables in the Springs Lakes area makes the hydrology inappropriate for septic systems and creates a situation where maintaining the required distance (greater than 2 ft.) is not possible, especially during the wet season (summer).

The human wastewater source of the nutrient pollution was also evidenced during our targeted reconnaissance sampling using stable nitrogen isotopes ($\delta^{15}N$) and the chemical tracer, sucralose. The stable nitrogen isotope ratios from macroalgae tissue and primary consumers (oysters and a hydroid) collected at sites within the Charlotte Harbor system indicated wastewater, rather than fertilizers, as the primary nitrogen source for this system. As the artificial sweetener sucralose is not naturally present in the environment or used by wildlife, its presence in the residential groundwater monitoring wells and surface waters indicated contamination from septic tank effluent.

Florida Department of Environmental Protection Numeric Nutrient Criteria and water quality standards were exceeded in the historical and reconnaissance data. Historical data for Charlotte County showed that over many years surface waters have intermittently exceeded current standards for nutrients and fecal coliforms resulting in impairments to these water bodies. Specifically, in the East and West Spring Lake Wastewater Pilot Program area, annual means of nitrogen were similar to criteria for surface waters, while groundwaters were in exceedance; fecal coliform levels were also high. In the reconnaissance sampling, standards were exceeded at all sites for nitrogen, at El Jobean and Ackerman for *Enterococci*, at El Jobean for phosphorus, and at Yacht Club for chlorophyll *a*.

The existing sampling configuration limited the applicability of the historical data towards deciphering effects of septic tank effluent on water quality within the East and West Spring Lake Wastewater Pilot Program area. For example, the locations of the original monitoring wells for this study were located in public right-of-ways, and thus were not effective in sampling septic system wastewater plumes that flow downgradient into the adjacent canals. However, the strategically placed county wells provided important data regarding the characterization and transport of septic tank effluent through groundwater into surface waters. We recommend continued monitoring of groundwater with more strategically placed sampling locations. Additionally, because the surface water samples were not collected during ebbing tides when nutrient loads from septic systems flowing into surface water are higher, this sampling did not allow for determination of tidal pumping. As such, the strategic reconnaissance surface water sampling revealed higher nitrogen concentrations via canal discharge points than was observed by the previous sampling due to appropriately timed collection. The project design also obscured any seasonal patterns that may have been observed in the septic system plumes. Accordingly, we recommend sampling strategically downstream of septic system plumes and at canal discharge points to capture the full effect of septic tank effluent, as well as to understand and document the reductions and seasonality in future nutrient loading.

We believe these recommendations will enable Charlotte County to effectively monitor water quality changes as they undergo the septic to centralized sewer conversion in the coming years. Charlotte Harbor has a well documented and growing hypoxia problem and impacts of human activities, such as nutrient loading, are exacerbating this issue. This research indicates that inadequately treated septic waste is a major source of nitrogen to this system, and that improved wastewater treatment, which currently removes an average of 90% of sewage nitrogen, would reduce nutrient loads from septic systems and help mitigate environmental impacts associated with current and future population growth. Given the relatively low development densities in Charlotte County, there is an opportunity to moderate the stress of non-point source pollution from septic tank effluent before Charlotte Harbor experiences higher levels of fecal contamination and harmful algal blooms similar to those in the Indian River Lagoon. Furthermore, this will move Charlotte County closer towards attainment of their long-range goals for the "Quality of Life" strategic focus on "blue water" and the "Water Resources" strategic focus to "ensure quality of natural water resources and provide a safe and reliable water supply".

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Acronyms Used in Text

BOD	biochemical oxygen demand
CCHMN	Coastal Charlotte Harbor Monitoring Network
CCMP	Comprehensive Conservation and Management Plan
CCUD	Charlotte County Utilities Department
cfu	colony forming units
CHNEP	Charlotte Harbor National Estuary Program
DIN	dissolved inorganic nitrogen
DO	dissolved oxygen (mg/L)
DOH	Department of Health
EPA	United States Environmental Protection Agency
FAC	Florida Administrative Code
FDEP	Florida Department of Environmental Protection
FIU EARL	Florida International University Environmental Analysis Research Laboratory
GDC	General Development Corporation
HAB	harmful algal bloom
HBOI-FAU	Florida Atlantic University-Harbor Branch Oceanographic Institute
mg/L	milligrams per liter
µg/L	micrograms per liter
MPN	most probable number
MSBU	Municipal Service Business Units
MW	monitoring well
NELAC	National Environmental Laboratory Accreditation Conference
NELAP	National Environmental Laboratory Accreditation Program
NH_3	ammonia
NNC	Numeric Nutrient Criteria
P	phosphorus
PPB	part per billion
PPM	parts per million
‰	per mille, per mil, parts per thousand
QAPP	Quality Assurance Project Plan
RAMP	Regional Ambient Monitoring Program
RESTORE	Resources and Ecosystems Sustainability, Tourist Opportunities, and Revived Economies of the Gulf Coast States Act
SD	standard deviation
SRP	soluble reactive phosphorus
STE	septic tank effluent
SWIM	Surface Water Improvement and Management
SWF RAMP	Southwest Florida Regional Ambient Monitoring Program
SWFWMD	Southwest Florida Water Management District
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus
TMDL	Total Maximum Daily Load
TN	total nitrogen
TP	total phosphorus
WRF	water reclamation facility

1. Introduction

Charlotte Harbor, located in southwest Florida, is a very special place, an estuary where three large rivers, the Caloosahatchee, Myakka, and Peace converge and flow into the Gulf of Mexico. Charlotte County takes its name from the harbor, which was named by English explorers in 1775 as a tribute to Queen Charlotte Sophia, wife of King George III. Over the last century, more people discovered the natural beauty of this area and the residential population grew steadily, until General Development Corporation (GDC), the largest land development company in Florida, spurred rapid growth (**Fig. 1**). Founded in 1954 and based in Miami, Florida, GDC promoted inexpensive Florida home sites worldwide in the 1950s and 1960s, and established several new communities in Florida, including Port Charlotte. Between 1950 and 2000, Charlotte County's population increased from 4,286 to 141,627. Today, Charlotte County encompasses a total area of 858 square-miles and has an estimated population of 173,115 with a population density of 235/square mile (**Fig. 1**).

With the steady urbanization in Charlotte County, as well as surrounding communities (**Fig. 2**), the demands for land, water, housing, transportation, boating, and recreational fishing presented challenges for sustainable growth on the watersheds of Charlotte Harbor. Management actions were needed to balance the unique natural resources with human needs. In 1995 the Charlotte Harbor National Estuary Program (CHNEP) was established and Charlotte Harbor was designated as an "estuary of national significance" in an effort to protect the greater Charlotte Harbor estuary by improving water quality and preserving ecological integrity of the 4,700 square-mile watershed extending from Venice to Bonita Springs, (**Fig. 3**). Regardless of these efforts, in 1999 Charlotte Harbor was listed as expressing high levels of eutrophic conditions,

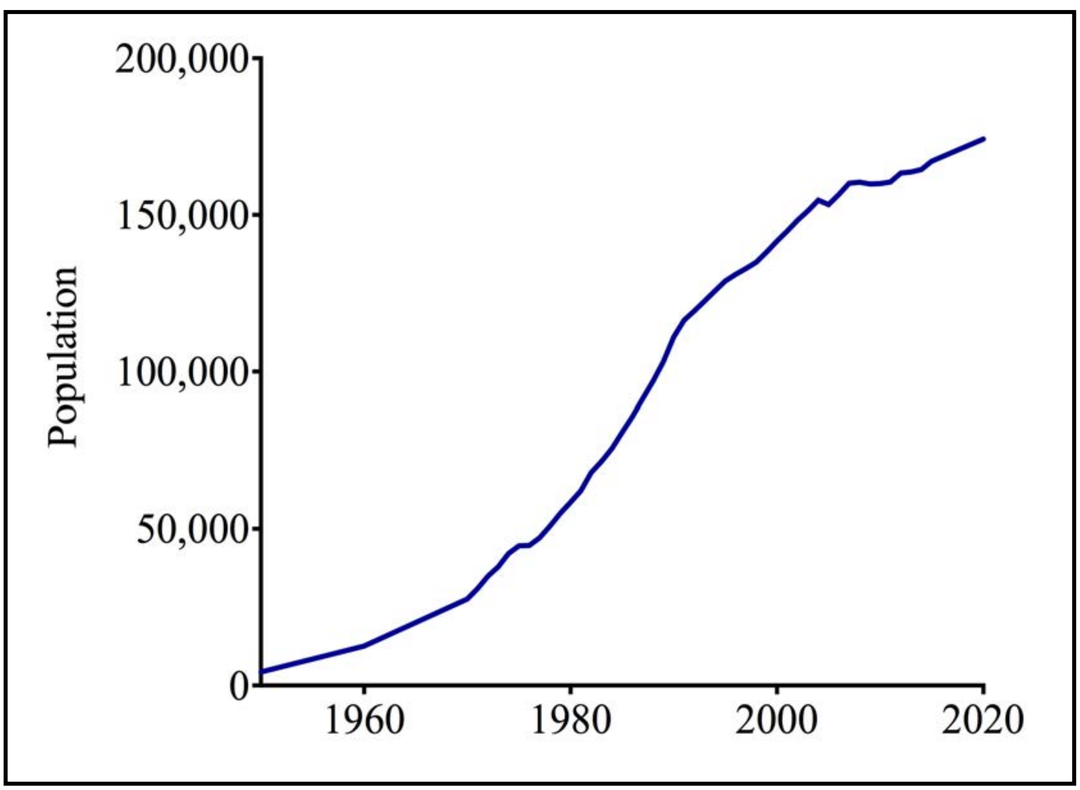

Figure 1. Charlotte County annual population from 1954 to 2016, showing projected population size for 2020.

including elevated levels of chlorophyll *a* and low dissolved oxygen (DO; Bricker et al., 1999). This sort of eutrophication can lower the biodiversity of a system directly, through depletion of oxygen, or indirectly, though compounding effects of excess nutrients (Howarth et al., 2000). Additionally, segments of Charlotte Harbor are listed on the United States Environmental Protection Agency (EPA) 303d list as impaired for nutrients, dissolved oxygen (DO), chlorophyll *a*, bacteria in shellfish, and mercury in fish tissue. The CHNEP completed its Comprehensive Conservation and Management Plan (CCMP) in 2001, and it marked the beginning of cooperative actions needed to protect and restore the estuary and its watershed. The success of the CCMP is highly dependent on participation of numerous partners, which involves all seven counties bordering the CHNEP, including Charlotte County.

Water quality degradation is a major management and planning issue for the CHNEP and its partners, including Charlotte County, because maintaining water quality is critical for economic, environmental, and human health. Threats to water quality in the Charlotte Harbor area include nutrients, bacteria, viruses, harmful algal blooms (HABs), dissolved oxygen, toxic and/or bio-reactive organic compounds, and water clarity (Hammet, 1990; Lipp et al., 2001; CHEC, 2003; Turner et al., 2006; Schermerhorn, 2008; CHNEP, 2013; Tetra Tech, 2015; Straugler, 2016). Furthermore, there is evidence that HABs, including red tides (Brand and Compton, 2007; Yentch et al., 2008) and red drift macroalage (Lapointe and Bedford, 2007) are on the rise as a result of increasing nitrogen loading.

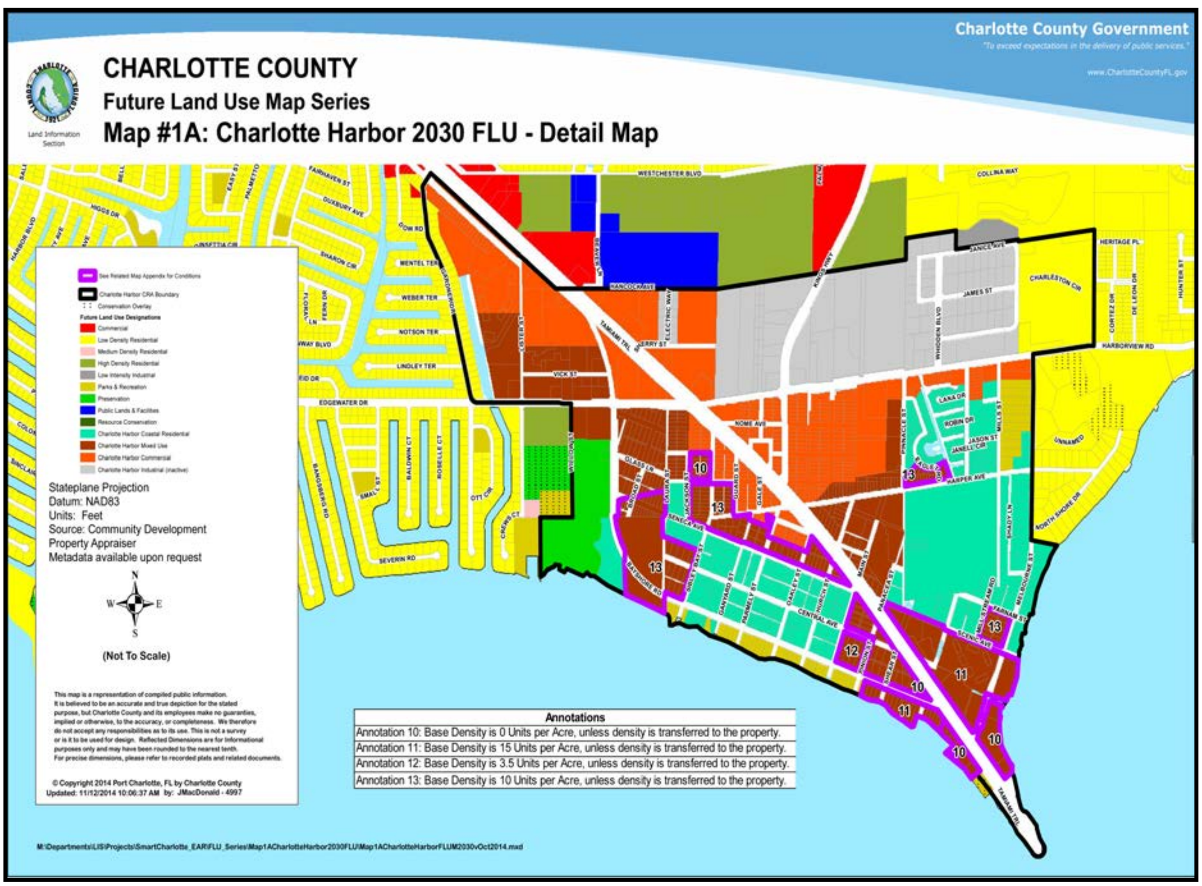

Figure 2. Charlotte County future land use, showing zoning and expected population densities; of note, there are many residential, waterfront properties (Map #1A Charlotte Harbor 2030 FLU-Detail Map © 2014 Port Charlotte, FL by Charlotte County, updated 11/12/2014 by JMacDonald-4997).

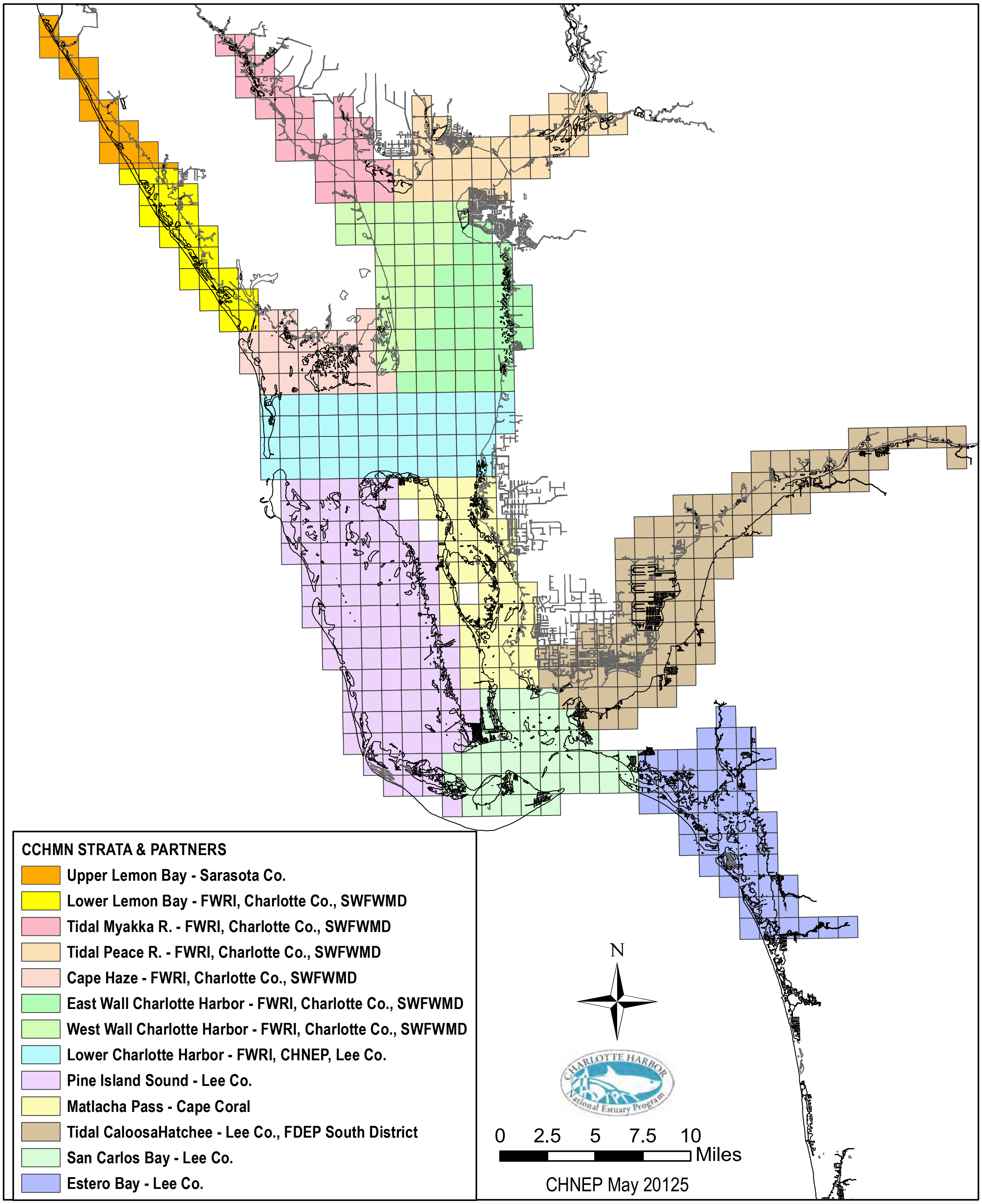

Figure 3. Coastal Charlotte Harbor Monitoring Network (CCHMN) strata and partners including Florida Fish and Wildlife Commission's Fish and Wildlife Research Institute (FWRI), Southwest Florida Water Management District (SWFWMD), Florida Department of Environmental Protection (FDEP) South District, city of Cape Coral, Lee County, and Charlotte County.

Charlotte County is actively addressing water quality impacts to Charlotte Harbor from land-based, non-point sources of pollution under multiple directives. These include: 1) Goal 2 of the Resources and Ecosystems Sustainability, Tourist Opportunities, and Revived Economies of the Gulf Coast States Act (RESTORE) Council Initial Comprehensive Plan to *"Restore Water Quality"* (Charlotte Harbor Project Number 48-032013), 2) Charlotte 2050 (Charlotte County Board of Commissioners, 2010), 3) CHNEP CCMP's Priority Actions (CHNEP, 2013), 4) the Joint Florida Gulf National Estuary Programs Southwest Florida Regional Ecosystem Restoration Plan (JFGNEP, 2013), and 5) the Southwest Florida Water Management District (SWFWMD) Surface Water Improvement and Management (SWIM) Plan (2006).

In the past, nutrient criteria for most of Florida's water bodies were descriptive in nature adding to the vagueness of whether or not these criteria were being met. In 2012, Florida Department of Environmental Protection (FDEP) first adopted specific numeric nutrient criteria (NNC) for the State's estuaries and coastal areas, including Charlotte Harbor (**Table 1**; Janicki Environmental, 2011). These numeric criteria are annual, arithmetic means and are not to be exceeded more than once in a three-year period (62-302.532 Florida Administrative Code; FAC). For surface waters, NNC and other standards vary based on the classification of the water body, with the classification ranging from the most stringent (Class I) to least (Class V). Class I waters are potable water supplies, Class II waters are suitable for shellfish propagation or harvesting, Class III waters are suitable for recreation and maintenance of a healthy population of fish and wildlife, Class IV are suitable for agricultural use, and Class V are suitable for navigation, utility, and industrial use (62-302.400 FAC). All Charlotte County surface waters are considered to be Class III except for certain segments that are Class II within Charlotte Harbor, portions of the Myakka River, and Gasparilla South. Additionally, the lower Myakka River is also classified as an "Outstanding Florida Water" (OFW) meaning FDEP has deemed this resource worthy of special protection because of its natural attributes (62-302.700 FAC). This classification has profound implications regarding water quality because activities or discharges that significantly degrade an OFW must be "clearly in public interest" and pass through a stringent approval process. Within the Charlotte Harbor system, there are three segments with individual NNC: Charlotte Harbor Proper, Tidal Myakka River, and Tidal Peace River (**Table 1**).

Table 1. Numeric Nutrient Criteria (NNC) for total phosphorus (mg/L), total nitrogen (mg/L), and chlorophyll *a* (µg/L) for Charlotte Harbor Proper, Tidal Myakka River, and Tidal Peace River (62-302.530(47)(b) FAC).

Analytes	Charlotte Harbor Proper	Tidal Peace River	Tidal Myakka River
Total phosphorus	0.19	0.31	0.50
Total nitrogen	0.67	1.02	1.08
Chlorophyll *a*	6.10	11.70	12.60

Charlotte Harbor is an economically important ecosystem that is home to many species of aquatic organisms, plants, birds, and other life. One of the more notable inhabitants is the critically endangered smalltooth sawfish (*Pristis pectinata*). Charlotte Harbor is one of the two identified areas in southwest Florida designated as critical smalltooth sawfish nursery habitat due to the environmental conditions of the estuary combined with the presence of red mangroves (*Rhizophora mangle*; Norton et al., 2012). Unfortunately, habitat degradation and destruction due to urbanization has been recognized as a threat to this imperiled species (Adams, 2000). Degradation of water quality in nursery areas could effect the distribution and health of red mangroves, further decreasing the amount of suitable habitat available. For example, nutrient enriched red mangroves have been shown to experience increased predation by insects (Onuf et al., 1977), which could result in a decrease of survival for young mangroves. The seasonal hypoxia that occurs in Charlotte Harbor (Turner et al., 2006) is another threat to juvenile smalltooth sawfish, as they have an affinity for well-oxygenated water (Poulakis et al., 2011). Thus, improving water quality would be beneficial to the conservation of smalltooth sawfish.

Water quality parameters reveal insight into the health of coastal ecosystems. For example, dissolved nutrient concentrations are essential for assessing the level of eutrophication within a water body. Specifically, high concentrations of nitrogen and/or phosphorus can be indicative of contamination in water via sewage or agricultural runoff. Nitrate and ammonia, the most reactive forms of nitrogen, are useful for understanding the source and degree of nutrient pollution; concentrations above 14 µg/L of ammonia or nitrate are adequate to support macroalgae blooms (Lapointe et al., 1993). At high levels (0.53 to 22.8 mg/L), ammonia can cause direct mortality to some fish and invertebrates, though the magnitude of effects is also related to pH and temperature (Oram, 2015). The ratio of nitrogen to phosphorus gives insight into whether the system is nitrogen or phosphorus limited and to what degree human activities have altered this balance. The limiting nutrient in a system defines what is needed for plant growth or an algae bloom. The naturally occurring ratio in estuarine systems is typically 16 nitrogen atoms to 1 phosphorus atom and is referred to as the Redfield ratio. If the ratio is less than 16:1, then nitrogen will limit primary production; if the ratio is higher, phosphorus will be limiting. Due to natural phosphate-rock formation (bone formation) and mining activities, Charlotte County has a natural high background level of phosphorus (Kauffman, 1969), and thus it is more likely that nitrogen is limiting in this system. Biochemical oxygen demand (BOD) is the amount of dissolved oxygen required for aerobic organisms to break down organic matter in a water sample and is an indicator of the amount of organic compounds in the water. Pristine waters have BOD concentrations below 1 mg/L, moderately impacted waters range from 2-10 mg/L, and raw sewage ranges from 200–600 mg/L. The presence of enteric bacteria in water, such as fecal coliforms and *Enterococcus* sp., is another indicator of contamination by fecal matter. This suite of important parameters can help decipher the long-term challenges facing water quality in Charlotte County.

Bioindicators are organisms that can reveal insight into the health of an ecosystem. Macroalgae are useful as bioindicators of the nutrient sources in an ecosystem because they quickly assimilate nutrients from the surrounding water into their tissues. Within a short timeframe of weeks to months, macroalgae can uptake water-borne nutrients to serve as a barometer for water conditions (Lapointe, 1985) and the stable nitrogen isotope signal of macroalgae is an ideal bioindicator of human wastewater pollution (Risk et al., 2009). Additionally, organisms that filter-feed on aquatic phytoplankton, or primary consumers, can be similarly useful in deciphering the major nutrient sources of a system (Cabana and Rasmussen, 1966; **Fig. 4**). For example, oysters (*Crassostrea virginica*) make an ideal bioindicator for nutrient pollution because they are common, non-mobile, filter-feeding organisms (Fertig et al., 2007). Through filter-feeding oysters incorporate particulate nutrients into their tissue and thus, can be used to reveal the nutrient sources of the system. The stable nitrogen isotope ratio ($\delta^{15}N$) of macroalgae or primary consumers can be used to identify environmental nitrogen sources, such as wastewater, which have been shown to increase along with human population (**Fig. 4**).

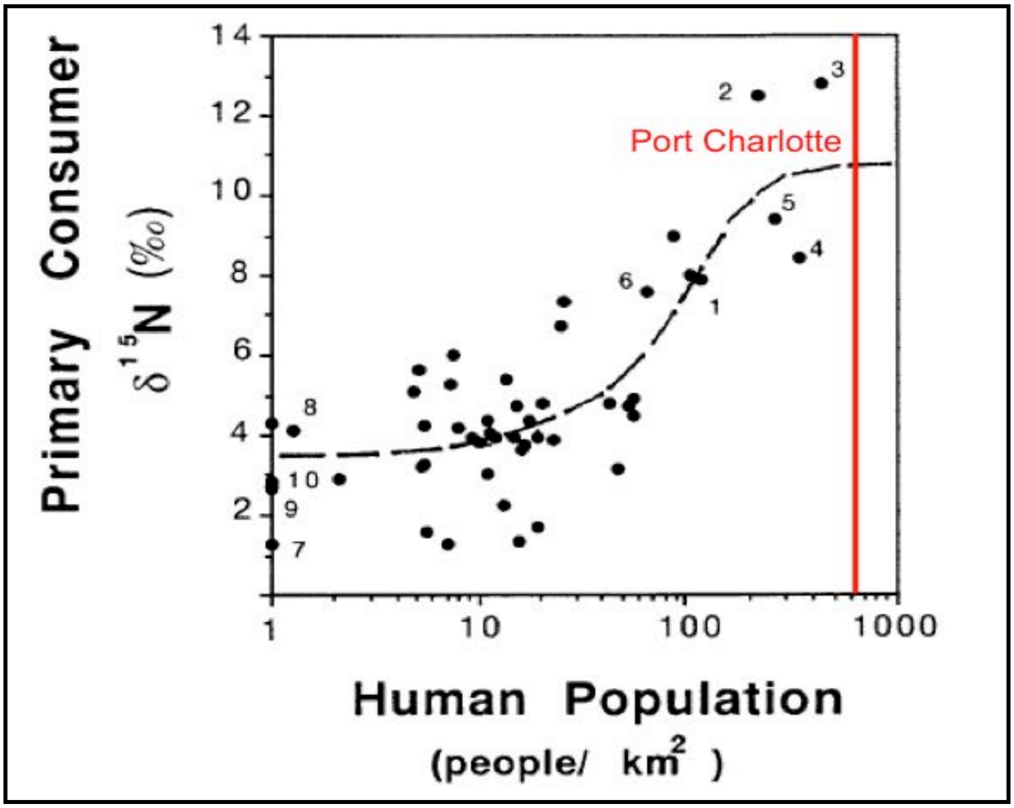

Figure 4. Primary consumer nitrogen stable isotope ($\delta^{15}N$) values compared to human population growth (adapted from Cabana and Rasmussen, 1996), showing the approximate population of Port Charlotte in 2016.

Septic tank effluent (STE) is one of the known sources of pollution to Charlotte Harbor (Lipp et al., 2001; CHEC, 2003; CHNEP, 2013; Frick et al., 2015; Straugler, 2016). Recent modeling of nutrient pollution shows septic systems to be the second largest source of nitrogen contamination to Florida waters, just behind agriculture (Badrazzaman et al., 2012). Properly functioning septic systems consist of two treatment steps that take place within a septic tank and a drainfield, respectively. Wastewater first flows from the building into the tank. Solids settle out in the tank and the supernatant, or effluent, flows through a one-way valve and a subsequent series of perforated pipes leading into the drainfield where most of the treatment occurs (**Fig. 5**; Mallin et al., 2013). Over time, the STE percolates through a deep layer of soil to allow further treatment before reaching the groundwater. As most of Florida's soil types are unsuitable for proper septic system function and the water table is high, especially in coastal areas, the effluent ends up flowing directly into the groundwater without adequate percolation and treatment, especially during the wet season when seasonally high water tables occur (Bicki and Brown, 1990). This results in untreated wastewater with high levels of nitrogen, phosphorus, fecal microbes, and organic wastewater contaminants routinely reaching the groundwater (Bicki et al., 1984; Woodward-Clyde Consultants, 1994). Research in the Florida Keys has demonstrated that septic systems enrich shallow groundwater with dissolved nutrients, coliform bacteria, and viruses (Griffin et al., 1999; Lapointe et al., 1990; Paul et al., 1995 a,b).

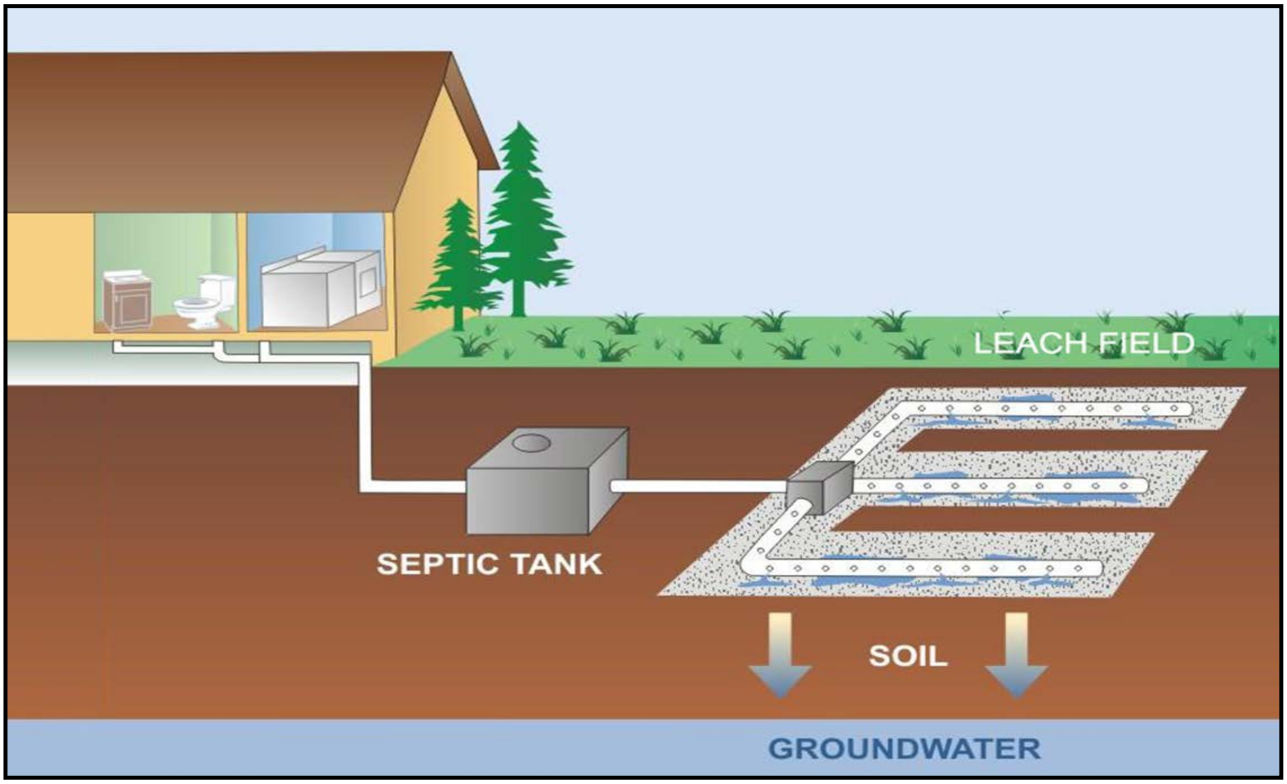

Figure 5. Diagram of a typical septic system showing the septic tank, leach field, and potential groundwater contamination by septic tank effluent via the soil. In Florida the requirements include, 12 in. of ground cover on top of the leach field, a drainfield depth of approximately 12in., a 24 in. separation between the drainfield and the seasonal high water table, and a 50-75 ft. setback from surface waters (64E-6.002 FAC; image from Mallin et al., 2013).

According to Charlotte County Utilities Department (CCUD, 2010), Charlotte County currently has more than 45,000 septic systems based on new construction permits. Many of these were installed in the 1960s and 1970s prior to a policy change in 1983 requiring a 24-inch separation of the drainfield from the seasonal high water table (via import of suitable fill) and a 50-75 ft. setback from surface waters (64E-6.002 FAC). Prior to 1983, Florida only required a six-inch separation (in existing soils without the introduction of fill) and a 25-50 ft. setback. Additionally, the Sarastota Bay National Estuary Program determined that raw waste required a setback of 900 ft. from surface waters in order to meet the 3 mg/L standard for wastewater and CHNEP has made it a priority action to sewer all developed areas within this distance (CHNEP, 2013). Even the stronger policies implemented in 1983 have the potential to be inadequate in low-lying coastal areas with strong tidal influences, like those adjacent to Charlotte Harbor, especially during the wet season when the water table is at its highest (Bicki and Brown, 1990). In the Florida Keys, rain events significantly increase the flow of groundwater (Lapointe et al., 1999) and tidal pumping increases nutrient loading during ebbing tides (Lapointe et al., 1990). It is likely that in Charlotte County nutrient transport via STE similarly fluctuates with tidal changes.

Beginning in June 2009, CCUD initiated the discussion, prioritization, and subsequent implementation of a large-scale, multi-phase septic to centralized sewer conversion initiative (CCUD, 2010). The resulting East and West Spring Lake Wastewater Pilot Program began in

2011 and completion is expected in 2017. Depending on funding, the anticipated priority for the remaining Municipal Service Business Units (MSBUs) is outlined in **Fig. 6**. Initial data collection and analysis for nutrients, total suspended solids, and BOD at 68 groundwater and 21 surface water sites suggest a strong STE influence (Tetra Tech, 2013). This large-scale effort is progressive and provides an often-missed opportunity to document pre- and post-construction water quality conditions. Long-term monitoring programs like this that capture the baseline conditions prior to conversion are extremely valuable to observe the level of improvement and also function as datasets that policy and decision-makers can use to make better science-based decisions about the land-based impacts to economically important estuaries and coastal waters. This is especially important in state with a tourist-driven economy, such as Florida.

This research addresses the first phase of a long-term project concerning the interactions between septic systems, groundwater, and surface waters in the Charlotte Harbor watershed and surrounding communities in Charlotte County. Specifically, Phase I entailed the following tasks: 1) datamining and synthesis of existing water quality data, 2) identification and initial coordination with laboratories and volunteer networks, 3) reconnaissance field trips for sampling across the study area at select canal and groundwater sites for dissolved nutrients and chlorophyll *a* in areas that, based on previous data, may be at or above TMDL limits or NNC, 4) measurement of human source tracers, such as stable nitrogen isotopes in macroalgae and/or oysters and sucralose concentrations in water, and 5) recommendations for a long-term monitoring program to document effects of improved wastewater collection and treatment. This initial study was intended to enable Florida Atlantic University-Harbor Branch Oceanographic Institute (HBOI-FAU) to design a long-term sampling network that will build upon, rather than duplicate, current monitoring efforts in the study area.

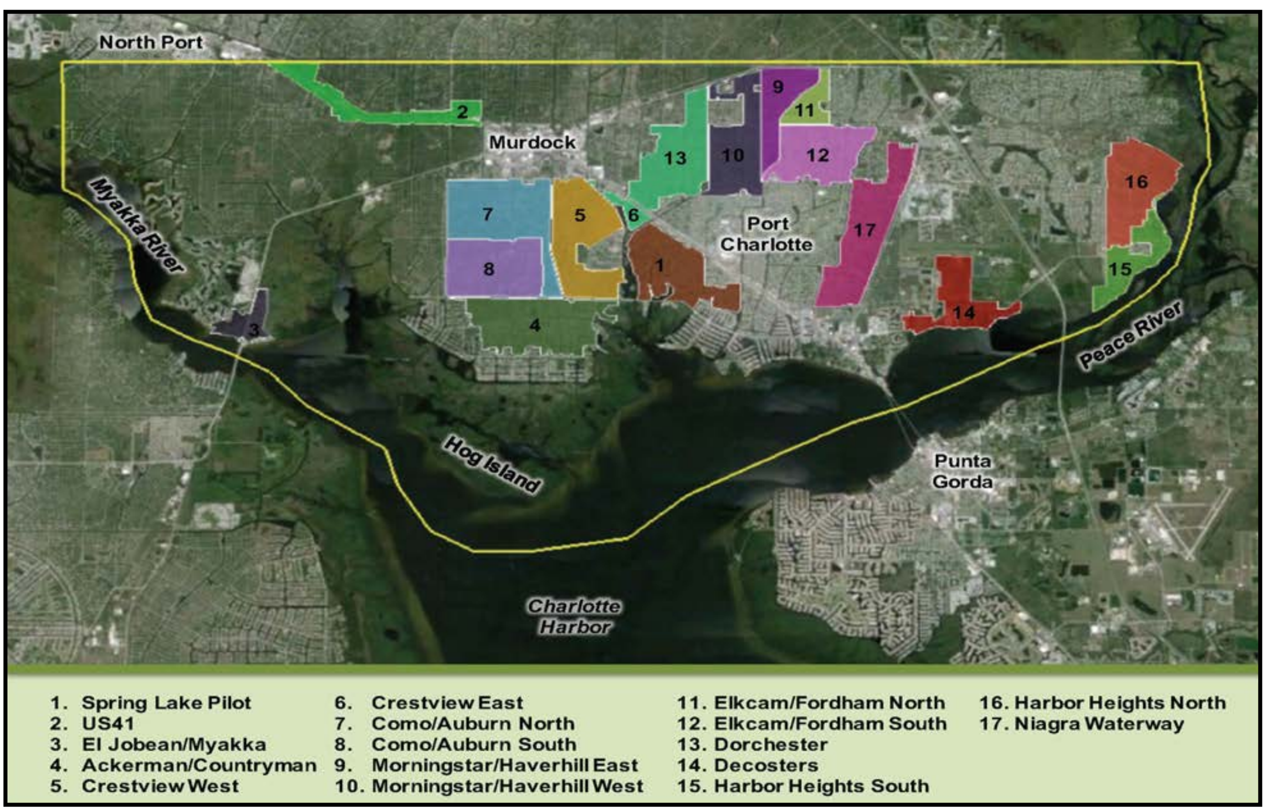

Figure 6. Charlotte County Proposed Municipal Service Business Units (MSBU) in the primary septic to sewer conversion project area by priority.

2. Historical Environmental Data for Charlotte County

Data from CCUD monitoring programs along with publicly available data were used as the basis to assess the current ecological state of water bodies within Charlotte County and obtain a better understanding of needs for future monitoring.

2.1 Data sources and parameters

Extensive datamining was conducted to discover available historical water quality data relating to Charlotte County. This included data from CCUD's water quality-monitoring program and other publicly available data from SFWMD, United States Geological Service (USGS), CHNEP, the Florida Water Resource Monitoring Catalog (WATER-CAT), and FDEP Storage and Retrieval and Water Quality Exchange (STORET) database. A list of agencies where data was obtained and the environmental parameters available from each source is summarized in **Appendix 1.1**.

As each sampling agency maintains their own monitoring program with differing sampling frequencies and analytes, the environmental parameters available varied by site, water body, and year. Long-term historical surface water samples included in the analysis were collected from canals and the Charlotte Harbor estuary, including Charlotte Harbor, Peace River, and Myakka River. Unfortunately, the long-term historical groundwater data obtained from different agencies for Charlotte County (1965-2015) was sparse (**Table 2**). As a part of the East and West Spring Lake Wastewater Pilot Program, surface water samples from canals (n=21) and groundwater samples from monitoring wells (n=39) were collected every two months in the project area, and these data were analyzed to assess more localized water quality over a fairly recent timeframe. (**Fig. 7**). Additionally, the depth from the ground to the seasonal high water table at the well sites was monitored from 2013-2016 (n=947).

Table 2. Historical water quality parameters for groundwater in Charlotte County, FL showing sample year(s), average value, minimum value (Min), maximum value (Max), and number of samples (n).

Parameter	Year (s)	Average	Min	Max	n
Total Nitrogen (mg/L)	1986-1995	0.56	0.31	0.98	12
Total Phosphorus (mg/L)	1995	0.10	0.01	0.55	11
Ammonia (mg/L)	1995	0.01	0.00	0.05	20
Color (PCU)	1995	6.70	5.00	10.00	6
Nitrate + Nitrite	1992-1995	0.04	0.02	0.16	25
pH	1965-1995	7.90	5.06	9.50	88

All of the historical data were compiled by type, including surface water, groundwater, and stormwater. Annual arithmetic means (and standard deviation) were calculated with GraphPad Prism version 7.00a for Mac OS (GraphPad Software, La Jolla, California, USA) and compared against the respective NNC or standards for each parameter to assess long-term trends in water quality. Particular attention was paid to STE response variables such as nitrogen, phosphorus, chlorophyll *a*, and fecal coliform concentrations.

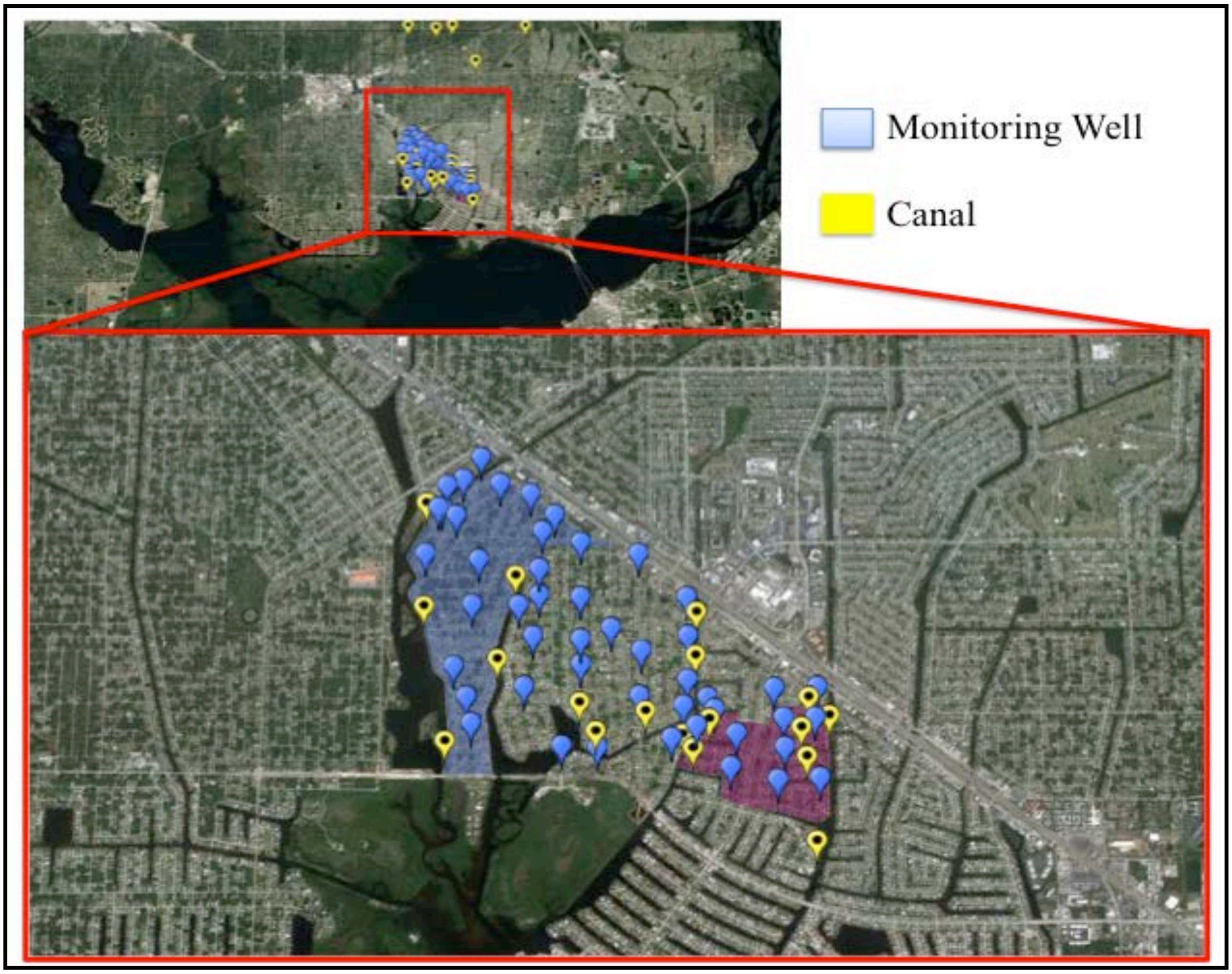

Figure 7. Charlotte County Utilities Department (CCUD) sampling locations for surface water (canals) and groundwater (monitoring wells) within the Spring Lakes area of Charlotte County, FL.

2.2 Historical nutrient and bacteria concentrations

2.2.1 Surface water

The analysis of fifty years of environmental data reveals a history of degraded water quality within Charlotte County. Generally speaking, many of the historical data points exceeded the average NNC criteria for the tidal Peace and Myakka rivers (1.06 mg/L), regardless of the environmental parameter and year considered (**Fig. 8**). Total nitrogen (TN), nitrate + nitrite, ammonia, and fecal coliforms (**Fig. 8 a, c, and e**) had peaks around the mid 1970s and 1980s, and early 2000s that might reflect El-Niño events reported for these same years and reveal the potential impacts of increased rainfall on water quality within Charlotte County.

Total phosphorus (TP) concentrations exhibited fewer fluctuations than the TN, nitrate + nitrite and ammonia concentrations (**Fig. 8b**). TP data exhibited an average of 0.4 mg/L with a high percent of data exceeding the NNC criteria from 1995-2005, which might be correlated with the stormwater events and intensive phosphorus mining. Moreover, many household products such

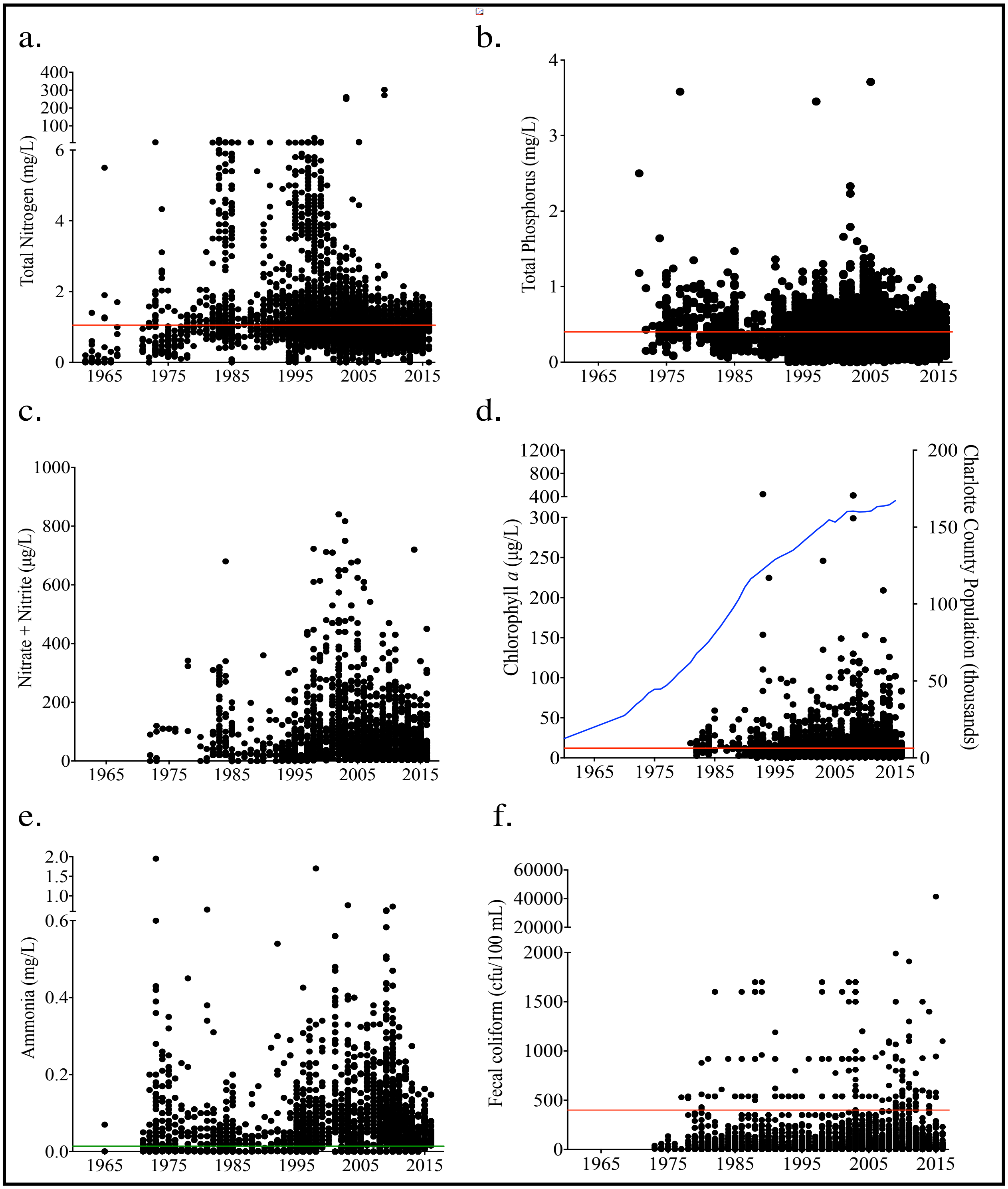

Figure 8. Long-term historical water quality parameters of surface water in Charlotte County, FL showing the averaged Numeric Nutrient Criteria (NNC; red lines) for the Tidal Peace and Myakka rivers, including: a) total nitrogen (TN; mg/L; n=7,211) showing the NNC (1.02 mg/L Tidal Myakka River and 1.08 mg/L Tidal Peace River), b) total phosphorus (TP; mg/L; n=13,953) showing the NNC (0.31 mg/L Tidal Myakka River and 0.50 mg/L Tidal Peace River), c) nitrate + nitrite (mg/L; n=6,967), d) chlorophyll *a* concentrations (n=10,805) showing the NNC (11.7 µg/L Tidal Myakka River and 12.6 µg /L Tidal Peace River), e) ammonia (mg/L; n=8,813) levels showing the threshold for macroalgae blooms (green line; 0.014 µg/L; Lapointe et al., 1993), and f) fecal coliform units (n=26,838) showing the surface water quality criteria (400 MPN). Values above the indicator lines are in exceedance of Florida water quality criteria.

as detergents contain phosphorus compounds, and can contribute to the pollution of coastal waters. Chlorophyll *a* data from 1975-2015 shows a generally increasing trend and when plotted with the population of Charlotte County, an apparent correlation is observed between increasing population growth and chlorophyll *a* concentrations (**Fig. 8d**).

Proceeding from a "blue water" vision for water quality within Charlotte County, we focused our analysis on the Spring Lakes area monitored since 2012 by CCUD as part of the East and West Spring Lake Wastewater Pilot Program (**Fig. 9**). Within this "Spring Lakes" area, the annual means for total nitrogen (1.0 and 0.96 mg/L) were statistically similar to the NNC (1.08 mg/L) for both 2015 and 2016 (**Fig. 9a**), however many individual samples exceeded the criteria (up to 1.54 mg/L). There was variation in TN by site and sampling date (**Appendix 1.2a,b**). The annual means for TP (0.32 and 0.16 mg/L) were below the NNC (0.5 mg/L) for 2012-2016 with some samples exceeding the criteria value (up to 0.90 mg/L; **Fig. 9b**). Nitrate + nitrite levels also remained similar for 2012-2016 (0.019 to 0.043 mg/L) with some high values for 2013 and 2015 (up to 0.4 mg/L; **Fig. 9c**). The ratio of TN:TP observed for 2015 and 2016 was less than 16:1, especially in 2016, indicating the system is increasingly nitrogen-limited (**Fig. 9d**). Ammonia values were well above the macroalgae bloom threshold (0.014 µg/L; Lapointe et al., 1993) for both 2015 and 2016 (**Fig. 9e**), indicating favorable conditions for macroalgal blooms as previously described for the lower Charlotte Harbor area by Lapointe and Bedford (2007). Detailed data for each parameter can be found in **Appendix 1.3**.

Fecal coliform levels have been recognized as an indicator of seasonal STE contamination in the Charlotte Harbor area (Lipp et al., 2001). Fecal coliform concentrations were above the surface water quality criteria for 2012-2016 (greater than 400 cfu/100 mL; **Fig. 9f**) with a trend of increasing maximum values (up to 600-800 cfu/100 mL) and variation over time. Although the removal of fecal coliforms by soil can be effective in certain soil conditions, poor soil types and close proximity of the drainfield to water table can compromise removal efficiency (Bicki and Brown, 1990). Indeed, the fecal coliform levels appear to be influenced by seasonal variation associated to higher values during summer when the water table is higher.

Analysis of these water quality parameters support the hypothesis that the surface waters within the Charlotte County watershed are impacted by STE, as evidenced by high nutrient fecal coliform concentrations. The increases in chlorophyll *a* levels over time are also a strong indicator of increasing eutrophication within Charlotte County.

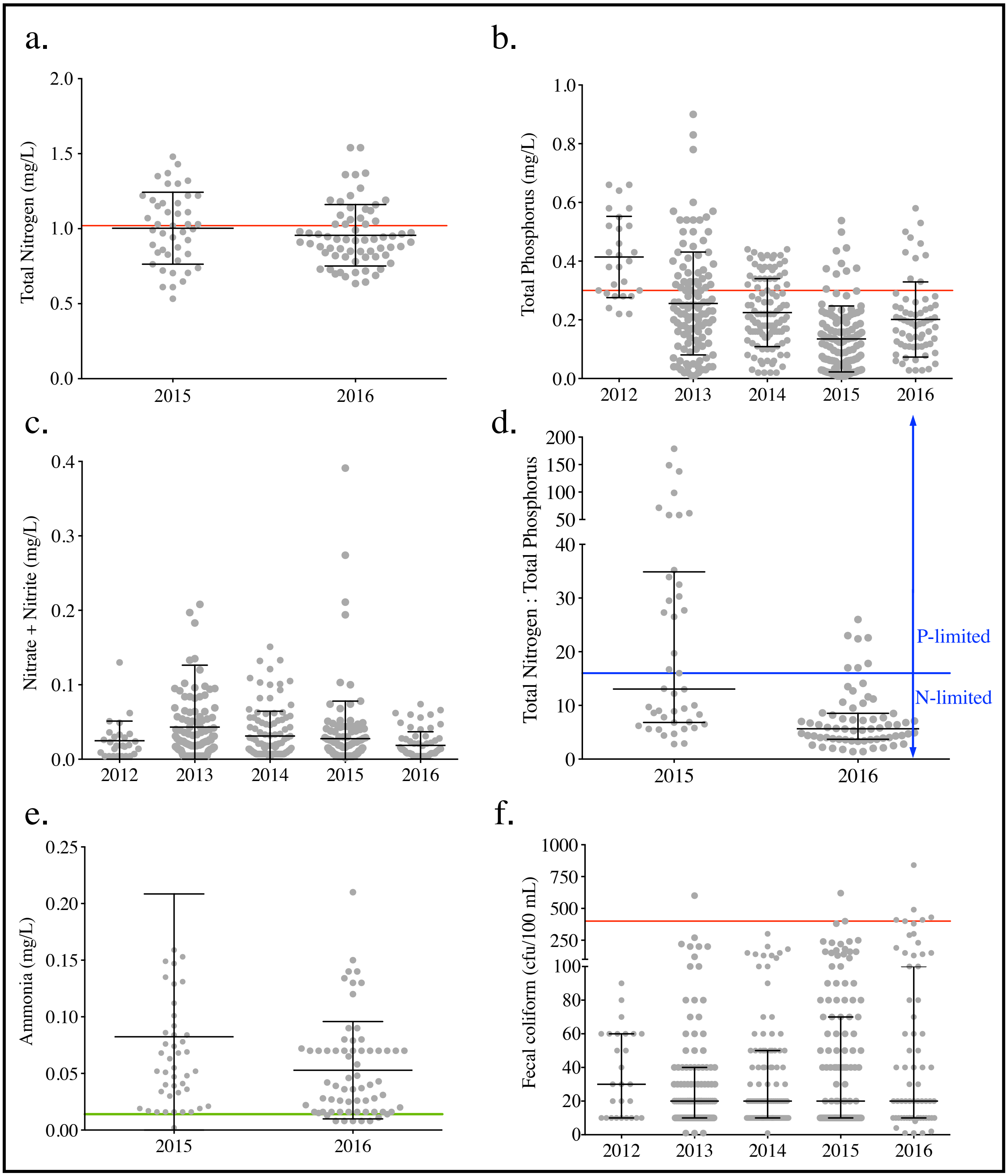

Figure 9. Localized historical surface water quality parameters for the East and West Spring Lake Wastewater Pilot Program area of Charlotte County, FL (collected from 21 canal sites) showing the respective surface water Numeric Nutrient Criteria (NNC; red lines) for the Tidal Peace River, including: a) mean total nitrogen (TN; mg/L; NNC=1.02 mg/L), b) mean total phosphorus (TP; mg/L; NNC=0.31 mg/L), c) mean nitrate + nitrite values (mg/L), d) mean molar TN:TP ratio showing the Redfield ratio (16; blue line), e) ammonia (mg/L) levels showing the threshold for macroalgae blooms (green line; 0.014 µg/L; Lapointe et al., 1993), and f) median fecal coliform units showing the surface water quality criteria (400 MPN). Values above the indicator lines are in exceedance of Florida water quality criteria.

2.2.2 Groundwater

When examined seasonally, a trend of decreasing distance between the ground surface and the water table can be observed during the summer throughout the data set (**Fig. 10**). In fact, 71% of the monitoring wells were in violation of the FAC requirements; the actual water table adjacent to drainfields would potentially be higher due to the mounding that occurs from STE. Additionally, the water table in Charlotte County is often less than 2 ft. below ground, making compliance with regulations almost impossible and suggesting that the hydrology in this region is not conducive to properly functioning septic systems.

Highly elevated levels of nutrient and bacteria concentrations were found in groundwater. Mean TN and TP were above the respective NNC surface water criteria (**Fig. 11a,b**), indicating that in the study area groundwaters are likely a source of nutrient enrichment to adjacent surface waters.

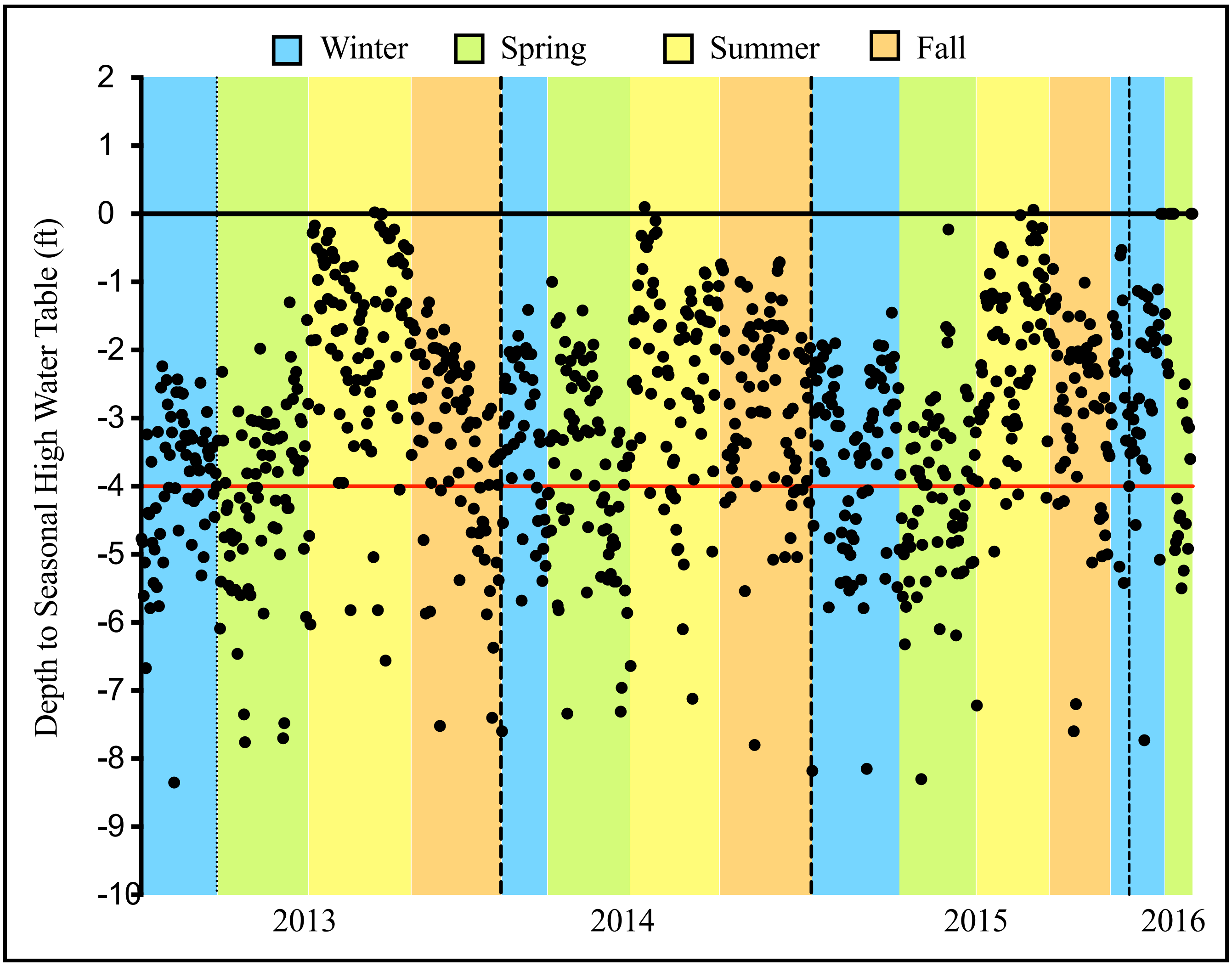

Figure 10. Depth from ground surface to the seasonal high water table (ft.) displayed by season and year at groundwater monitoring well sites in Charlotte County (n=947), showing requirements according to Florida Administrative Code (FAC); ground level is at 0 (represented by the x-axis), the minimum required depth from the ground to the drainfield is 1 ft. of cover, and the minimum depth between the bottom of the drainfield to the seasonal high water table is 2 ft. (red horizontal line). All points above the red line are in violation of the FAC (62E-6).

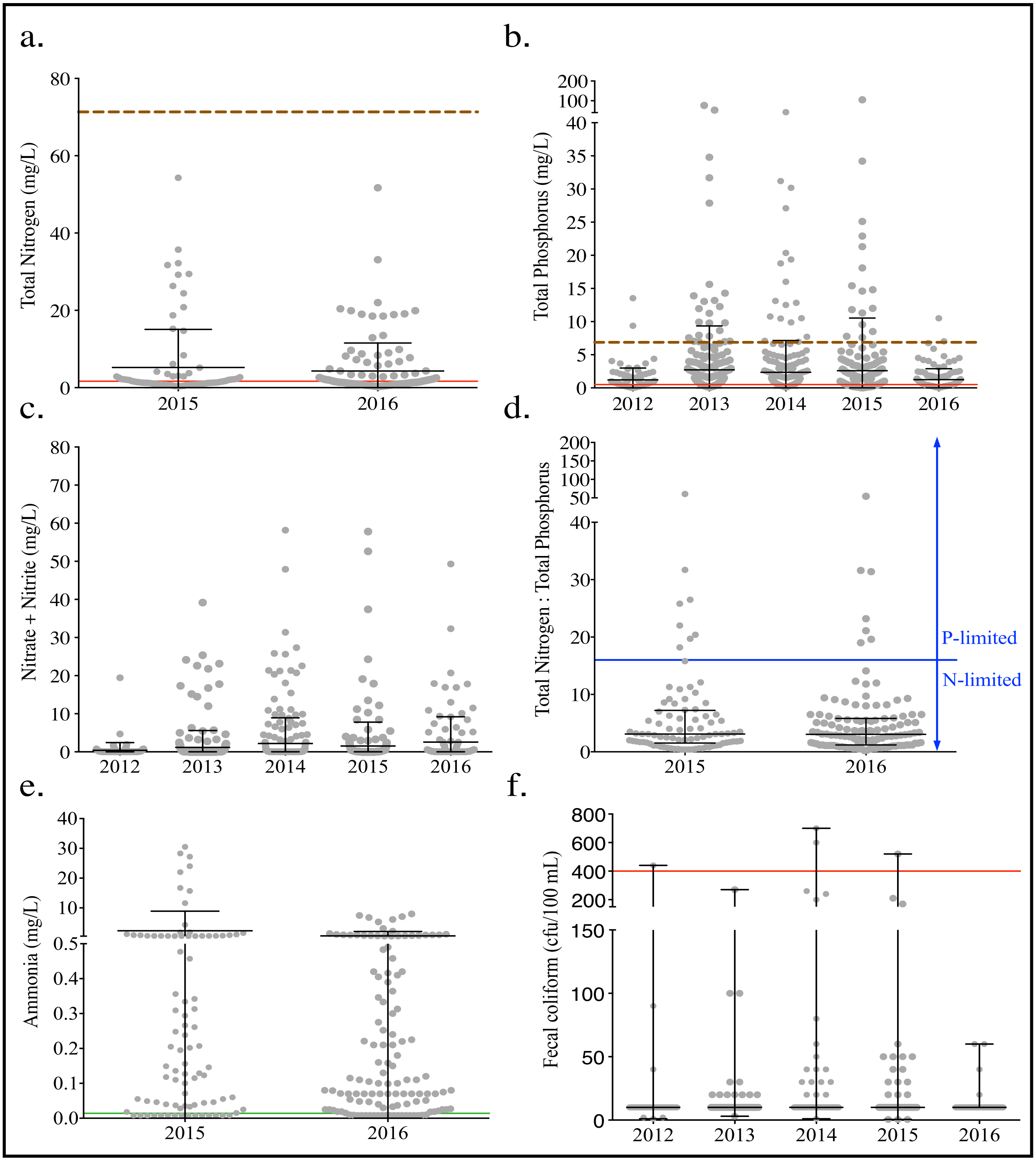

Figure 11. Localized water quality parameters of groundwater collected from monitoring wells (n=39) in the East and West Spring Lake Wastewater Pilot Program area, Charlotte County, FL, including: a) mean total nitrogen (TN; mg/L) showing the Numeric Nutrient Criteria (NNC; 1.02 mg/L Tidal Peace River, b) mean total phosphorus (TP; mg/L) showing the NNC (0.31 mg/L Tidal Peace River), c) nitrate + nitrite values (mg/L), d) mean molar TN:TP ratio showing the Redfield ratio (16; blue line), e) ammonia (mg/L) levels showing the threshold for macroalgae blooms (green line; 0.014 µg/L; Lapointe et al., 1993), and f) fecal coliform units showing the surface water quality criteria (400 MPN). Red lines denote NNC values for respective parameters and brown dotted lines denote the respective nutrient values of septic tank effluent collected from a low-pressure sewer lift station at the intersection of O'Hara and Midway roads (#23; OMLS).

TN was variable by monitoring well and sampling date (**Appendix 1.4a,b)**. Total phosphorus exhibited a value similar to that of raw sewage (6.9 mg/L; **Fig. 11b**). Nitrate + nitrite concentrations had a range of 1.2 to 2.6 mg/L (**Fig. 11c**). The TN:TP molar ratio was below the Redfield ratio of 16:1, indicating these groundwaters are highly phosphorus-enriched and nitrogen-limited (**Fig. 11d**). Concentrations of ammonia were above the threshold for macroalgae blooms (Lapointe et al., 1993) during 2012-2016 (**Fig. 11e**). Fecal coliform levels were high in groundwater samples and many samples approach the surface water quality criteria (400 cfu/ 100 mL), indicating that groundwater is a likely source of contamination to adjacent surface waters (**Fig. 11f**). There were many occurrences of high spikes in data points for all of parameters. Detailed information on parameters for groundwater can be found in **Appendix 1.5**.

2.2.3 Stormwater

Stormwater sampling could help to evaluate the correlation between the nutrient and fecal coliform concentrations in rain-driven stormwater runoff with elevated concentrations in the surface water. Unfortunately, no publicly available stormwater data from monitoring programs within Charlotte County was available. Presented here are the available stormwater data from the East and West Spring Lake Wastewater Pilot Program.

Eight stormwater sampling events were conducted from September 2015 to May 2016. Overall, samples exhibited higher total nitrogen, total phosphorus, and ammonia concentrations for the events in fall 2015 (ranging from 1.3 to 1.6 mg/L, from 0.6 to 1.2 mg/L, and from 0.1 to 0.2 mg/L, for total nitrogen, total phosphorus, and ammonia levels, respectively) compared to the events in spring and summer 2016 (**Figure 12a,b,e**). However, nitrate + nitrite was less variable seasonally (**Figure 12c**). Thus, surface water samples are also subjected to higher nutrient-contamination levels via stormwater input. Additionally, stormwater samples exhibited higher fecal coliform maximum levels than the surface and groundwater samples (up to 48,000 cfu/100 mL) with peaks in September and January (**Figure 12f**). Detailed information on all parameters for stormwater can be found in **Appendix 1.6**.

2.3 Summary

This analysis of the historical data regarding water quality in Charlotte County and the East and West Spring Lake Wastewater Pilot Program area has yielded a comprehensive assessment of the on-going challenges facing this area. In surface waters, the mean observed concentrations for TN were often similar to or in exceedance of the NNC and the median values for fecal coliforms indicated STE contamination. Furthermore, a reduced separation was observed between the ground surface and the seasonal high water table during the summer, often less than the required minimum distance of 24 in. (2 ft.). The monitoring wells suggest that 71% of the septic tank drainfields in study area would be in violation of the FAC requirements, which has serious implications for the contamination of groundwater by STE during the wet season (Bicki and Brown, 1984).

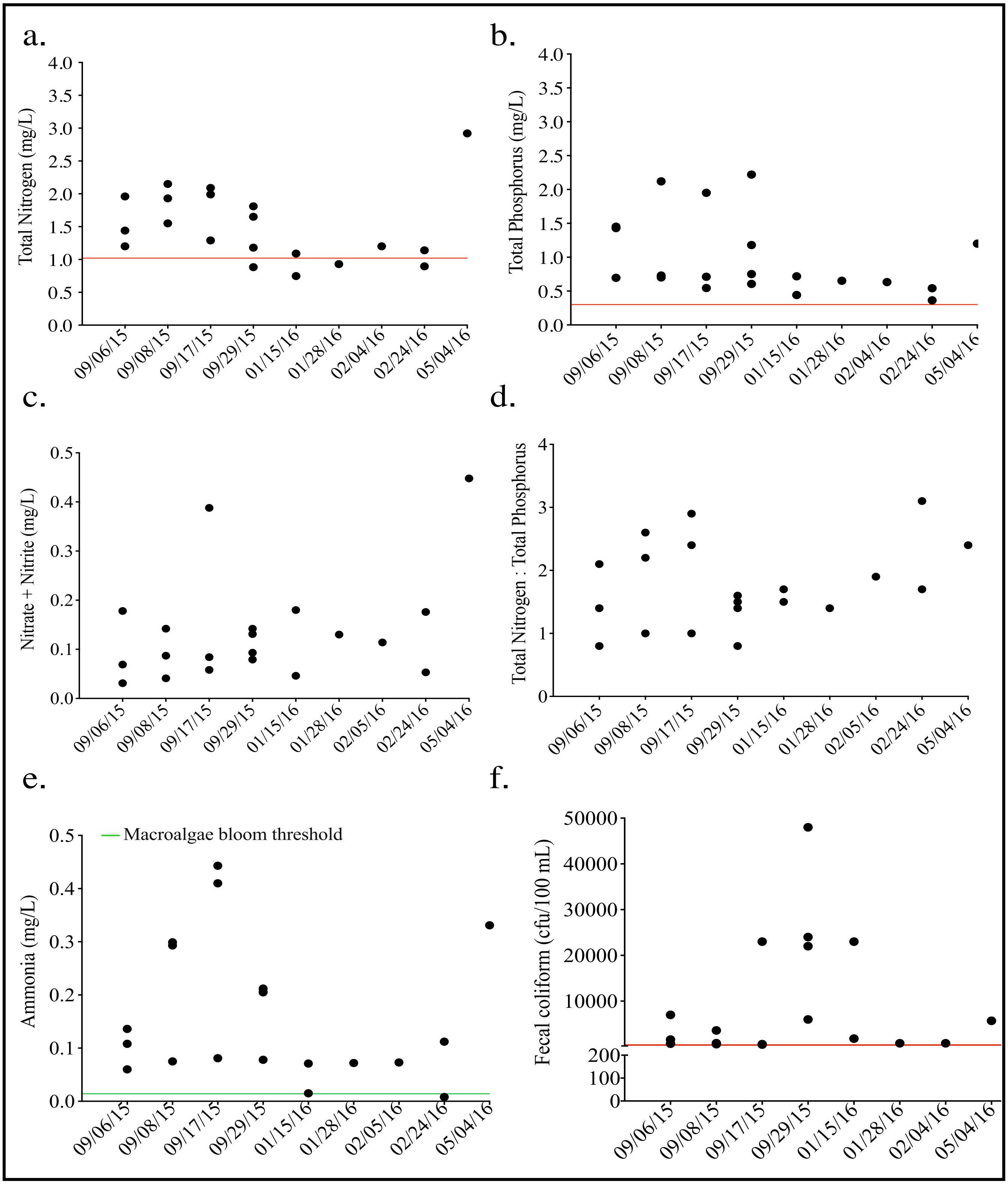

Figure 12. Localized water quality parameters of stormwater from 8 sampling events in the East and West Spring Lake Wastewater Pilot Program area of Charlotte County, FL, including: a) total nitrogen (TN; mg/L) showing the Numeric Nutrient Criteria (NNC) for surface water (1.08 mg/L Tidal Peace River), b) total phosphorus (TP; mg/L) showing the average NNC for surface water (0.50 mg/L Tidal Peace River), c) nitrate + nitrite values (mg/L), d) TN:TP molar ratio, e) ammonia (mg/L) levels showing the threshold for macroalgae blooms (green line; 0.014 µg/L; Lapointe et al., 1993), and f) fecal coliform showing the surface water quality criteria (400 MPN). Red lines denote NNC for respective parameters.

3. Reconnaissance Sampling in Charlotte County

As demonstrated in Section 2, multiple entities either have collected or are currently collecting nutrient and bacteria data in the greater Charlotte County area. Some parameters, especially ammonia and human source tracking indicators, such as stable nitrogen isotopes ($\delta^{15}N$) and sucralose, were not well represented in these datasets. To address these data gaps, 11 sampling events were conducted during a reconnaissance sampling effort between June–August 2016 to help guide the long-term monitoring plan recommendation process. This strategic weekly surface water sampling was conducted during the summer on ebbing tides in an expanded area that included El Jobean, Ackerman, Spring Lakes, and Yacht Club (**Fig. 13**). Samples for stable nitrogen isotopes and sucralose were also collected to help guide the long-term monitoring process. Detailed information on all locations sampled during Phase I can be found in **Appendix 2.1**. The reconnaissance data were summarized using GraphPad Prism version 7.00a for Mac OS.

HBOI-FAU contracted the following labs for analytical support services: University of Georgia Center for Stable Isotope Analysis Stable Isotope Ecology Laboratory (UGA-SIEL), Nutrient Analytical Services – Chesapeake Biological Laboratory at the University of Maryland (NAS-CBL), and Florida International University- Southeast Environmental Research Center Environmental Analysis Research Laboratory (FIU-EARL). Additionally, Charlotte County contracted Benchmark EnviroAnalytical, Inc. for field and analytical services. Benchmark EnviroAnalytical, Inc. conducted water quality sampling in Charlotte County on a weekly schedule (06/30/2016-08/31/2016). Additionally, HBOI-FAU completed two days of reconnaissance sampling and exploratory fieldwork (June 24 and July 25, 2016).

3.1 *Methods*

Calibrated YSI Models 1030 and ProODO hand-held meters were used to determine pH, salinity, conductivity, temperature, and DO at the time water samples were collected from each site (**Appendix 2.2**). Surface and groundwater samples were collected in duplicate by HBOI-FAU into acid-washed 0.5 L high-density polyethylene (HDPE) bottles and were immediately stored on ice in a dark cooler until processing at the HBOI laboratory. The samples were filtered (25 mm GF/F filters) and frozen until analysis. Frozen samples were shipped to NAS-CBL for analysis on a Technicon Auto-Analyzer II (nitrate, TN, soluble reactive phosphorus (SRP), and TP) or a Technicon TRAACS 800 (ammonium and nitrate + nitrite). Detection limits were 0.029 mg/L for ammonium, 0.001 mg/L for nitrate + nitrite, 0.006 mg/L for SRP, 0.288 mg/L for TN, and 0.016 mg/L for TP. The resulting data were used to characterize ambient dissolved nutrient concentrations and ratios at the Charlotte County reconnaissance sites.

3.1.1 Surface water sampling

Surface water was collected at four sites representing the confluence of residential canals and Charlotte Harbor (El Jobean, Ackerman, Spring Lakes, and Yacht Club; **Fig. 14b**). Under the lead of CCUD, the four surface water stations were sampled weekly by Benchmark EnviroAnalytical, Inc. during ebbing tides between the weeks of June 27, 2016 - August 25, 2016 for nutrients, chlorophyll *a*, bacteria (fecal coliforms and *Enterococcus*), total dissolved solids, BOD, and environmental parameters (pH, salinity, conductivity, temperature; **Appendix**

2.2). In addition to the weekly surface water monitoring by Benchmark EnviroAnalytical, Inc., HBOI-FAU collected surface water samples at the cage sites during an outgoing tide on June 24 (dissolved nutrients and aqueous isotopes) and July 25 (sucralose), 2016.

3.1.2 Groundwater sampling

Preliminary nutrient and wastewater tracer analyses were conducted on groundwater (monitoring wells 66, 67, and 68) in the East and West Spring Lake Wastewater Pilot Program area (**Fig. 13**). The three groundwater stations sampled by HBOI-FAU represented areas where nutrient concentrations, especially nitrogen, were exceptionally high during the East and West Spring Lake Wastewater Pilot Program. Dissolved nutrient analyses were conducted on groundwater samples collected at three wells on June 24 and July 25, 2016 by HBOI-FAU. Groundwater samples were collected using a Masterflex ® ES portable peristaltic pump with two ft. of silicon tubing connected via silicon junction to 17 feet of Tygon tubing. Monitor well depth was calculated and the well volume was turned over a minimum of three complete times prior to sample collection. All aqueous isotope samples were collected in duplicate and immediately preserved on ice until processing at HBOI-FAU.

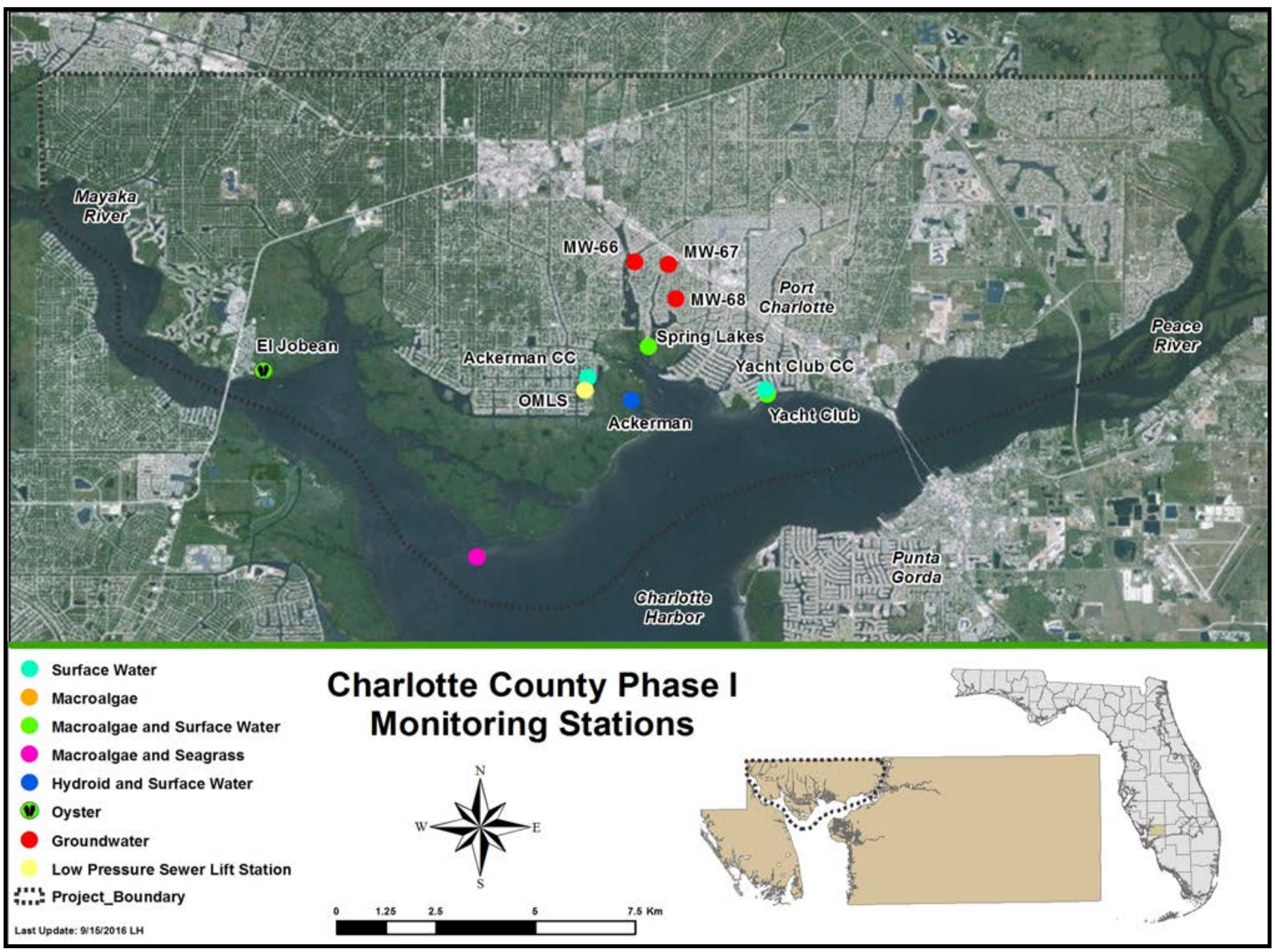

Figure 13. Reconnaissance sampling groundwater monitoring well (MW) and surface water sites and a low-pressure sewer lift station at the intersection of O'Hara and Midway roads (#23; OMLS) incorporated into the Phase I monitoring by Florida Atlantic University- Harbor Branch (HBOI-FAU) and Charlotte County.

Raw wastewater effluent associated with a low-pressure sewer lift station at the intersection of O'hara and Midway roads (#23; OMLS) was also collected on by HBOI-FAU on July 25, 2016 to measure the nutrient, aqueous nitrogen isotopes, and sucralose concentrations emanating from residential homes into a centralized collection site. Benchmark EnviroAnalytical, Inc. conducted weekly sampling at OMLS starting July 28, 2016 and continued throughout reconnaissance sampling (**Fig. 14c)**.

In the laboratory, aqueous stable nitrogen isotopes ($\delta^{15}N/-NH_4$, and $\delta^{15}N/-NO_3$) analyses were conducted on groundwater and OMLS samples. At HBOI-FAU, the samples were filtered using 47 mm GF/F filters and frozen until shipped to UGA-SIEL. At UGA-SIEL the water samples were run through ammonia diffusion, which involved increasing the pH of the dissolved sample, converting the ammonium to gaseous ammonia, which is captured on an acidified filter in the bottle headspace. Nitrate-specific N was quantified by first boiling-off the volatile ammonia, adding a reducing agent to convert oxidized N to NH_4, then proceeding with the standard diffusion and ammonia capture on an acidified filter. The filter was then analyzed as a typical solid sample on a Carlo Erba Isotope Ratio Mass Spectrometer for $\delta^{15}N$ -NH_4 and $\delta^{15}N$ -NO_3.

3.1.3 Stable nitrogen isotopes

Stable nitrogen isotope ratios from the tissue of macroalgae, hydroids, and oysters were used to identify nitrogen sources (e.g. fertilizers vs. wastewater vs. atmospheric deposition) in the study area. For this analysis, the four weekly sampling stations were also used by HBOI-FAU as macroalgae cage deployment sites (established on June 24, 2016; **Fig. 13**). Because macroalgae may be difficult to consistently find at the specific locations, cages were placed *in situ* at the desired sites and filled with red macroalgae (*Gracilaria tikvahiae*) grown at HBOI-FAU by the Marine Botany Laboratory (**Fig. 14a,d**). The cages were deployed on June 24, 2016 and cleaned (debris and fouling removed) once each week by Florida Sea Grant staff and volunteers. The cages were left in the field for approximately four weeks to allow time for the macroalgae to incorporate the ambient carbon and nitrogen signals of the environment. The cages and macroalgae were retrieved on July 25, 2016. During the second reconnaissance trip, when it was determined that one cage was missing (Ackerman) and that the *Gracilaria tikvahiae* in some of the cages was not thriving, the HBOI-FAU field team also opportunistically collected green macroalgae (*Chaetomorpha gracilis*) and eastern oysters *(Crassostrea virginica*) from El Jobean (**Fig. 14e)**; shoal grass (*Halodule wrightii*) and naturally occurring *Gracilaria tikvahiae* from Hog Island (26.92060, -82.15636); and a hydroid from Ackerman (**Fig. 14f**).

The samples were transported back to HBOI-FAU in plastic bags on top of ice covered with a towel to protect the tissue. Samples were cleaned of epiphytes and debris, rinsed briefly in deionized water to remove excess salt, and dried in a Fisher Scientific Isotemp® oven at 65°C for 48 h. The dried organisms were ground to a fine powder using a mortar and pestle and stored in plastic screw- top vials. The dried samples were analyzed for stable nitrogen isotopes ($\delta^{15}N$) and nutrient concentrations (C:N:P).

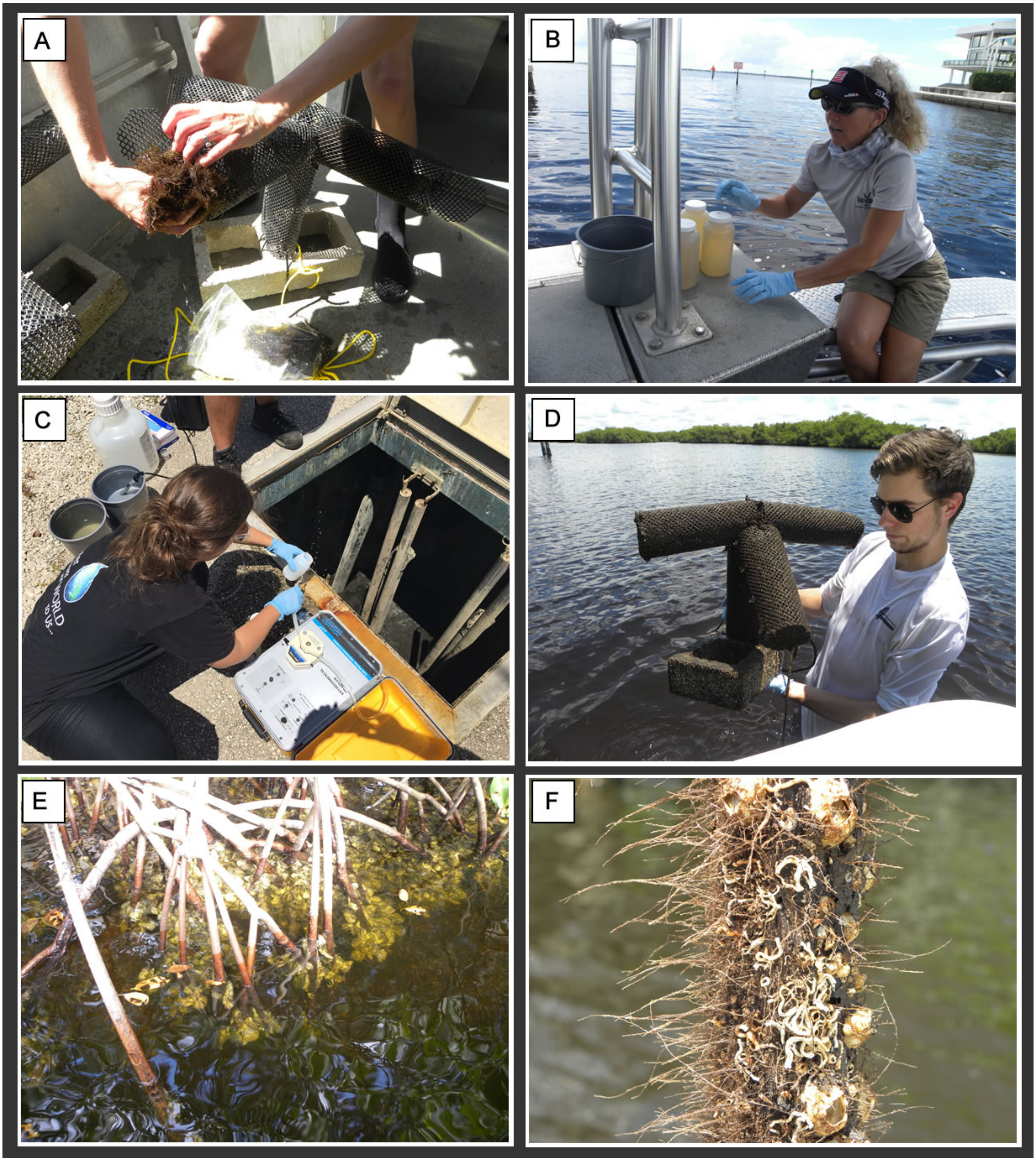

Figure 14. Images from reconnaissance sampling in Charlotte County: A) Prior to deployment, mesh cages were filled with red algae (*Gracilaria tikvahiae*), B) surface water collection in Charlotte Harbor estuary, C) water sample collection from the O'hara-Midway low-pressure sewer lift station #23 (OMLS), D) retrieval of *Gracilaria tikvahiae* cages, E) eastern oysters (*Crassostrea virginica*) and mangrove roots in Charlotte Harbor, and F) a hydroid collected from the Ackerman site.

Sub-samples were shipped to the UGA-SIEL for stable carbon and nitrogen isotope analysis and to NAS-CBL for %P analysis. At UGA-SIEL, samples were analyzed for stable carbon and nitrogen isotopes and %C and %N on a Thermo Delta V Environmental Analysis - Isotope Ratio Mass Spectrometer coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo Conflo III Interface (see the following for methods: http://sisbl.uga.edu/ratio.html#top). National Institute of Standards and Technology reference materials 8549, 8558, 8568, and 8569 were used to routinely calibrate or check working standards produced by the methods described by Böhlke et al. (1993) and Böhlke et al. (2016). QA/QC results were incorporated into the raw data reports. The resulting stable nitrogen isotope data are expressed relative to atmospheric nitrogen in part per thousand (*per mille*, ‰) and were used to determine inferences regarding nutrient availability in relation to various natural and anthropogenic nitrogen sources (Risk et al., 2009; Lapointe et al., 2015). At NAS-CBL, %P was analyzed following the methodology of Asplia et al. (1976) on a Technicon Autoanalyzer II with an IBM-compatible, Labtronics, Inc. DP500 software data collection system (D'Elia et al., 1997). C:N:P data were compared to a modified Redfield ratio of 360:30:1 to characterize temporal and spatial variation in tissue nutrient status (Atkinson and Smith, 1983; Lapointe, 1987; Lapointe et al., 2015).

3.1.4 Sucralose Sampling

As mentioned in sections 3.1.1 and 3.1.2, sucralose samples were collected on an outgoing tide on July 25, 2016 at the four sites in Charlotte Harbor, three monitoring wells (66, 67, and 68) in the Spring Lakes area, and OMLS. Individual samples were collected in 125 mL HDPE bottles, placed on ice immediately after collection, frozen when returned to the HBOI-FAU laboratory, and then shipped to FIU-EARL for processing. At FIU-EARL, sucralose was analyzed in accordance with Batchu et al. (2015).

3.2. Results

3.2.1 Surface Water

Overall, average (n=11) surface water nutrient concentrations decreased from west to east in the study area. The El Jobean site had the highest average concentrations of TN and TP, with values decreasing at the more eastern sites (**Fig. 15a,b**). The average TN concentrations at all four monitoring sites were above the NNC for the tidal Myakka and Peace rivers. Additionally, TN concentrations were about 10 % higher than those from the historical East and West Spring Lake Wastewater Pilot Program dataset; this is most likely because the reconnaissance sampling was conducted on an ebbing tide when STE leaching from groundwater to surface water would most discernable. TN:TP ratios indicate that nitrogen is limiting (**Fig. 15c**). In contrast, chlorophyll *a* concentrations generally increased from west to east among the sampling sites, with the highest average value at Yacht Club (**Fig. 15d**). The average chlorophyll *a* at Yacht Club exceeded the NNC and values for Ackerman and Spring Lakes fell within the 95% SD. The concentrations of ammonia, nitrate + nitrite, and the dissolved inorganic nitrogen (DIN):SRP ratio generally decreased from west to east, with no pattern observed for soluble reactive phosphorus (SRP; **Fig. 16a,b,c,d**). Average BOD concentrations at these four sites ranged from 4.4-6.9 mg/L with maximum values as high as 12.1 (**Table 3**); for reference, clean tidal creeks have a BOD of about 2mg/L (Mallin et al., 2006).

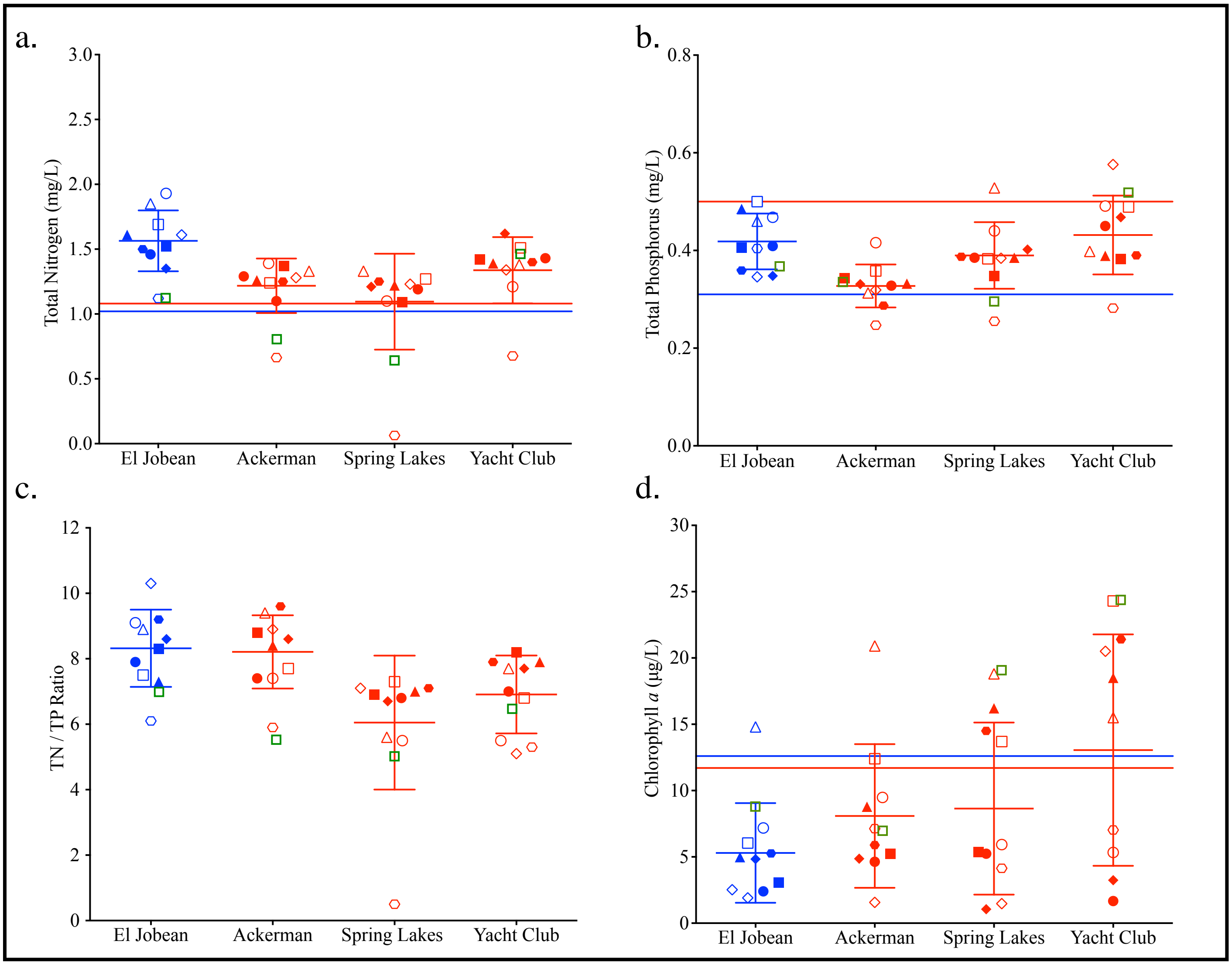

Figure 15. Nutrient concentrations (arithmetic mean ± SD) observed in weekly reconnaissance sampling of surface waters (canals) within Charlotte County, FL from June 30 to August 31, 2016 showing the respective Numeric Nutrient Criteria (NNC) for surface water, including: a) total nitrogen (TN; NNC= 1.02 mg/L Tidal Myakka River and 1.08 mg/L Tidal Peace River), b) total phosphorus (TP; NNC=0.31 mg/L Tidal Myakka River and 0.50 mg/L Tidal Peace River), c) molar TN:TP ratios, and d) chlorophyll *a* levels (NNC= 12.6 Tidal Myakka River and 11.7 Tidal Peace River). Sampling dates are represented by differently, colored shapes: white triangle = week 1, white square = week 2, white circle = week 3, black triangle = week 4, black square = week 5, black circle = week 6, black diamond = week 7, white diamond = week 8, black hexagon = week 9, and white hexagon = week 10. Any values above the corresponding NNC line are in exceedance of the criteria. Green squares represent data collected by Florida Atlantic University-Harbor Branch Oceanographic Institute during reconnaissance sampling (June 24, 2016). Blue lines denote NNC values for the Tidal Myakka River and red lines denote NNC values for the Tidal Peace River.

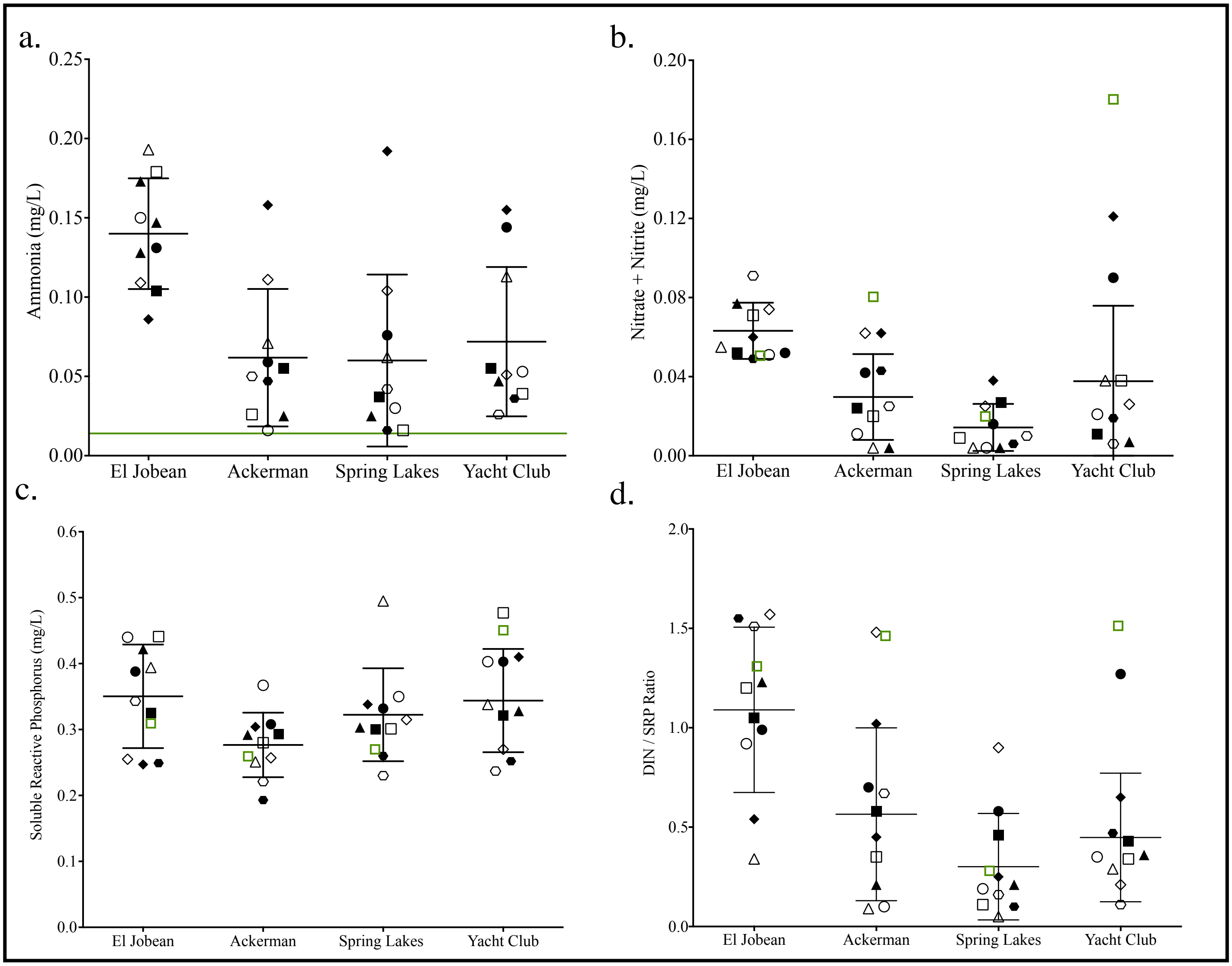

Figure 16. Water quality parameters (arithmetic mean ± SD) from reconnaissance sampling of surface waters (canals) within Charlotte County, FL collected weekly from June 30 - August 31, 2016, including: a) ammonia showing the threshold for macroalgae blooms (0.014 µg/L; Lapointe et al., 1993), b) nitrate + nitrite (mg/L), c) soluble reactive phosphorus (SRP), and d) the molar ratio of dissolved organic nitrogen to soluble reactive phosphorus (DIN / SRP). Sampling dates are represented by differently colored shapes: white triangle = week 1, white square = week 2, white circle = week 3, black triangle = week 4, black square = week 5, black circle = week 6, black diamond = week 7, white diamond = week 8, black hexagon = week 9, and white hexagon = week 10. Green squares represent data collected by Florida Atlantic University-Harbor Branch Oceanographic Institute during reconnaissance sampling (June 24, 2016).

3.2.2 Groundwater

Groundwater sampling at monitoring wells 66, 67, and 68 corroborated the high historical nutrient concentrations and confirmed the likelihood of STE nutrient contamination in these wells. TN concentrations ranged up to 15.57 mg/L, TP to 4.97 mg/L, nitrate plus nitrite to 13.26 mg/L, and ammonium to 9.15 mg/L (**Appendix 2.3)**. These high nutrient concentrations are typical of STE plumes. TN observed was higher than in the East and West Spring Lake Wastewater Pilot Program dataset; this is likely due to the strategic placement of these monitoring wells within suspected septic plumes.

Samples collected from OMLS showed very high nutrient concentrations typical of STE; mean TN was 71.33 mg/L, BOD was 39.23 mg/L, ammonia was 52.58 mg/L, and fecal coliforms were 245,000 cfu/100mL (**Table 3**). In comparison, samples collected from effluent of Charlotte County Eastport Water Reclamation Facility (WRF) had much lower nutrient concentrations, with TN averaging 13.2 mg/L and TP 3.2 mg/L. This comparison illustrates how WRFs in Charlotte County are removing an average of 90% of the nitrogen and 67% of the phosphorus compared to raw STE.

3.3 Bacterial Abundance

Bacteria samples (fecal coliforms and *Enterococcus*) were collected during the weekly surface water monitoring events overseen by CCUD. The current standards established by Florida Department of Health (DOH) Healthy Beaches Program for *Enterococcus* bacteria are: Good (0-35 *Enterococci* / 100 mL seawater), Moderate (36-70 *Enterococci* / 100 mL seawater), and Poor (71+ *Enterococci* / 100 mL seawater). Surface water standards for fecal coliforms are 400 MPN/100 mL (directly relatable to 400 cfu/100 mL) and the presence of fecal coliform bacteria in these samples indicates the presence of waterborne human pathogens and strongly suggests contamination via STE.

The highest average fecal coliform concentrations were observed at El Jobean and decreased from west to east among the sampling sites (**Fig. 17a**). El Jobean and Ackerman had samples that exceeded surface water quality criteria (400 MPN/100mL). A similar spatial trend was observed for *Enterococci* values, which averaged highest at El Jobean and decreased from west to east (**Fig. 17b**). Both the El Jobean and Ackerman sites had average values exceeding the standard for “poor” water quality according to the DOH Healthy Beaches program (>71 *Enterococci*/100 mL seawater).

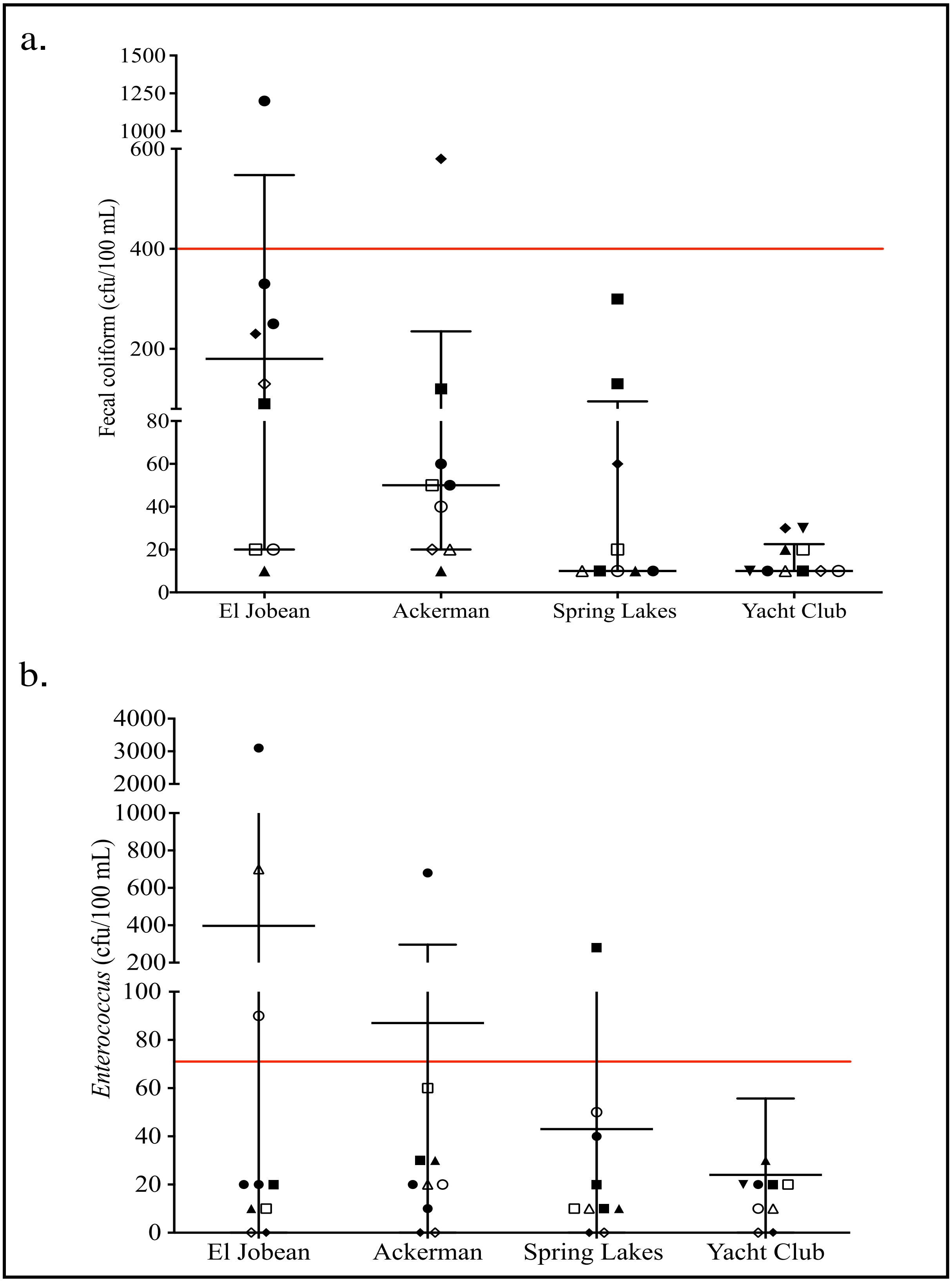

Figure 17. Bacterial abundance in surface waters during reconnaissance sampling in Charlotte County, including: a) fecal coliform, showing surface water quality criteria, and b) *Enterococcus* concentrations from sites within Charlotte County, showing the level at which the Department of Health Healthy Beaches Program considers water quality to be "Bad" (71 cfu/100mL; red line).

Table 3. Water quality parameters from reconnaissance sampling effort (06/30/2016-08/31/2016) at four sites in Charlotte Harbor (El Jobean, Ackerman, Spring Lakes, and Yacht Club) and the O'Hara-Midway low-pressure sewer lift station #23 (OMLS).

Parameter	Site	Ackerman	Spring Lakes	El Jobean	Yacht Club	OMLS
Enteroccoci (cfu/100 mL)	Mean	157	51	426	27	575000
	SD	271	83	964	30	328131
	Max	680	280	3100	110	960000
	Min	10	10	10	10	190000
	Number	10	10	10	10	6
Fecal Coliform (cfu/100 mL)	Mean	50	10	180	10	245000
	SD	291	98	1236	8	92682
	Max	850	300	4000	30	370000
	Min	10	10	10	10	160000
	Number	10	9	10	10	6
Chlorophyll *a* (µg/L)	Mean	8.1	8.6	5.3	13.1	na
	SD	5.4	6.5	3.8	8.7	na
	Max	20.9	18.8	14.8	24.3	na
	Min	1.57	1.05	1.91	1.67	na
	Number	10	10	10	9	na
Biological Oxygen Demand (mg/L)	Mean	5.155	4.250	6.891	4.389	39.233
	SD	3.782	4.052	4.761	4.244	53.558
	Max	11.9	10.8	12.1	11	148
	Min	0	0	0	0	12
	Number	10	10	10	10	6
Total Nitrogen (mg/L)	Mean	1.22	1.10	1.56	1.34	71.33
	SD	0.2	0.4	0.2	0.3	16.7
	Max	1.39	1.33	1.93	1.62	88
	Min	0.664	0.063	1.12	0.676	45
	Number	10	10	10	10	6
Nitrate+Nitrite (mg/L)	Mean	0.030	0.014	0.063	0.038	0.014
	SD	0.022	0.012	0.014	0.038	0.018
	Max	0.062	0.038	0.091	0.121	0
	Min	0.004	0.004	0.049	0.006	0
	Number	10	10	10	10	6
Ammonium (mg/L)	Mean	0.047	0.036	0.137	0.047	na
	SD	0.042	0.043	0.075	0.054	na
	Max	0.141	0.132	0.217	0.181	na
	Min	0	0	0	0	na
	Number	10	10	10	10	na
Total Kjeldahl Nitrogen (mg/L)	Mean	1.19	1.08	1.50	1.30	71.32
	SD	0.2	0.4	0.2	0.2	16.7
	Max	1.38	1.33	1.88	1.5	88
	Min	0.639	0.0528	1.03	0.67	45
	Number	10	10	10	10	6
Total Phosphorus (mg/L)	Mean	0.33	0.39	0.42	0.43	6.87
	SD	0.0	0.1	0.1	0.1	1.2
	Max	0.416	0.528	0.5	0.576	8
	Min	0.247	0.255	0.346	0.282	5
	Number	10	10	10	10	6

3.4 Sucralose concentrations

Sucralose concentrations at OMLS were greater than 60 µg/L, with lower, but elevated concentrations of approximately 10 µg/L in the groundwater monitoring wells, and concentrations less than 1 µg/L at the four surface water sites **(Appendix 2.3; Fig. 18a).** These concentrations are similar to those measured in a recent Martin County septic tank study and confirm contamination of surface waters with human-sourced pollutants from STE **(Fig. 18b)**.

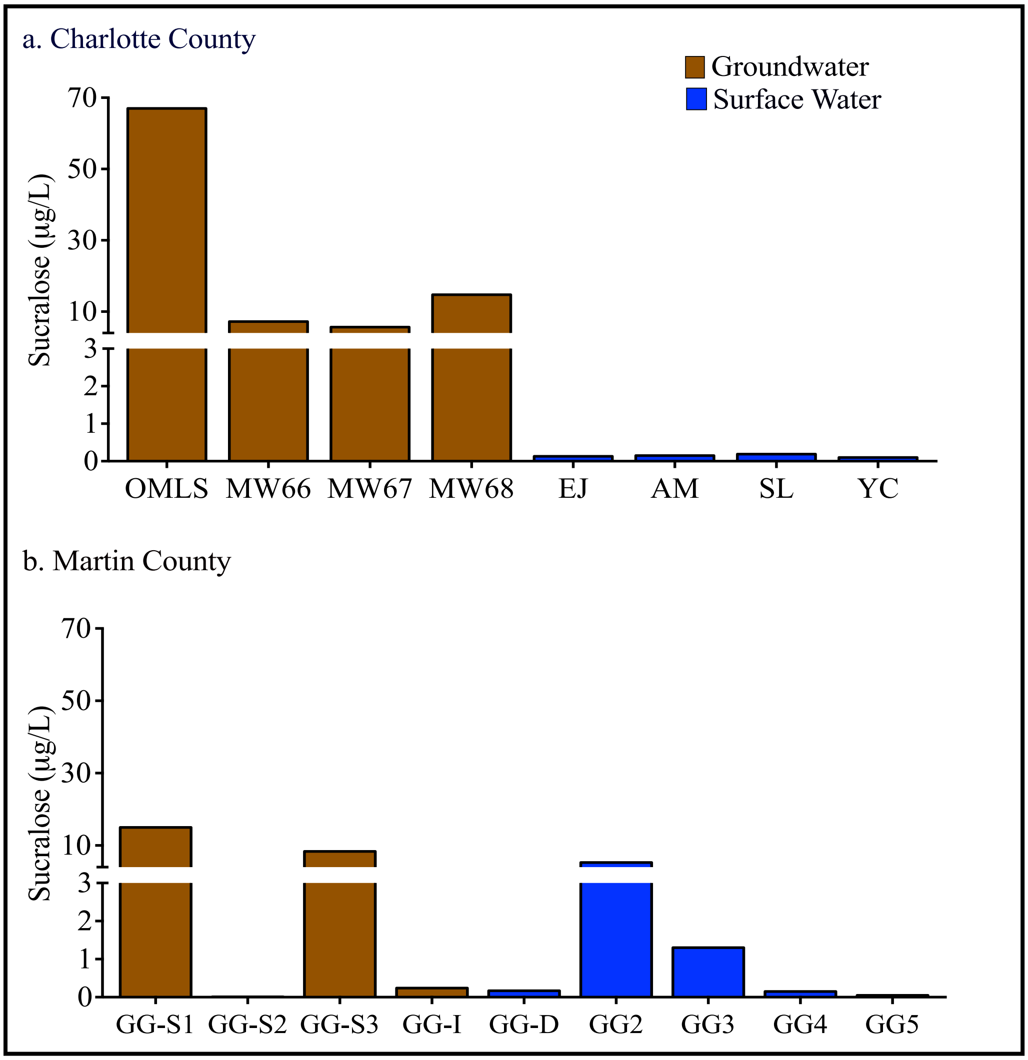

Figure 18. Sucralose concentrations (µg/L) from groundwater and surface water sites within a) Charlotte County, including O'hara-Midway low-pressure sewer lift station (OMLS) compared to sites with b) Martin County.

3.5 Aqueous Stable Isotopes

The aqueous $\delta^{15}N$ values from groundwater monitoring wells 66, 67, and 68 ranged from +15.14 to +20.45 ‰ for ammonia and from +10.15 to +15.30 ‰ for nitrate (**Appendix 2.3**). These values are consistent with values reported for groundwater impacted by STE.

3.6 Stable nitrogen isotopes and C:N ratios

Stable nitrogen isotope samples generally revealed a strong signal of sewage nitrogen ($\delta^{15}N > 3$ ‰). The naturally occurring organisms had some of the highest $\delta^{15}N$ values. For example, the hydroid sample from Ackerman had the highest $\delta^{15}N$ value, followed by the oysters from El Jobean (**Fig. 19a**). For the cage sites where *Gracilaria tikvahiae* was collected, Yacht Club had the highest $\delta^{15}N$ value, followed by Spring Lakes, El Jobean, and Hog Island. The *Chaetomorpha gracilis* sample from El Jobean was slightly lower in $\delta^{15}N$, than the cage samples, but still within the range of $\delta^{15}N$ for wastewater. The lowest observed $\delta^{15}N$ value was for the seagrass *Halodule wrightii* from the Hog Island site; notably, seagrasses assimilate nutrients via rhizomes from the sediments and are not as effective as macroalgae in monitoring water column nutrients. These high stable nitrogen isotope values are within the range reported for wastewater and are similar to those observed in Lee County, FL (**Appendix 2.4**). The C:N molar ratios of *Gracilaria tikvahiae* ranged from ~5-12 **(Fig. 19b**), which are very low values and indicative of high levels of nitrogen enrichment; values greater than 13 indicate nitrogen limitation in macroalgae (Lapointe, 1985; Lapointe et al., 1993). Higher C:N molar ratios were observed in *Halodule wrightii* collected from open waters of Charlotte Harbor near Hog Island.

3.7 Summary

Reconnaissance sampling was productive and enabled us to better understand the sources and levels of STE pollution within Charlotte County. NNC values in canal discharges were exceeded for TN and chlorophyll *a*. Median fecal coliform concentrations in surface water exceeded the state criteria at El Jobean and Ackerman; additionally, some individual samples from the Spring Lakes site and Yacht Club exceeded the criteria. Furthermore, ammonia, nitrate + nitrite, and SRP were above the threshold values for macroalgal blooms (Lapointe et al., 1993). Groundwater samples revealed a strong STE signal based on elevated nutrient concentrations, $\delta^{15}N$ values, and sucralose concentrations. These data combined with the elevated BOD values observed during the reconnaissance sampling, indicate that STE contaminates shallow groundwaters and surface waters in Charlotte County and represent a significant source of nutrient and bacterial pollution in Charlotte Harbor.

Observed surface water TN values were approximately 10% higher than those from the East and West Spring Lake Wastewater Pilot Program in both groundwater and surface water. This is most likely not indicating a recent increase in TN, but rather the effects of more strategic sampling that is accurately documenting existing conditions. This strategic sampling approach will be instrumental in understanding the results of any septic to centralized sewer program.

Human source tracers were effective in documenting STE pollution. Sucralose was detectable in groundwater and surface water. Macroalgae and oysters were effective bioindicators and stable nitrogen isotopes revealed a signal indicative of STE. Taking an ecosystem-based approach is advisable and thus, considering the effects on resident fish would also be informative. Fish are excellent indicator organisms for this purpose because $\delta^{15}N$ can be used to understand the long-term “ecological relevance” of waterborne wastewater pollution (Schlacher et al., 2005). Spatial patterns in $\delta^{15}N$ enrichment have been found to be consistent across taxa and ecosystem-based approaches reveal broad ecological effects of nutrient pollution (Connolly et al., 2013).

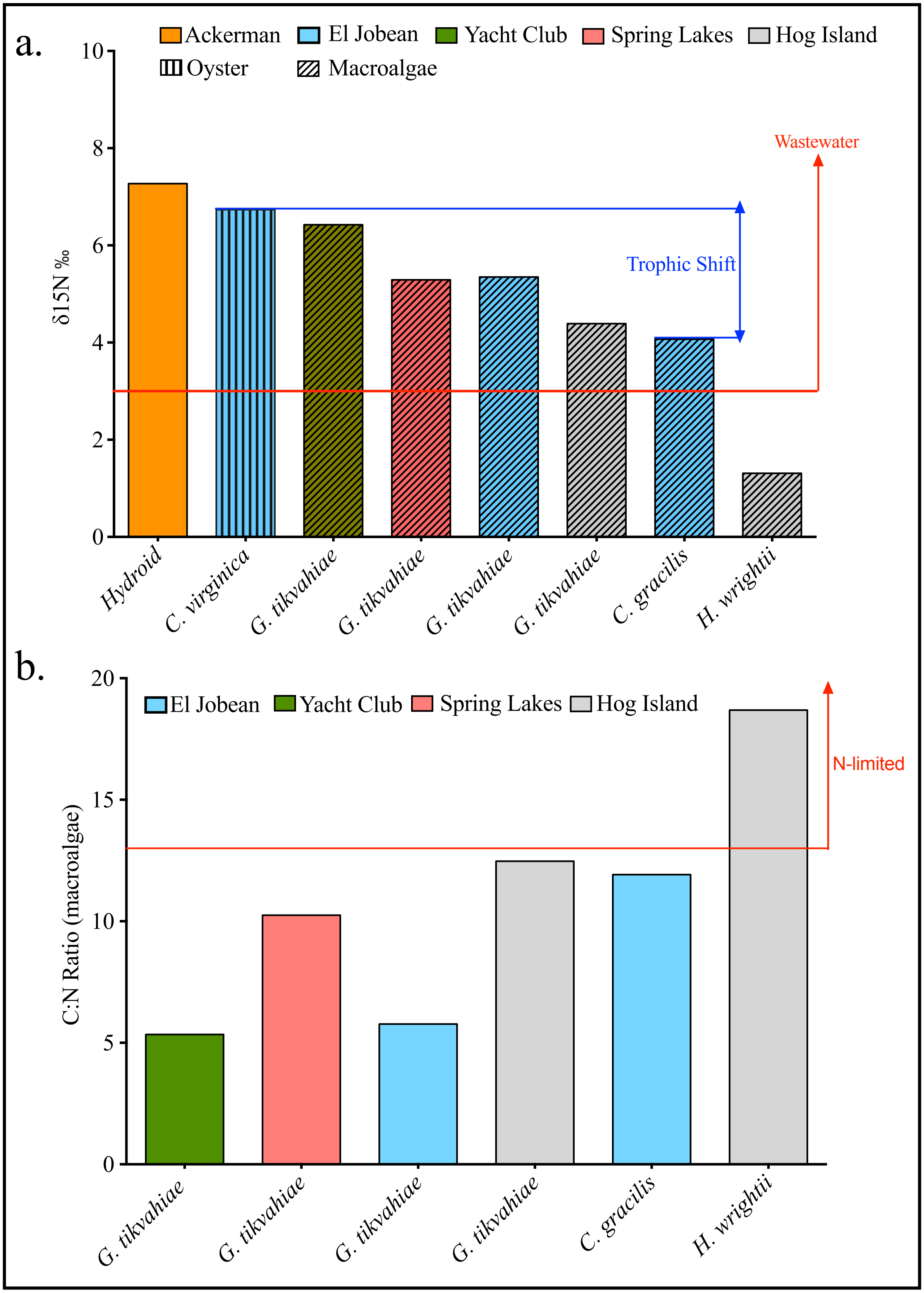

Figure 19. a) Stable nitrogen isotopes ($\delta^{15}N$) values from macroalgae and primary consumers collected at sites throughout Charlotte Harbor estuary compared to the value indicative of a wastewater signal and b) measured carbon to nitrogen (C:N) molar ratios showing the value above which macroalgae become nitrogen limited (Lapointe, 1985).

4. Existing Resources for Water Quality Monitoring in Charlotte County

Agency and volunteer-based monitoring efforts have been established in the project area for several decades. These existing programs provide valuable baseline data and various forms of partner support and should be considered in conjunction with the establishment of comprehensive long-term monitoring plan.

4.1 Established Monitoring Stations

Active monitoring stations in and adjacent to the project area provide an opportunity to incorporate existing datasets and, therefore, facilitate better understanding of pre-construction baseline conditions.

4.2 Established Volunteer Networks

Charlotte County has an extensive, long-standing volunteer network. Volunteers perform field tests and gather samples while obtaining a comprehensive understanding of water quality throughout the county and within Charlotte Harbor National Estuary. Through intensive training the volunteers gain knowledge of science based sampling, sample collection protocols, water quality parameters, and the significance of physical, biological, chemical, and bacteria levels in the water. The volunteer network is instrumental in assisting Charlotte County and local scientific entities gather the data needed to make long and short-term recommendations effecting water quality and the health of the estuary.

CHNEP and the surrounding waterways provide a variety of outdoor, recreational, and financial advantages to the Charlotte County community. Citizens with interests in wildlife, boating, fishing, swimming, and other recreational activities all benefit from the work and dedication of the Charlotte County water quality volunteer network. Volunteers bridge the gap between government and other scientific entities with the community by communicating information regarding local water quality with other community members.

The efforts of volunteers and volunteer coordinators from FDEP Aquatic Preserve, CHNEP, Florida Sea Grant, and CCUD have played an integral part in gathering water samples and environmental data collection. Without their efforts the dataset would be incomplete. Moving forward, volunteer networks will be called upon to continue and potentially expand their efforts.

4.3 Existing Charlotte County Resources

Charlotte County, specifically CCUD, contracted several laboratories throughout the course of the East and West Spring Lake Wastewater Pilot Program. It will be important that these and/or similar labs be used as the project progresses so that results are comparable. Contracted labs and their respective responsibilities include:

a) Benchmark EnviroAnalytical, Inc.
Certified by the National Environmental Laboratory Accreditation Program (NELAP) and accredited by Florida's Department of Health. Benchmark EnviroAnalytical, Inc. was contracted to perform analysis on groundwater and surface water throughout the Spring

Lakes area. Parameters analyzed in reconnaissance testing all met National Environmental Laboratory Conference Institute (NELAC) standards. Benchmark EnviroAnalytical, Inc. conducts the field sampling as well as the laboratory analysis for reconnaissance sites, including TN, nitrate + nitrite, ortho-phosphorus, TP, chlorophyll *a*, ammonium as NH_4, fecal coliforms, and others. Benchmark EnviroAnalytical, Inc. also completed the BOD analyses for the East and West Spring Lake Wastewater Pilot Program.

b) Charlotte Country East Port Laboratory
NELAP certified with the ability to analyze water samples using methods that are in compliance with NELAC standards. During the East and West Spring Lake Wastewater Pilot Program they have been utilized as an intermediary to hold samples during transfer between Johnson Engineering and Benchmark EnviroAnalytical, Inc. Additionally, they have analyzed samples from the East and West Spring Lake Wastewater Pilot Program for all parameters, excluding BOD. They have state certifications to process groundwater, surface water, and stormwater, and there is potential that they will be called upon for more analyses in future phases.

c) Tetra Tech
Contracted since the onset of East and West Spring Lake Wastewater Pilot Program in 2012. Tetra Tech gathers and analyzes data to make determinations and recommendations for Charlotte County's East and West Spring Lake Wastewater Pilot Program. As per the East and West Spring Lake Quality Assurance Project Plan (QAPP), Task Memo 2, some of the key Phase I tasks included creating a database for surface water and groundwater and evaluating groundwater depths in the monitoring wells to determine flow paths and identify potential monitoring septic plumes (Frick et al., 2015).

d) Johnson Engineering
Subcontracted by Tetra Tech, Johnson Engineering's QAPP was established to quantify the nutrient load reduction achieved by the Revitalize Impaired Waters of Charlotte Harbor- East and West Spring Lakes Project (Johnson Engineering, 2015). Johnson Engineering will conduct pre and post construction sampling at five sampling sites to assess stormwater effects. The QAPP was established to assure QAQC and adherence to appropriate FDEP standard operating procedures throughout the duration of the project. Sampled collected by Johnson Engineering are analyzed at the Charlotte Country East Port Laboratory.

5. Summary of Findings

This Phase I study provided a review and synthesis of water quality monitoring data from multiple data sources in the Charlotte County study area. In addition, reconnaissance sampling was also performed to provide additional insight as to how STE impacts groundwaters and surface waters within the study area. The results documented the current status of non-point source nutrient and bacterial pollution from STE in the Charlotte County area, as well as seasonal trends in some water quality variables. The major findings of the study included the following:

- Surface water monitoring data (2015-2016) from 21 sites showed that the average total nitrogen concentrations were similar to the NNC (1.02 mg/L) for the Tidal Peace River, indicating a need for reducing nitrogen loading.

- Surface water monitoring data (2012-2016) from 21 sites indicated a trend of increasing annual average and maximum fecal coliform concentrations.

- Groundwater monitoring data (2012-2016) from 68 wells in the Spring Lakes area indicated significant contamination by STE, evidenced by high maximum concentrations of TN (up to 54.3 mg/L), TP (105.0 mg/L), nitrate + nitrite (58.16 mg/L), ammonia (30.5 mg/L), BOD (30.23 mg/L), and fecal coliforms (2,940 cfu/100 mL); these concentrations are typical of poorly diluted plumes from STE. The use of septic systems leads to an increase in the overall mean concentrations of these contaminants in groundwaters and surface waters.

- Analysis of depth to the seasonal high water for the 68 groundwater monitoring wells showed a distinct seasonal pattern with a reduced separation between the ground surface and groundwater tables in the wet season. Many of the values were less than the minimum state standard (Chapter 62E-6 FAC) of 2 ft. separation from the seasonal high water table during the wet season. Considering that 2 ft. of elevation above seasonal high water tables is required for proper drainfield placement according to state requirements, it is likely the vast majority (~71%) of septic systems in the study area do not meet the minimum state standard.

- Reconnaissance sampling of canal discharges during summer of 2016 showed that mean TN concentrations exceeded the NNC at all four sites (El Jobean, Ackerman, Spring Lake, Yacht Club), chlorophyll *a* exceeded the NNC at Yacht Club, and fecal coliform and *Enterococcus* exceeded state criteria at El Jobean and Ackerman. Mean concentrations of ammonia, nitrate + nitrite, and SRP were above nutrient thresholds noted for macroalgal blooms in subtropical waters. Observed BOD values were indicative of a highly impacted system.

- Reconnaissance sampling using stable nitrogen isotopes and the artificial sweetener, sucralose, demonstrated a coupling between STE, groundwaters, and surface waters in the study area. Stable nitrogen isotope values in macroalgae, hydroids, and oysters were enriched (~5-7 ‰) to values typical of STE polluted coastal waters. The low C:N ratios (~5) of *Gracilaria tikvahiae* indicate very high nitrogen loading within the study area.

6. Recommendations for Long-Term Monitoring

The East and West Spring Lake Wastewater Pilot Program showed great vision on behalf of CCUD for initiating water quality monitoring in 2011 and this thorough dataset was instrumental for this analysis. Moving forward, we recommend continued long-term monitoring at a subset of these initial stations. By using fewer stations, more detailed data can be obtained from selected stations. As Charlotte County moves through the septic to sewer conversion, these sites must be monitored to gauge the progress towards achieving levels lower than the NNC. With this in mind, our recommendations for efficient and effective continued monitoring are as follows:

Groundwater Monitoring

- The initial placement of groundwater wells in the Spring Lakes area provides a good background, but does not capture the septic plumes which were characterized by the strategically placed monitoring wells (66, 67, and 68) used in the reconnaissance sampling. We recommend installation of new monitoring wells around septic systems in three other neighborhoods (El Jobean, Ackerman, and Yacht Club), the addition of reference wells in natural, non-impacted areas, and continued monitoring at the least and most impacted historical sites. We recommend using a smaller subset of wells and adjacent surface water sites (see surface water monitoring recommendations). The establishment of reference sites in other neighborhoods that may be converted to centralized sewer later would also allow for establishment of baseline data.

- Monitoring seasonal STE discharge of nutrients and bacteria into adjacent surface waters should be implemented. For example, a “delayed discharge” of nutrients from the previous winter was observed in groundwaters and surface waters during the following summer (Lapointe et al., 1990). With an estimated increase in county population of approximately 35,000 seasonal residents increasing from summer to winter (CCUD, personal communication, 2016), one can assume significant increases in STE loading to groundwaters in winter compared to summer. A delayed discharge of groundwater nutrients could account for some of the observed increase in nutrient and bacterial concentrations in surface waters during the summer reconnaissance sampling in the Charlotte County area. Therefore, we recommend groundwater monitoring on a monthly schedule and possible use of dye tracer tests to determine seasonal variation in groundwater transport rates.

- Site specific studies using piezometers and groundwater level monitoring around drainfields to determine the potential for “mounding” as a result of STE discharge. These studies could also examine the gradient of STE discharge and the phenomenon of tidal pumping that has been recognized as an important mechanism enhancing downgradient transport of contaminants into coastal waters (Lapointe et al., 1990; Lapointe and Krupa, 1995).

- <u>Recommended parameters for groundwater monitoring</u>: fecal coliforms, *Enterococcus*, *E. coli*, nutrients (TN, TP, SRP, ammonia, and nitrate), BOD, depth to seasonal high water table, sucralose, acetaminophen, and aqueous nitrogen isotopes (ammonium and nitrate)

Surface Water Monitoring

- Continue the Phase I reconnaissance water sampling at canal discharge sites on ebbing tides and expand to include El Jobean, Ackerman, Spring Lakes, and Yacht Club. Within each sub-watershed, expand spatial coverage to include points within the canal systems. The canal sites should be placed close to monitoring wells, so that groundwater results are relevant to adjacent surface water. This stratified sampling allows for observation of STE dispersion into the receiving waters via tidal pumping (Lapointe et al., 1990; Lapointe and Krupa, 1995).

- Expand stable carbon and nitrogen isotope monitoring of algae and oysters at all sub-watersheds with a seasonal component to capture effects of variation in rainfall. These studies should include additional sampling at reference sites without STE influence that reflect natural, background nutrient conditions.

- $\delta^{15}N$ enrichment via nutrient pollution is consistent across taxa (Connolly et al., 2013) and fish have been recognized as an excellent indicator organism of these effects (Schlacher et al., 2005); therefore the addition of fish response using stable isotopes and other molecular biomarkers would enable for more of an ecosystem-based monitoring approach. Recommended species include sheepshead minnow (*Cyprinodon variegatus*), pinfish (*Lagodon rhomboids*), gobies (*Gobiosoma* sp.), or other common, resident fish. Model effects of environmental variation and water quality on current and historical resident fish community structure.

- <u>Recommended parameters for surface water monitoring</u>: fecal coliforms, *Enterococcus*, *E. coli*, chlorophyll *a*, nutrients, BOD, and stable isotopes (nitrogen and phosphorus) of algae primary consumers, and fish

Geospatial Monitoring

- Catalog septic systems in Charlotte County and assign individual drainfield distance from groundwater and soil attributes. Florida is particularity susceptible to groundwater contamination due to the soil types and high water table, therefore cataloging nutrient loading and human source tracers in groundwater and surface water from STE into a spatial database to create a monitoring platform and GIS data sets to be used for mapping and modeling of critical areas of concern is recommended to monitor this issue.

- Model STE plumes and generate a heat map of affected areas. Develop maps overlaying relevant parameters (water tables, soil type, elevation, nutrients, and bacterial values) to show critical areas that contribute to STE loading of Charlotte Harbor. This study would also benefit from incorporating scenarios of fluctuating seasonal high water tables to model potential increased inundation of septic system drainfields.

- <u>Recommended parameters for geospatial monitoring</u>: depth to seasonal high water table, septic tank concentration areas, and STE plumes

7. Acknowledgements

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Appendix 1. Historical Data

Appendix 1.1. Current and historical data parameters available for water bodies within Charlotte County, FL by source, showing: a) local sources and b) state and national sources.

a.

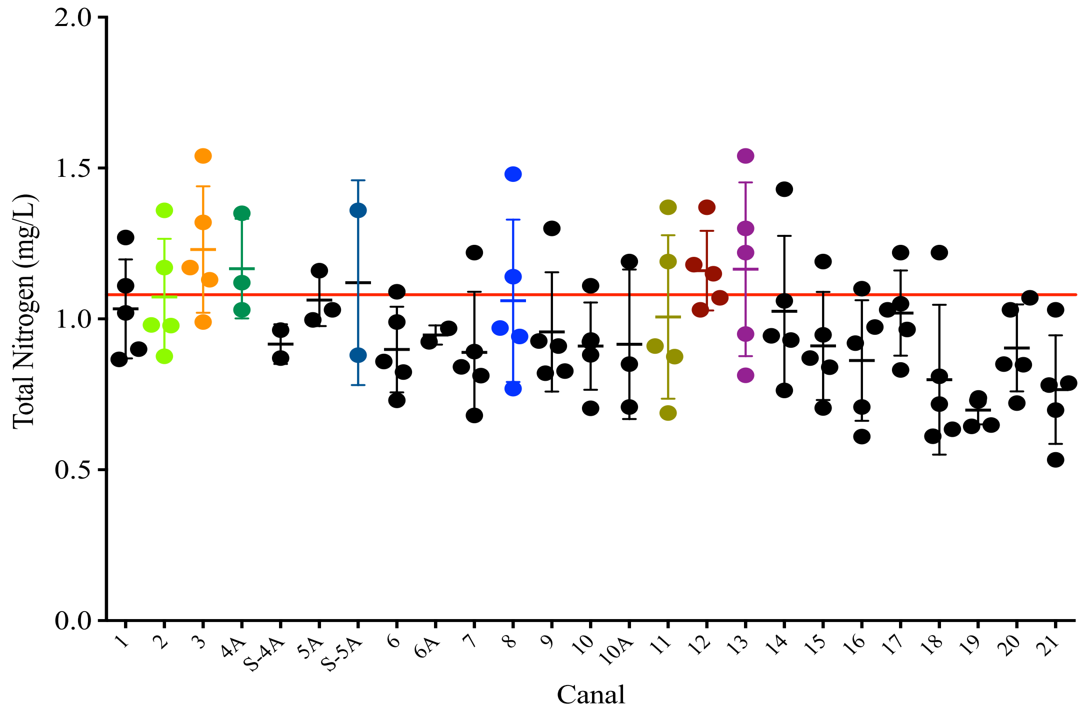
Parameter	Benchmark Laboratories	Charlotte County	Charlotte Harbor Aquatic Preserves	City of Punta Gorda Utilities	Peace River Manasota Regional Water Supply Authority	Sarasota County Environmental Services	North Shore
Date Range	2001 - 2010	2012 - 2016	1996 - 2016	1991 - 2007	1993 - 2013	2009 - 2016	2016
Number	116	84	6	7	218	4	12
Water Source	SW	SW, GW, Stormwater	SW	SW	SW	SW	SW, GW
Dissolved nutrients	X	X	X	X	X	X	X
Metal					X		
Other chemical							
Chloride / Fluoride					X		
Silica				X			
Temperature	X	X	X	X	X	X	X
Conductivity	X	X		X	X	X	
Secchi	X		X			X	
Color			X	X	X	X	
Cloud cover							
Hardness							
Alkalinity					X		
pH		X	X	X	X	X	X
Turbidity		X	X	X	X	X	
Level		X				X	X
Depth		X				X	X
Dissolved solids / TSS				X	X	X	X
Wind						X	
Total Organic Carbon					X		
DO		X			X	X	X
Salinity	X		X	X	X	X	
Chlorophyll *a*	X		X	X	X	X	
Pheophytin						X	
Fecal Coliform		X	X			X	X
Enterococcus		X					
Biochemical Oxygen Demand		X				X	X

b.

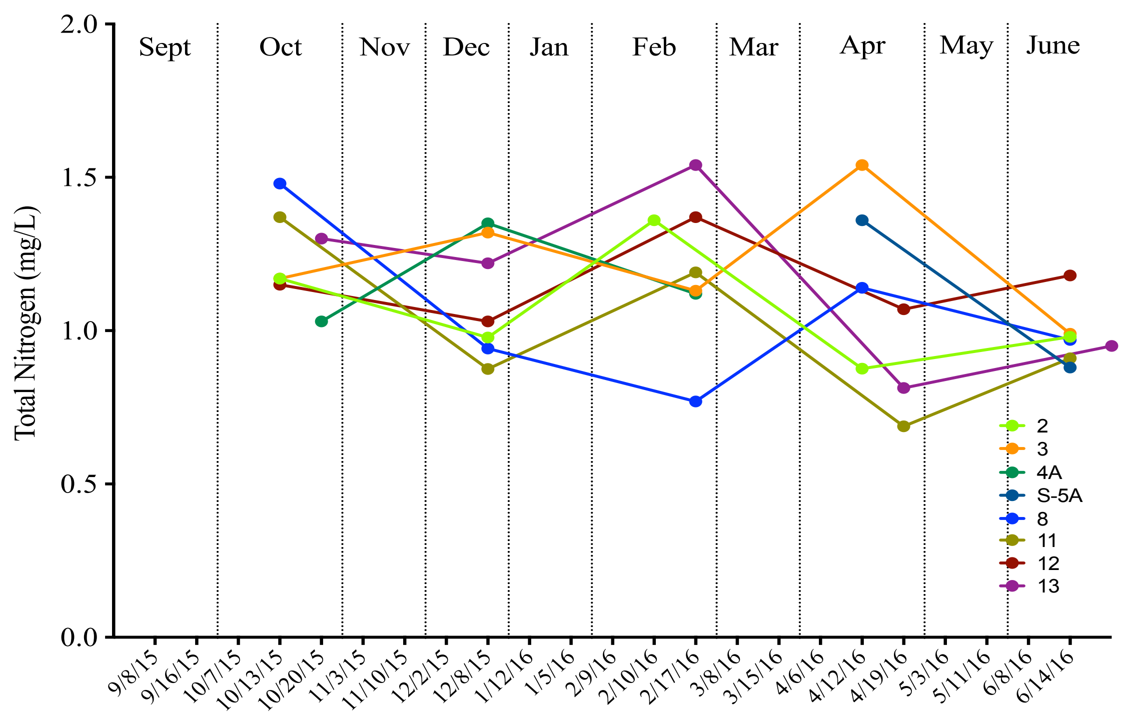
Parameter	Department of Environmental Protection	Department of Agriculture	Department of Health	Florida Fish and Wildlife Marine Research Institute	Florida LakeWatch	South Florida Water Management District	United States Geological Survey
Date Range	1974 - 2015	1979 - 2013	2000 - 2015	2000 - 2004	2005	1976 - 2016	1962 - 2015
Number	281	63	2	16	6	28	78
Water Source	SW, GW	SW	SW	SW	SW	GW, SW	SW, GW
Dissolved nutrients	X			X	X	X	X
Metal	X					X	X
Other chemical	X						X
Chloride / Fluoride	X					X	X
Silica				X			X
Temperature	X	X		X		X	X
Conductivity	X			X		X	X
Secchi				X	X		X
Color				X		X	X
Cloud cover							
Hardness	X						X
Alkalinity							X
pH	X	X		X		X	X
Turbidity	X	X		X		X	
Level	X					X	X
Depth	X				X	X	X
Dissolved solids / TSS	X					X	X
Wind		X					
Total Organic Carbon	X					X	X
DO	X	X		X		X	X
Salinity		X		X		X	
Chlorophyll *a*	X			X	X	X	X
Pheophytin	X						
Fecal Coliform	X	X	X			X	
Enterococcus			X				
Biochemical Oxygen Demand	X			X			X

Appendix 1.2. Total nitrogen (TN) in surface water in the East and West Spring Lake Wastewater Pilot Program area of Charlotte County, FL showing: a) TN by sample site and b) TN by sample site by date.

a.



b.

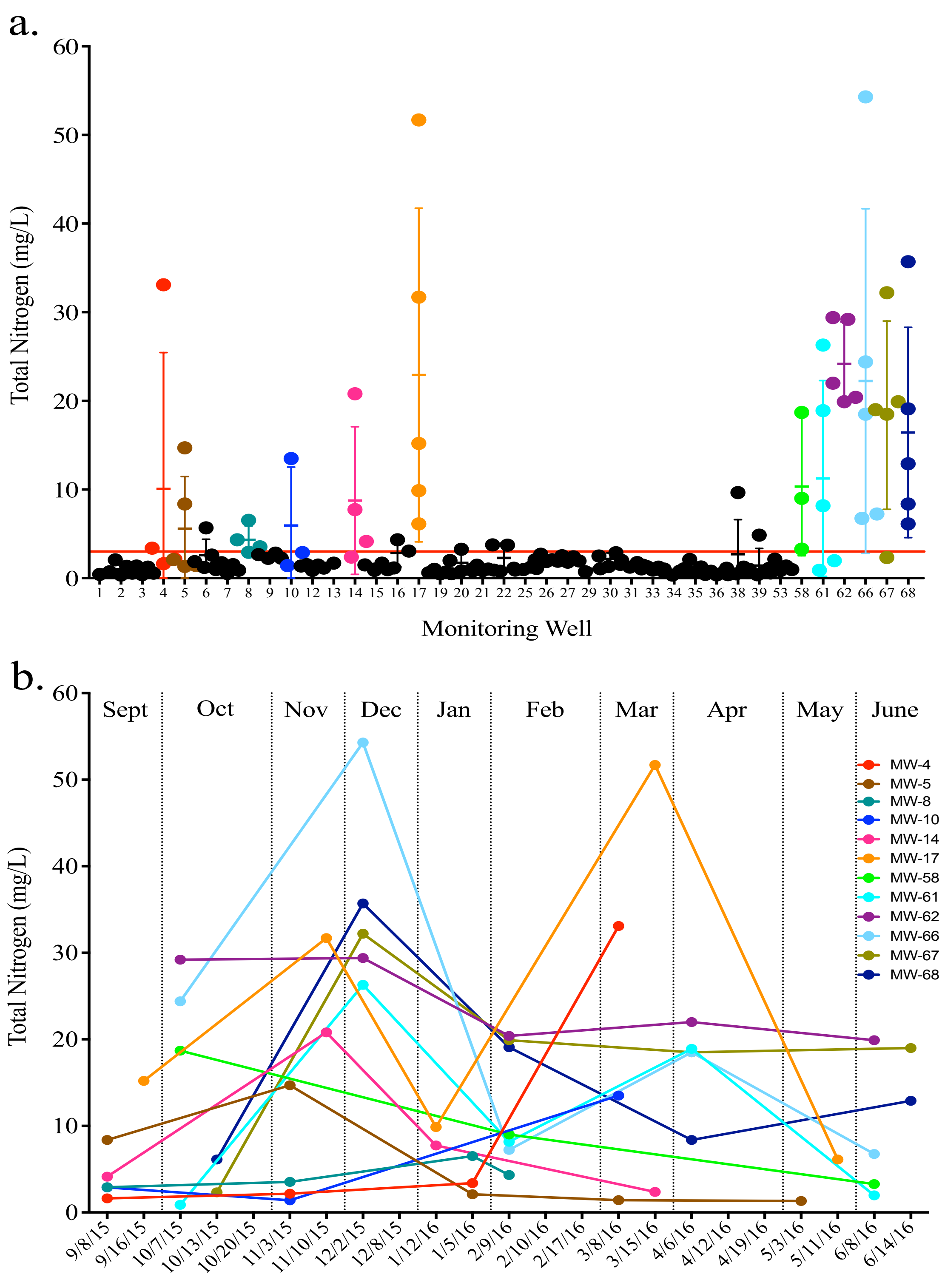


Appendix 1.3. Environmental parameters of surface water (canals) in the Spring Lakes area of Charlotte County, FL obtained from publicly available data.

Parameter	Year	2012	2013	2014	2015	2016
Fecal Coliform (cfu/100 mL)	Number	27	120	111	124	66
	Mean	36	60	46	58	397
	SD	25	182	107	87	1,234
	Max	90	1,400	1,050	620	8,600
	Min	10	1	10	10	10
Biological Oxygen Demand (mg/L)	Number	na	113	100	123	44
	Mean	na	3.0	5.9	5.0	5.4
	SD	na	4.0	10.2	5.6	3.6
	Max	na	20.0	60.9	22.6	11.7
	Min	na	0.5	0.5	0.1	0.5
Total Nitrogen (mg/L)	Number	na	na	na	44	66
	Mean	na	na	na	1.00	0.96
	SD	na	na	na	0.24	0.20
	Max	na	na	na	1.48	1.54
	Min	na	na	na	0.53	0.63
Nitrate + Nitrite (mg/L)	Number	27	120	111	124	66
	Mean	0.02	0.04	0.03	0.03	0.02
	SD	0.03	0.08	0.03	0.05	0.02
	Max	0.13	0.83	0.15	0.39	0.07
	Min	0.004	0.004	0.004	0.002	0.004
Ammonia (mg/L)	Number	na	na	na	44	66
	Mean	na	na	na	0.08	0.05
	SD	na	na	na	0.13	0.04
	Max	na	na	na	0.85	0.21
	Min	na	na	na	0.002	0.008
Nitrate (mg/L)	Number	na	126	105	na	na
	Mean	na	0.03	0.03	na	na
	SD	na	0.08	0.03	na	na
	Max	na	0.82	0.15	na	na
	Min	na	0.00	0.01	na	na
Nitrite (mg/L)	Number	na	125	105	124	na
	Mean	na	0.01	0.01	0.01	na
	SD	na	0.01	0.00	0.01	na
	Max	na	0.04	0.03	0.07	na
	Min	na	0.004	0.005	0.002	na
Total Kjeldahl Nitrogen (mg/L)	Number	na	na	na	44	66
	Mean	na	na	na	0.98	0.93
	SD	na	na	na	0.23	0.23
	Max	na	na	na	1.47	1.54
	Min	na	na	na	0.53	0.08
Total Phosphorus (mg/L)	Number	27	120	111	122	68
	Mean	0.19	0.19	0.17	0.32	0.16
	SD	0.14	0.15	0.11	0.17	0.09
	Max	0.40	0.66	0.53	0.90	0.41
	Min	0.01	0.01	0.01	0.02	0.02

Parameter	Year	2012	2013	2014	2015	2016
Temperature (°C)	Number	na	26	104	120	37
	Mean	na	23.9	25.4	25.9	22.6
	SD	na	4.0	4.3	4.3	1.4
	Max	na	29.2	32.2	33.0	25.7
	Min	na	17.6	14.0	16.2	19.5
Conductivity (µS/cm)	Number	na	26	104	120	37
	Mean	na	22779	16759	19688	17373
	SD	na	36442	13472	14622	15455
	Max	na	188300	37500	38300	81800
	Min	na	496	353	16	69
Dissolved Oxygen (mg/L)	Number	na	118	104	120	37
	Mean	na	4.4	8.0	4.4	5.3
	SD	na	1.5	35.1	1.9	1.0
	Max	na	8.3	362.0	11.2	7.6
	Min	na	0.7	0.8	1.1	3.3
Dissolved Oxygen (%)	Number	na	na	8	120	37
	Mean	na	na	68.6	59.2	69.1
	SD	na	na	15.8	24.7	12.6
	Max	na	na	91.5	153.8	92.8
	Min	na	na	50.5	14.7	43.2
pH	Number	na	26	104	120	37
	Mean	na	7.4	7.5	7.5	7.5
	SD	na	0.3	0.3	0.4	0.3
	Max	na	8.1	8.0	8.4	8.2
	Min	na	7.1	6.2	6.6	6.6
Turbidity (NTUs)	Number	na	26	102	120	37
	Mean	na	5.1	4.3	6.6	8.6
	SD	na	4.9	4.3	7.0	14.1
	Max	na	23.2	34.4	68.2	78.1
	Min	na	1.3	0.7	1.3	1.3

Appendix 1.4. Total nitrogen (TN) measured from groundwater monitoring wells in the East and West Spring Lake Wastewater Pilot Program area of Charlotte County, FL showing: a) TN by monitoring well and b) TN by date for each monitoring well.



Appendix 1.5. Environmental parameters of groundwater from the Spring Lakes area of Charlotte County, FL obtained from publicly available sources.

Groundwater	Year	2012	2013	2014	2015	2016
Fecal Coliform (cfu/100 mL)	Number	98	288	271	233	122
	Mean	84	12	22	15	11
	SD	400	17	87	38	7
	Max	2,940	270	1,060	520	60
	Min	10	3	10	1	10
Biological Oxygen Demand (mg/L)	Number	na	278	266	248	87
	Mean	na	1.6	15.7	5.2	2.1
	SD	na	2.4	117.8	10.3	3.3
	Max	na	19.6	1548	63	11.1
	Min	na	0.50	0.50	0.00	0.50
Total Nitrogen (mg/L)	Number	na	na	na	88	122
	Mean	na	na	na	5.23	4.29
	SD	na	na	na	9.8	7.3
	Max	na	na	na	54.3	51.7
	Min	na	na	na	0.35	0.23
Nitrate + Nitrite (mg/L)	Number	98	288	272	233	122
	Mean	0.39	1.16	2.20	1.52	2.56
	SD	2.0	4.4	6.7	6.3	6.6
	Max	19.4	39.2	58.2	57.8	49.3
	Min	0.004	0.004	0.007	0.004	0
Ammonia (mg/L)	Number	na	na	na	87	122
	Mean	na	na	na	2.3	0.6
	SD	na	na	na	6.6	1.5
	Max	na	na	na	30.5	8.0
	Min	na	na	na	0.008	0.008
Nitrite (mg/L)	Number	na	287	272	232	na
	Mean	na	0.026	0.217	0.028	na
	SD	na	0.06	2.16	0.12	na
	Max	na	0.5	30.1	1.5	na
	Min	na	0.003	0.005	0.003	na
Nitrate (mg/L)	Number	na	288	272	na	na
	Mean	na	1.1	2.0	na	na
	SD	na	4.4	6.3	na	na
	Max	na	39.0	57.4	na	na
	Min	na	0.004	0.007	na	na
Total Kjeldahl Nitrogen (mg/L)	Number	na	na	na	88	122
	Mean	na	na	na	3.9	1.8
	SD	na	na	na	7.8	1.8
	Max	na	na	na	35.3	9.9
	Min	na	na	na	0.35	0.23
Total Phosphorus (mg/L)	Number	98	288	272	233	122
	Mean	1.2	2.7	2.3	2.6	1.2
	SD	1.8	6.6	4.8	8.0	1.6
	Max	13.5	76.2	41.9	105	10.5
	Min	0.02	0.08	0.04	0.01	0.008

Groundwater	Year	2013	2014	2015	2016
Temperature (°C)	Number	287	272	238	65
	Mean	26.5	26.2	26.4	23
	SD	2.3	2.6	2.6	1.4
	Max	32.2	30.2	30.1	27
	Min	20.4	20.7	20.4	19
Conductivity (µS/cm)	Number	286	272	238	65
	Mean	720	708	712	632
	SD	696	757	834	505
	Max	6390	6410	7840	3030
	Min	37	5	4	27
Dissolved Oxygen (mg/L)	Number	287	272	65	na
	Mean	1.87	1.14	1.37	na
	SD	13.0	1.2	1.0	na
	Max	220.0	6.9	4.8	na
	Min	0.02	0.11	0.29	na
pH	Number	287	272	238	65
	Mean	7.0	6.8	6.7	7.1
	SD	3.3	0.3	0.3	0.3
	Max	62.0	7.6	7.5	7.8
	Min	5.9	6.1	5.5	6.5
Turbidity (NTUs)	Number	285	246	212	64
	Mean	21.7	17.9	12.9	10
	SD	34.7	34.9	16.8	12.9
	Max	299.0	400.0	94.1	70
	Min	0.4	0.4	0.6	0.8
Distance from Ground to Water Level (ft)	Number	325	276	270	76
	Mean	3.2	3.0	3.2	3
	SD	1.6	1.5	1.6	2
	Max	8.4	7.8	8.3	8
	Min	-0.02	-0.10	-0.06	0.00

Appendix 1.6. Historical values for environmental parameters measured from stormwater in the Spring Lakes area in Charlotte County, FL obtained from publicly available sources.

Stormwater	Sampling range	09-29-15 to 05-04-16	09-06-15 to 09-29-15
Total Phosphorus (mg/L)	Number	8	12
	Mean	0.663	1.195
	SD	0.255	0.626
	Max	1.200	2.220
	Min	0.363	0.545
Total Nitrogen (mg/L)	Number	8	12
	Mean	1.263	1.662
	SD	0.688	0.397
	Max	2.920	2.150
	Min	0.747	0.883
Nitrite & Nitrate (mg/L)	Number	8	12
	Mean	0.16	0.12
	SD	0.13	0.10
	Max	0.45	0.39
	Min	0.05	0.03
Total Kjeldahl Nitrogen (mg/L)	Number	8	12
	Mean	1.10	1.55
	SD	0.57	0.37
	Max	2.47	2.11
	Min	0.70	0.79
Ammonia (mg/L)	Number	8	12
	Mean	0.11	0.20
	SD	0.11	0.13
	Max	0.33	0.44
	Min	0.01	0.06
Total Suspended Solids (mg/L)	Number	8	12
	Mean	9.3	38.3
	SD	12	103
	Max	34.4	362.0
	Min	0.4	0.8
Fecal Coliform (cfu/100 mL)	Number	6	12
	Mean	6343	11033
	SD	8491	15072
	Max	23000	48000
	Min	780	500
Biological Oxygen Demand (mg/L)	Number	8	12
	Mean	3.4	4.3
	SD	1.3	2.3
	Max	5.2	8.8
	Min	2.0	2.0

Stormwater	Sampling range	09-06-15 to 09-29-15	09-29-15 to 05-04-16
Temperature (°C)	Number	12	8
	Mean	24	15
	SD	9	7
	Max	32	21
	Min	7	21
Specific Conductivity (µs/cm)	Number	12	8
	Mean	0.4	0.9
	SD	0.2	1.5
	Max	0.9	4.6
	Min	0.1	0.0
pH	Number	6	7
	Mean	8.39	6.99
	SD	0.48	0.44
	Max	8.94	7.69
	Min	7.71	6.50
Dissolved Oxygen (%)	Number	12	8
	Mean	76.0	75.1
	SD	5.2	16.9
	Max	82.5	102.8
	Min	64.2	48.5
Turbidity (NTU)	Number	11	8
	Mean	9.0	10.7
	SD	16.7	12.2
	Max	58.6	35.9
	Min	1.7	1.7

Appendix 2. Reconnaissance Data

Appendix 2.1. Reconnaissance sampling locations for sites incorporated into the Phase I Charlotte County Water Quality Monitoring Project. Station types are divided up by the type of sample collected at the station where MA = macroalgae, SW = surface water, GW = groundwater, SG = seagrass, OY = oyster, HY = hydroid, and LPS = water collected from a low-pressure sewer lift station.

Station	Station Type	Latitude	Longitude
Yacht Club	MA, SW	26.95800	-82.09023
Yacht Club CC	SW	26.95910	-82.09054
Spring Lake	MA, SW	26.96922	-82.11701
Ackerman	BR, SW	26.95659	-82.12079
Ackerman CC	SW	26.96193	-82.13073
El Jobean	MA, SW, OY	26.96355	-82.20372
Hog Island	MA, SG	26.92060	-82.15636
MW-66	GW	26.98893	-82.12009
MW-67	GW	26.98835	-82.11244
MW-68	GW	26.98034	-82.11079
Ohara-Midway lift station (OMLS)	LPS	26.95899	-82.13138

Appendix 2.2. Field measured parameters for reconnaissance surface water sites within Charlotte County, FL.

Parameter	Site	Ackerman	Spring Lakes	El Jobean	Yacht Club
Temperature (°C)	Mean	30.9	30.6	30.1	30.6
	SD	1.9	2.2	1.9	2.1
	Max	34.2	34.4	33.2	34.8
	Min	27.9	27.2	27.0	28.4
	Number	10.0	10.0	10.0	9.0
Conductivity (µS/cm)	Mean	12416	12524	10729	16698
	SD	5104	4828	4743	4974
	Max	17700	18100	18000	25100
	Min	1090	1880	2990	9570
	Number	5104	4828	4743	4974
Dissolved Oxygen (mg/L)	Mean	7.0	3.8	3.4	3.8
	SD	9.8	0.5	0.6	0.7
	Max	34.8	4.6	4.2	4.7
	Min	3.2	3.2	2.7	2.6
	Number	11	11	11	10
Dissolved Oxygen (%)	Mean	53	53	43	56
	SD	17	18	15	22
	Max	65	71	55	75
	Min	44	40	33	35
	Number	11	11	11	9
Salinity	Mean	7.6	8.1	6.0	9.8
	SD	2.1	2.3	3.0	3.4
	Max	10.5	11.2	10.7	15.3
	Min	4.7	5.0	1.6	5.4
	Number	9	9	9	8
pH	Mean	7.4	7.4	7.5	7.3
	SD	0.5	0.7	0.6	0.6
	Max	8.0	8.2	8.1	8.0
	Min	6.5	6.2	6.3	6.4
	Number	10	10	10	9
Turbidity (NTUs)	Mean	3.4	2.8	3.3	3.0
	SD	0.8	0.5	0.8	0.6
	Max	4.6	3.7	5.1	3.9
	Min	2.0	2.1	2.4	2.1
	Number	10	10	10	9

Appendix 2.3. Nutrients and sucralose concentrations in groundwater in monitoring wells (MW) and the O'Hara-Midway lift station (OMSL) by date for reconnaissance sites within Charlotte County, FL; some parameters were not analyzed (na) or below detection limits (BDL). Also, showing aqueous stable isotope values for 06/24/2016.

Date	Site	δ15N - NH4 (o/oo)	δ15N - NO3 (o/oo)	Ammonium (mg/L)	Nitrate+ Nitrite (mg/L)	SRP (mg/L)	TDN (mg/L)	TDP (mg/L)	TDN:TDP	Sucralose (µg/L)
6/24/16	MW-66	BDL	11.53	0.10	0.18	2.81	1.61	4.26	0.38	na
	MW-67	20.45	15.30	1.46	13.26	1.98	15.36	4.96	3.10	na
	MW-68	15.14	10.15	3.79	5.87	2.47	11.25	4.97	2.32	na
7/25/16	MW-66	na	na	0.03	5.14	1.43	6.72	3.27	2.05	7.21
	MW-67	na	na	0.32	4.01	2.28	5.36	4.58	1.17	5.05
	MW-68	na	na	9.15	3.45	2.22	15.57	4.13	3.77	14.74
	OMLS	na	na	50.34	0.01	5.33	53.86	6.56	8.21	67.01

Appendix 2.4. Stable nitrogen isotope values and nitrogen to phosphorus (N:P) ratios from macroalgae collected at different sites located in Lee County, FL during August and September 2005.