2015 MARTIN COUNTY
WATERSHED TO REEF SEPTIC STUDY

FINAL REPORT

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Executive Summary

The St. Lucie Estuary (SLE) is a heavily impacted system, receiving freshwater inputs from an artificially large watershed associated with a network of canals constructed in the 1900s to alleviate flooding and increase development potential. The health of the SLE and the underlying causes of its impairments have been a controversial topic for decades. SLE water quality is affected by inputs from the watershed (ground and surface waters, including multiple tidal creeks) and the primary canals (C-44, C-23, and C-24). Unlike the C-23 and C-24, which delivers water only from the SLE watershed, inputs from the C-44 originate in both the watershed and Lake Okeechobee. While the authors have previously documented deleterious affects of prolonged, high-volume releases to the SLE, their results simultaneously highlight findings of local septic tank contributions. From a management perspective, it is important to distinguish between direct watershed impacts versus those originating from Lake Okeechobee.

To address this management concern, a one-year watershed study was designed to investigate the interactions between on-site sewage treatment and disposal systems (OSTDS), groundwater, and surface water in the SLE and nearshore reefs. The study entailed: 1) well installation and groundwater sampling in two residential areas (Old Palm City, Golden Gate Estates) identified by Martin County as high priority septic to sewer conversion sites and undeveloped reference sites for dissolved nutrients, aqueous isotopes, sucralose and acetaminophen, 2) surface water sampling throughout the SLE (including upstream of the water control structures in the C-44 and C-23 canals) and nearshore reefs for dissolved nutrients, aqueous nitrogen isotopes, sucralose and acetaminophen, and 3) analysis of macroalgae and phytoplankton for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and elemental composition (C:N:P).

Results suggest significant contributions of nitrogen (N) and phosphorus (P) from the SLE watershed, primarily from OSTDS. Groundwater N and P concentrations were significantly higher in the residential communities than the reference sites. Groundwater nitrate, an indicator of sewage contamination, was also significantly higher at the residential sites and identified through aqueous stable nitrogen isotope analysis to have a wastewater N source. The presence of the artificial sweetener, sucralose, solely in the residential wells further supported the notion of wastewater contamination in the residential areas.

In the surface water, the highest nutrient concentrations were primarily documented in the residential networks (Old Palm City and/or Golden Gates Estates) and, to a lesser extent, the primary canals. In the Golden Gates Estates network, dissolved nutrients exemplified a dilution curve between the residential sites (most concentrated) and the SLE (least concentrated). As with the groundwater, there were multiple lines of evidence of septic tank contamination in the surface waters of the SLE and its watershed (see section 5.0 Conclusions). Stable nitrogen isotopic ratios, which are used to identify N sources, indicate a predominantly wastewater source in the surface water (especially the Golden Gates Estates retention pond and All American Ditch), macroalgae, and phytoplankton collected during the study. The presence of sucralose provided additional evidence that, in addition to groundwater, wastewater was reaching surface waters of the SLE. The wastewater signal was seen throughout the SLE and extended downstream to the nearshore reefs north and south of St. Lucie Inlet.

Table of Contents

1.0 Introduction

Coastal and estuarine ecosystems are highly productive, providing invaluable ecological services to human populations. Despite their importance, coastal ecosystems are being degraded on a global scale as a result of expanding human activities, ultimately lowering their ability to sustain future societal needs. Humans have greatly increased the concentrations of nitrogen (N) and phosphorus (P) in freshwaters flowing into the coastal zone (Nixon, 1995; Vitousek *et al*., 1997; MEA, 2005), exacerbating eutrophication and habitat loss (NRC, 2000; Bricker *et al*., 2007). As a result, nutrient enrichment is now a major agent of global change in coastal waters, linking an array of problems along coastlines, including eutrophication, biodiversity loss, harmful algal blooms (HABs), ‘‘dead zones,’’ emerging marine diseases, fish kills, and loss of seagrass and coral reef ecosystems (NCR, 2000; Howarth and Marino, 2006; Rockström *et al*., 2009).

In Florida, studies in the Florida Keys documented how on-site sewage disposal systems (OSTDS; septic tanks and shallow injection wells) enrich shallow groundwaters with dissolved nutrients, coliform bacteria and viruses that are transported into nearshore surface waters via submarine groundwater discharge (SGD; Griffin *et al*., 1999; Lapointe *et al*., 1990; Paul *et al.*, 1995a,b). Additional studies within canals and tributaries along the 251 km Indian River Lagoon (IRL) showed similar results. In Jupiter (Lapointe and Krupa, 1995a) and Tequesta (Lapointe and Krupa, 1995b), located at the southern end of the IRL, dissolved nutrients and two bacterial indicators of sewage pollution—fecal coliform and total coliform—were also transported via SGD into downstream waters of the Loxahatchee River, the IRL, and likely nearshore reefs adjacent to Jupiter Inlet. Just north of the Loxahatchee River, the St. Lucie River (SLR) was also found to have high nutrient concentrations and fecal and total coliform counts in its tidal creeks adjacent to high-density residential areas relying on OSTDS for on-site sewage disposal (Lapointe *et al*., 2012). These results prompted recommendations for increased stormwater retention, minimization of freshwater releases from Lake Okeechobee, and enhanced treatment of both stormwater and sewage to mitigate future stormwater-driven water quality perturbations in this system. Further north, in the central IRL, simultaneous groundwater, surface water, and stable nitrogen isotope studies along major canals and the St. Sebastian River in Indian River County corroborated the results of these previous studies in showing strong evidence of sewage contamination in the study area (Tarnowski, 2014; Lapointe, *unpublished data*).

Located on Florida’s east central coast in St. Lucie and Martin Counties, the SLR is one of the largest IRL tributaries. The SLR and its core estuary, known as the St. Lucie Estuary (SLE), is classified as Class III waters suitable for recreation, propagation, and maintenance of a healthy, well-balanced population of fish and wildlife (62– 302.530 Florida Administrative Code). The upper SLE includes the lower-salinity North and South forks of the SLR, which converge to form the higher-salinity Middle Estuary. The Middle Estuary flows east through the Lower Estuary to the IRL where the majority flows through St. Lucie Inlet, and to a lesser extent (~9%), north in the IRL towards Fort Pierce Inlet (Smith, *in review*).

A heavily impacted system, the SLE receives freshwater inputs from an artificially large watershed as the result of a network of canals constructed in the early to mid 1900s to alleviate flooding and increase development potential (FDEP, 2009; SFWMD, FDEP, and FDACS, 2009). Hydrological alterations began in 1924, when the South Fork of the SLE was connected to Lake

Okeechobee via the C-44 canal (St. Lucie canal; Blake, 1980). Additional modifications, extending to the headwaters of the North Fork, were made during the early and mid 1900s to increase the receiving capacity of this system (Herren *et al*., 2011). The health of the SLE and the underlying causes of its impairments have been a controversial topic for decades. SLE water quality is affected by inputs from the watershed (ground and surface waters) and the primary canals (C-44, C-23, and C-24) and, in turn, the sediments that can sequester incoming N and P from these sources (Howes, 2008). Unlike the C-23 and C-24, which deliver water only from the SLE watershed, inputs from the C-44 originate in both the watershed (C-44 basin) and, periodically, Lake Okeechobee via freshwater releases up to 10,000 cfs aimed at controlling lake water levels (Doering, 1996, Lapointe *et al*., 2012). While Lapointe *et al*. (2012) previously documented the deleterious affects of prolonged, high-volume releases to the SLE, the 2005-2006 study simultaneously highlighted local septic tank contributions. Tidal creeks, such as Warner Creek in Martin County and Poppelton Creek in the city of Stuart, were found to be a significant sources of fecal coliform, total coliform, and Enterococcus bacteria; both showing high to low count gradients from upstream (residential areas) to downstream (SLE). From a management perspective, the ability to distinguish between water quality impacts from the groundwater and rainwater runoff emanating from the SLE watershed, including the local tidal creeks, versus impacts directly related to periodic discharges from Lake Okeechobee is key because the combination of sources interacts to exacerbate the chronically poor water quality conditions of the SLE. Within the local watershed, it is also important to understand where the nutrients are emanating from (e.g., atmosphere, fertilizers, wastewater) so that management efforts can be initiated to reduce nutrient inputs into the system (Budrazzaman *et al*, 2012). While water samples analyzed for dissolved nutrients allow one to ascertain the general degree of nutrient pollution, water samples analyzed for stable isotopes of N, both $\delta^{15}N - NH_4$ and $\delta^{15}N - NO_3$, allow researchers to identify sources of dissolved inorganic N. For example, earlier studies by Lapointe *et al*. (2015a) showed that stormwater runoff emanating from urban and agricultural lands within the SLE watershed had depleted mean $\delta^{15}N$ values within the accepted range for fertilizers (-2 to +2 $^{o}/_{oo}$).

In addition to aqueous isotopes of N, macroalgae and phytoplankton are effective indicator organisms commonly used to assess the relative importance of N sources and gauge the type and degree of N versus P limitation. Macroalgae are especially ideal ‘‘bio-observatories’’ for assessing nutrient availability as they are typically attached to the benthos and integrate nutrient availability over temporal scales of days to weeks (Lapointe, 1985). Documentation of stable nitrogen isotope ($\delta^{15}N$) ratios in macroalgal tissue has been widely used to discriminate among natural (upwelling, N-fixation) and anthropogenic (sewage, fertilizer) nutrient sources (Risk *et al*., 2008). Because natural N-fixation source values are close to 0 $^{o}/_{oo}$ (Heaton, 1986; France *et al.*, 1998), offshore upwelled nitrate is ~ 2.0 $^{o}/_{oo}$ (Knapp *et al*., 2008), atmospheric N typically ranges from -3 $^{o}/_{oo}$ to +1 $^{o}/_{oo}$ (Paerl and Fogel, 1994) and synthetic fertilizer N ranges from -2 $^{o}/_{oo}$ to +2 $^{o}/_{oo}$ (Bateman and Kelly, 2007), all these N sources are depleted relative to enriched values of +3 $^{o}/_{oo}$ to +19 $^{o}/_{oo}$ for human sewage (Heaton, 1986; Costanzo *et al*., 2001; Table 1). Accordingly, enriched $\delta^{15}N$ values > +3 $^{o}/_{oo}$ have been reported for macroalgae in a wide variety of sewage-polluted coastal waters (Costanzo *et al*., 2001), including the IRL (Lapointe *et al*., 2015b) and nearshore reefs off urban areas of east-central Florida (Barile, 2004), southeast Florida (Lapointe *et al.*, 2005), and southwest Florida (Lapointe and Bedford, 2007). In addition, measurement of C:N:P contents of macroalgae provides a measure of nutrient quantity and

stoichiometry that is useful in assessing the relative importance of N versus P-limitation (Atkinson and Smith, 1983; Lapointe *et al*., 1992). This is particularly appropriate for assessing OSTDS groundwater-borne sewage pollution that can deliver nutrient pollution at high N:P ratios as a result of selective adsorption of P onto soil particles (Bicki *et al*., 1984; Lapointe *et al*., 1990; Weiskel and Howes, 1992).

In dense residential communities relying primarily on OSTDS, high cumulative P inputs to groundwater can supersaturate the soil and reduce its ability to selectively absorb P (Bicki *et al*., 1984). When this occurs, groundwater and, ultimately, surface water P concentrations will be higher, thus lowering the N:P ratio. Low N:P ratios (< 44:1) have been documented to promote blooms of the potentially toxic cyanobacterium *Microcystis aeruginosa* (Parrish, 2014). In the past ten years, the SLE has experienced two toxic blooms of *M. aeruginosa*; one in 2005 (N:P = +9:1; Lapointe *et al*., 2012) and the other in 2013 (N:P = +32.7). In August 2013, the cyanobacterium also had highly enriched ($+8.6^{o}/_{oo}$) $\delta^{15}N$ values, pointing to wastewater N as a primary N source fueling the bloom. *Microcystis aeruginosa* toxicity is directly related to nitrate concentrations in the water column (Horst *et al.*, 2014a,b), providing further evidence of wastewater inputs during both blooms.

In addition to stable nitrogen isotopes and C:N:P, source tracking studies are increasingly using the artificial sweetener sucralose and pharmaceuticals, such as acetaminophen, to track sources in ground and surface waters to high density residential areas primarily relying on OSTDS. Sucralose has been used in food and beverage products the United States as such brand names as Splenda, Zerocal, Sukrana, SucraPlus Candys, Cukren, and Nevella since 1988. This non-caloric sweetener is an ideal tracer because it is not broken down by any treatment process (including the body) and is transported conservatively through wastewater treatment plants (WWTP) and OSTDS (Oppenheimer *et al*., 2011). Sucralose has been used with success since 2012 in local studies along the Loxahatchee River (Loxahatchee River District), Indian River County canals and St. Sebastian River (Tarnowski, 2014), and Martin County (Florida Department of Environmental Protection [FDEP]) to pinpoint areas of OSTDS leaching. Acetaminophen is a widely used over-the-counter pain medication and fever-reducer. Also known as paracetamol in some contexts, such as on prescription bottles of painkillers that incorporate this medicine, it is simply abbreviated as APAP, for acetyl-para-aminophenol.

To address the concern of potential watershed impacts on the quality of the SLE, a one-year watershed to reef study was designed to better understand the interactions between OSTDS, groundwater, and surface waters in the SLE and nearshore reefs. The study entailed: 1) well installation and groundwater sampling in two residential areas identified by Martin County as high priority septic to sewer conversion sites and undeveloped reference sites for dissolved nutrients, aqueous N isotopes, sucralose and acetaminophen, 2) surface water sampling throughout the SLE (including sites upstream of the water control structures in the C-44 and C-23 canals) and nearshore reefs for dissolved nutrients, aqueous isotopes, sucralose and acetaminophen, and 3) collection and analysis of macrophytes and phytoplankton for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis and elemental composition (C:N:P).

2.0 Materials and Methods

Four sampling events were executed during the ebbing tide in 2015. Two Dry season events took place on April 7, 8, 9, 10, 17, 20 (Dry 1) and May 11, 12, 13, 14 (Dry 2) and two Wet season collections were performed on August 5, 6, 7 (Wet 1) and September 21, 23, 24 (Wet 2). During each event, a total of 11 groundwater, 18 surface water, and 8 macroalgae sampling stations within primary canals, the SLE, and nearshore reefs were visited, each with unique parameters of interest (Fig. 1, Tables 1-2). Upstream to downstream sampling networks were created within and adjacent to two older communities previously identified by Martin County Utilities Department as high-priority sites for septic to sewer conversion. In Old Palm City, fixed ground and surface water sites were selected along All American Ditch (OPC1-3) and the culvert-connected tidal creek draining into the South Fork (OPC4-5; Fig. 2). In Golden Gates Estates, fixed ground and surface water sites were selected in and adjacent to the community retention pond (GG1-2) downstream to the confluence of Willoughby Creek and the Lower Estuary (GG5; Fig.3). Sites were also selected upstream of the water control structures on the C-44 and C-23 (C44W and C23W, respectively) and on the nearshore reefs north (BTR) and south (SLR-N and SLR-S) of St. Lucie Inlet. To provide multiple lines of evidence, several different analyses were conducted (Table 2). The analyses outlined in Table 2 were performed for each of the four sampling events by either the University of Georgia's Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL) or FDEP's Central Laboratory (Table 2).

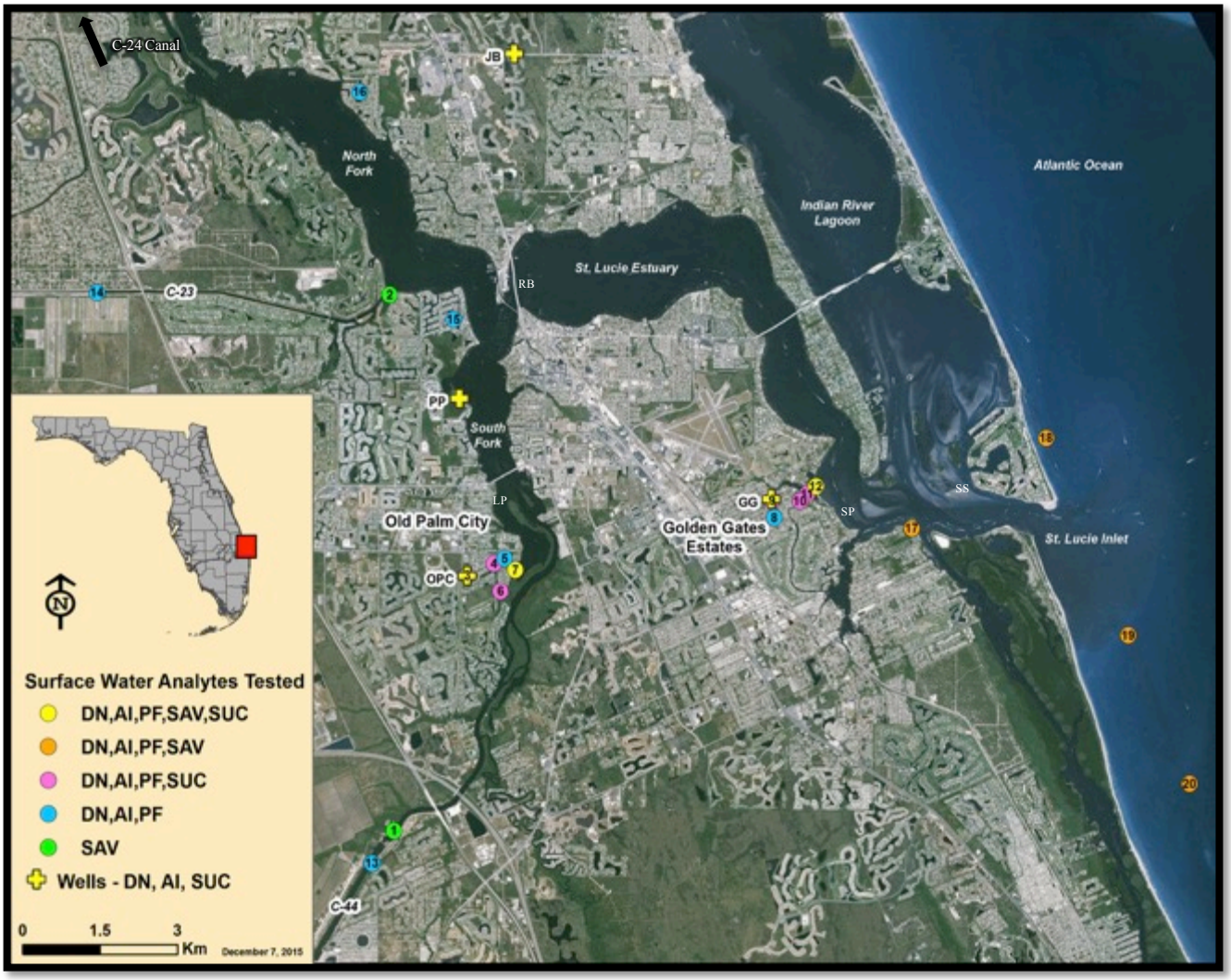

Fig. 1. Martin County Septic Project Site Map. Analytes measured at each site are color-coded as indicated by the key. DN = dissolved nutrients, AI = aqueous isotopes, PF = phytoplankton, SAV = submerged aquatic vegetation, SUC = sucralose and acetaminophen. Martin County Department of Health Enterococcus monitoring stations are identified as white letters; LP = Leighton Park, RB = Roosevelt Bridge, SP = Sandsprit Park, and SS = Stuart Sandbar.

Table 1. Martin County Septic Project site location information and measured analytes. Analyte codes: DN = dissolved nutrients, AI = aqueous isotopes, PF = phytoplankton, SAV = submerged aquatic vegetation, SUC = sucralose, and ACT = acetaminophen.

Site	Code	Latitude	Longitude	Analytes
1	C44E	27.11401	-80.28311	SAV
2	C23E	27.20477	-80.28341	SAV
3	OPC1	27.15715	-80.26855	DN,AI,PF,SUC
4	OPC2	27.15914	-80.26323	DN,AI,PF,SUC
5	OPC3	27.16000	-80.26116	DN,AI,PF
6	OPC4	27.15448	-80.26204	DN,AI,PF,SUC
7	OPC5	27.15803	-80.25907	DN,AI,PF,SAV,SUC
8	GG1	27.16661	-80.20834	DN,AI,PF
9	GG2	27.16958	-80.20876	DN,AI,PF,SUC
10	GG3	27.16941	-80.20329	DN,AI,PF,SUC
11	GG4	27.17062	-80.20183	DN,AI,PF,SUC
12	GG5	27.17188	-80.20013	DN,AI,PF,SAV,SUC
13	C44W	27.10853	-80.28744	DN,AI,PF
14	C23W	27.20544	-80.34067	DN,AI,PF
15	DP	27.20058	-80.27090	DN,AI,PF
16	BR	27.23919	-80.28900	DN,AI,PF
17	CR	27.16468	-80.18144	DN,AI,PF,SAV
18	BTR	27.17982	-80.15498	DN,AI,PF,SAV
19	SLR_N	27.14629	-80.13917	DN,AI,PF,SAV
20	SLR_S	27.12107	-80.12741	DN,AI,PF,SAV

Table 2. Parameters studied during the 2015 Martin County Septic Study. Pace = Pace Analytical, FAU = Florida Atlantic University, UGA= University of Georgia, FDEP = Florida Department of Environmental Protection Central Laboratory.

General Analysis	Media Collected (# Stations)	Analytes	Collector	Laboratory
dissolved nutrients	ground (11), surface (18) water	ammonium, nitrate, phosphate, total nitrogen, total phosphorus	Pace, FAU	UGA
stable aqueous isotopes	ground (11), surface (18) water	δ^{15}N-ammonium, δ^{15}N-nitrate	Pace, FAU	UGA
dissolved artificial substances	ground (11), surface (8) water	sucralose, acetaminophen	Pace, FAU	FDEP
stable tissue isotope	macroalgae (8), phytoplankton (18)	δ^{13}C, δ^{15}N	FAU	UGA
elemental tissue composition	macroalgae (8), phytoplankton (18)	%C, %N, %P	FAU	UGA

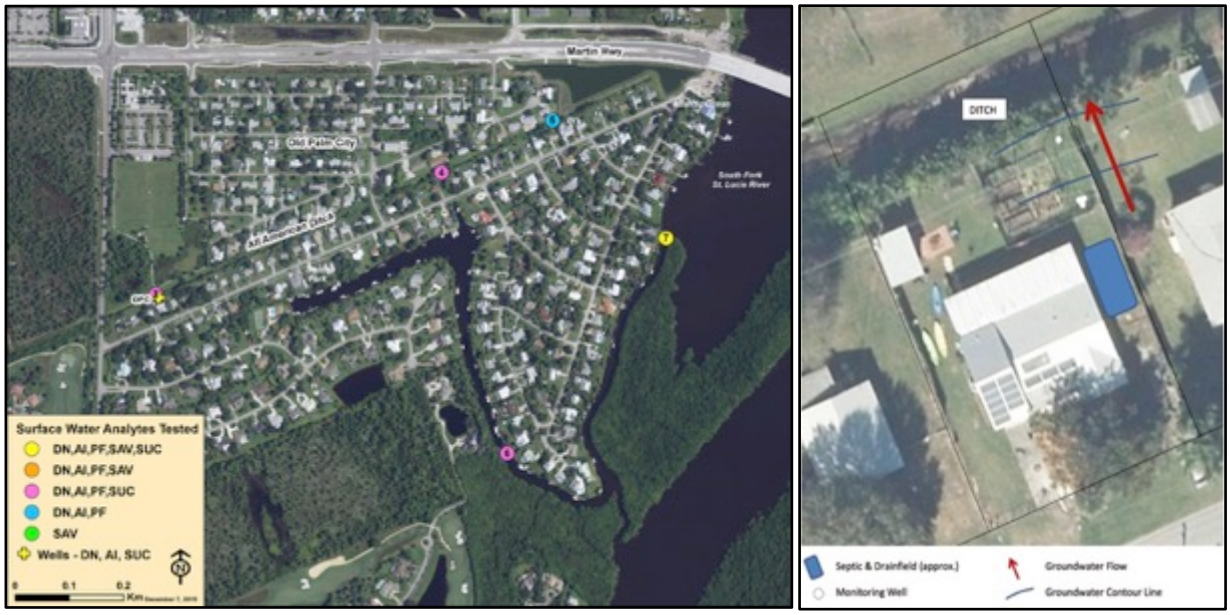

Fig. 2. Close-up of Old Palm City's All American Ditch ground and surface water sampling sites and direction of groundwater flow. Surface water analytes measured at each site are color-coded and are as indicated by the key. DN = dissolved nutrients, AI = aqueous isotopes, PF = phytoplankton, SAV = submerged aquatic vegetation, SUC = sucralose and acetaminophen.

Fig. 3. Close-up of Golden Gates Estate ground and surface water sampling sites and direction of groundwater flow. Surface water analytes measured at each site are color-coded and are as indicated by the key. DN = dissolved nutrients, AI = aqueous isotopes, PF = phytoplankton, SAV = submerged aquatic vegetation, SUC = sucralose and acetaminophen.

2.1 Freshwater Inputs and Enterococcus Bacteria Counts

Although not part of the direct sampling plan, freshwater inputs to the SLE system, including rainfall and discharges through the C-44, C-23, and C-24 canals, and bacterial counts at four sites within the study area were followed throughout the study.

2.1.1 Rainfall: Rainfall data were downloaded from the NOAA's National Centers for Environmental Information (http://www.ncdc.noaa.gov/data-access) for the duration of the project (January 1 – September 30, 2015). The station (GHCND:US1FLMT0018, STUART 1.0 ESE FL US) was centrally located (27.1883, -80.2279) within the study area; just north of the airport in Stuart, Florida. Daily total precipitation (mm) was plotted to better understand rainwater inputs prior to sampling events.

2.1.2 Canal Discharges: Three primary Central and South Florida canals discharge into the SLE; the C-44, C-23, and C-24. The C-44 drains both Lake Okeechobee and the watershed (C-44 basin) with pollutants from both mixing in the C-44 prior to release through the S-80 structure. The other two primary canals, the C-23 and C-24, exclusively drain the SLE watershed so all pollutants entering into the SLE through these systems emanate from the watershed. Discharge (flow) rates from the water control structures nearest to the SLE along the C-44, C-23, and C-24 canals were downloaded from South Florida Water Management District's online database, DBHYDRO. Flow data for the S-80 structure (Key: DJ238) at the confluence of the C-44 canal and the South Fork of the SLR were monitored by the U.S. Army Corps of Engineers, data for the S-48 structure (Key: JM106) on eastern end of the C-23 canal near the confluence of the north and south forks of the system were monitored by the South Florida Water Management District (SFWMD), and data for the S-49 structure (Key: JW223) along the east end of the C-24 canal that empties into the North Fork were also monitored by SFWMD. Data were obtained for January 1 – September 30, 2015 and, like rainfall, charted against the sampling event dates. The U.S. Army Corps of Engineers and South Florida Water Management District were informed of the study and requests were made by Martin County to stop releases during the sampling events.

2.1.3. Bacterial Health: Martin County Department of Health (DOH) provided *Enterococcus* bacteria count data collected between January 1 and September 30, 2015. Counts (number of colony-forming units [cfu] *Enterococcus*/100 mL river water) were reported for four DOH monitoring stations: 1) Roosevelt Bridge, 2) Sandsprit Park, 3) Leighton Park, and 4) the Stuart sandbar (near Sailfish Point and St. Lucie Inlet). Leighton Park is closest to the project's Old Palm City sampling network, Sandsprit Park is closest to the Golden Gates Estates sampling network, and the Stuart sandbar is closest to the crossroads with the IRL. The water quality scale based on count data set by Florida Department of Health includes good (0 to 35 cfu/100mL), moderate (36 to 104 cfu/100mL), and poor (>105 cfu/100mL).

2.2. Groundwater

To better understand the potential effects of OSTDS on surface waters in the SLE, nine wells were drilled at two residential sites; Old Palm City (OPC) and Golden Gates Estates (GG; Figs.

2-3). The OPC well cluster consisted of two shallow (3.7 m), one intermediate (7.4 m), and one deep (17.5 m) well behind a single-family residential home along the All American Ditch (Fig. 2). The GG well complex included three shallow (3.7 m), one intermediate (7.3 m), and one deep (17.4 m) well behind a duplex adjacent to the community retention pond (Fig. 3). Two existing reference or control wells, one managed by South Florida Water Management District (SFWMD) and the other by the Martin County Utilities Department, were also incorporated into the study to investigate anthropogenic effects of residential septic systems on water quality. The SFWMD-managed well (PCP-C; 9.0 m) was located at Pendarvis Park along the South Fork and the Martin County managed well (W4B; 14.9 m) was installed along Jensen Beach Boulevard adjacent to the Savannas Preserve State Park in Jensen Beach (Fig. 1; Appendix A).

2.2.1 Monitoring Well Siting

The well construction and installation work was conducted between February 17 and 24, 2015 by AMPS, Inc.. Prior to construction, the contractor applied for and received well construction permits for all monitor wells from the Martin County Health Department. Underground utility locates were conducted prior to construction by Sunshine Utility Locators. All wells were constructed in accordance with the Monitoring Well Design and Construction Guidance Manual (FDEP, 2008). An on-site geologist was present during drilling to oversee collection of field samples and data and to observe drilling operations. Daily drilling, lithologic and well logs were recorded for each of the wells. The South Florida Water Management District authorized access to the Pendarvis Park reference well in Palm City. The second reference well located in Jensen Beach is owned by Martin County (Fig. 1, Appendix A).

The well construction and testing program included drilling, testing and well installation at the two residential sites, Old Palm City and Golden Gates Estates, respectively (Figs. 2-3). These sites represent priority areas within the county for septic tank elimination based on previous county studies (LBFH, 2001; Keene, 2015). The locations of well clusters were selected based on the direction of groundwater movement, proximity to receiving waters and OSTDS, and a minimum of at least two full time residents at each property. This study was coordinated with and supports other like water quality programs in Martin County. Exact locations and total depths of the wells were determined in the field.

Generally, the program included installation of a three well cluster (one each shallow, intermediate and deep well) down gradient of the residential septic drainfield. A second shallow well was located half the distance between the installed well cluster and the receiving water body. A third shallow well was located nearest the water at the end of the property at the Golden Gates Estates site only based given a larger property size compared to the All American Ditch site **(**Figs. 2-3**)**. The study also involved collecting water samples for analysis from two existing reference or control wells. These wells were not located within the study areas and were not in close proximity to any OSTDS (Appendix A).

2.2.2 Monitoring Well Construction

The wells were constructed of schedule 40 2-inch PVC casing and screen at selected intervals below ground level (bgl) and all wells were constructed in separate boreholes. All drilled depths

for the shallow wells were aimed at well installation within a similar geologic layer. The wells were installed by either hollow stem auger (4.5-inch ID [6-inch OD] for shallow and intermediate wells) or mud rotary (3-inch ID [3-7/8-inch OD] drill bit for deep wells) drilling methods. Bentonite fluid was used in drilling the deep wells. Well casing, screen complete with bottom well point and centralizers (used on intermediate and deep well casings) were installed to intended depths and in some cases less than target depth based on flowing sands (Appendix B) All monitoring well and screen joints were threaded and connected with manufacturers "O" rings. The shallow and intermediate wells were installed with 2-inch ID pre-packed well screens (length 2 ft and 5 ft, respectively. Utilizing pre-packed [gravel pack sandwiched between an interior and exterior 0.02 inch slotted PVC screen] ensures that gravel pack surrounds the well screen. Additional 6/20 washed silica gravel pack was emplaced (poured) around each well to approximately 10 ft above the well screen. Deep wells were gravel packed from total depth to 10 ft above well screen (10 ft long). The gravel pack was installed by pumping the gravel pack down the tremie line to the screened interval. Gravel pack depths were confirmed with a tremie line. The remaining annulus was then grouted to land surface using neat cement. The wells were developed using compressed air via the airlift method after the grout was allowed to cure for a minimum of 12 hours. The wells were developed until the discharge water was free or as free of suspended sediment as reasonable given unconsolidated nature of shallow sediments.

The lithology underlying both residential sites was comprised of fine to medium grained sand with varying amounts of silt and shell topped with a thin veneer of organic soil at the surface. Lithologic samples were collected using the split spoon technique in two foot increments using the mud rotary drilling method from surface to total depth of approximately 60 ft below land surface (bls) in each deep well. These undisturbed geological samples were captured in new clear plastic tubes (24 in long x 1.5 in diameter) and then capped at both ends after recovery for geological description, sample identification and storage. Lithologic samples were also collected in the intermediate and shallow wells during augering operations at five-foot increments. These samples were logged and identified appropriately in standard sample bags. All samples were retained for reference.

Drill rig and components as well as construction materials were contained in a work area approximately 20 ft x 20 ft on site to minimize disturbance to property. All water used in drilling operations was from the county's potable water supply via a hydrant equipped with a temporary meter. A truck mounted polyurethane water tank was used to transport water to each site. All well materials were steamed cleaned before installation and all drill rig components were steam cleaned at each site to eliminate the potential for cross contamination. Workers wore latex gloves during the installation process. Appendix B includes photographs of well drilling, testing and completion operations.

All monitor wells were completed and capped with a vented PVC cap recessed below grade and enclosed in a protective box. Each well was sealed at the surface with roughly 15 in x 15 in x 4 in cement pad with a steel well cover secured by hex-head bolts and watertight seal. Appendix C describes well locations and elevations. Following well completion, water level data and field test samples were collected from each well. All wells were functioning as intended.

2.2.3 Groundwater Sampling

The 11 wells were sampled by Martin County Utilities Department through a subcontract with Pace Analytical. During each of the four sampling events, Pace Analytical collected groundwater in accordance with FDEP Standard Operating Procedure (SOP) FS 2200 Groundwater Sampling, revised March 1, 2014 (available at http://www.dep.state.fl.us/water/sas/sop/sops.htm). The SOP was designed to ensure that the collected samples are representative of water in the aquifer or target formation and that the samples are not altered or contaminated by the sampling and handling procedures.

Before sample collection began, water level measurements (to the nearest 3.05 mm) were collected using an electronic sounding probe to determine water table elevations and to calculate required well purging volumes. The "conventional purge" (Wells with Fully Submerged Screen and Pump or Intake Tubing Placed at the Top of the Water Column) method was used on all wells. Clean polyethylene tubing was connected to a variable-speed peristaltic pump, and the bottom of the tubing was placed just below the top of the water column. Purging rates were adjusted to minimize drawdown of each well. One well volume was removed before the first set of stabilization parameters was recorded. Thereafter, approximately $^1/_4$ of a well volume was purged between stabilization parameter readings.

Completion of well purging is indicated by the stabilization of the parameters within the following tolerances:

Temperature:	± 0.2° C
pH:	± 0.2 Standard Units
Specific Conductance:	± 5.0% of reading
Dissolved Oxygen:	≤20% Saturation
Turbidity:	≤20 NTU

Groundwater Sampling Logs, DEP Form FD 9000-24, were completed for each well. These forms include sampling event and well information, well volume calculations, field parameter data, observations of the field sampling staff, and sampling data.

Equipment used for field measurements was calibrated prior to the start of purging and sampling. Equipment primarily used in the field included Oakton 300 series pH & conductivity meters, YSI 550A DO meters, and HF Scientific turbidimeters.

After stabilization criteria were met, groundwater samples were collected using the following procedures.

2.2.3.1 Dissolved Nutrient and Aqueous N Isotopes: Samples for these analytes were collected from the same pump after a 0.45 µm high capacity cartridge filter (Jensen Inert Products disposable groundwater filter) was fitted to the discharge tube of the pump. A new cartridge filter was used for each well. From each well, three field-filtered 1 L replicates for both forms of N aqueous isotopes ($\delta^{15}N$ -NH_4 and $\delta^{15}N$ -NO_3) were collected in six high-density polyethylene (HDPE) bottles provided by the UGA-SIEL. All 66 samples (six 1 L bottles x 11 wells) were

placed in wet-ice filled insulated coolers to chill, and then maintained at temperatures below 4°C through shipping. The coolers, containing completed chain-of-custody forms, were sealed, and shipped via FedEx to the UGA-SIEL in Athens for analysis. Once received, the samples were thawed, homogenized, and ~100 mL of sample was removed from three of the 1 L bottles for dissolved nutrients analysis. The remaining water was analyzed for aqueous N isotopes.

For dissolved nutrients, the ~100 mL subsamples were divided for either persulfate digestion (TN/TP) or direct analyses (NO_x, NH_4, and SRP). To digest TN/TP, 5 mL of sample were digested with 1 mL persulfate reagent, autoclaved until all N was oxidized to nitrate and all P was oxidized to orthophosphate. Once digested, all nutrient forms (NH_4, NOx, SRP, TN, and TP) were analyzed on an Alpkem 300 series nutrient autoanalyzer using EPA standard methods (4500-NH_3 G, 4500-NO_3^- F, and 4500-P F).

To analyze aqueous N isotopes, UGA-SIEL ran the water samples 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 NH4, then proceeding with the standard diffusion and ammonia capture on an acidified filter. The filter was then analyzed as a typical solid sample on our Carlo Erba Isotope Ratio Mass Spectrometer (IRMS) for $\delta^{15}N$ -NH_4 and $\delta^{15}N$ -NO_3.

2.2.3.2 Sucralose and Acetaminophen: A single 1 L amber glass bottle provided by the FDEP Central Laboratory was filled directly from the peristaltic pump used to purge each of the 11 wells. In addition to these 11 unique samples, associated field blank and duplicate samples were collected during each of the four sampling events. All samples were placed in insulated coolers with wet ice to chill, and then maintained at temperatures below 4 degrees °C through shipping. The coolers, with accompanying chain of custody forms, were returned overnight via FedEx to the FDEP Central Laboratory in Tallahassee the same day the samples were collected. At the laboratory, water samples are first filtered with through a 0.75 μm glass fiber filter. A 250 mL aliquot of filtered sample is then passed through a graphitized carbon-based, solid-phase extraction (SPE) column. After extraction, the absorbed analytes are eluted from the SPE column with a mixture of 80% methylene chloride: 20% methanol. The extract is reduced to near dryness and brought to a final volume of 1 mL with 10% acetonitrile in deionized water. Analytical standards are prepared in the same final solvent mixture. Each extract is analyzed twice by high performance liquid chromatography/tandem mass spectrometry (LC/MSMS), once in the negative ion mode for the determination of sucralose and once in the positive ion mode for acetaminophen. The sample method detection limits are 0.01 μg/L for sucralose and 0.002 μg/L for acetaminophen. This analytical procedure is based on US Geological Society (USGS) method O-2060-01 (Furlong *et al*., 2001), and its details are described in FDEP standard operating procedure LC-001/LC011[2] located on FDEP's website (http://www.dep.state.fl.us/labs/library/lab_sops.htm).

2.3 Surface Water and Tissue

Surface water, tissue, and sediment samples were collected by Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) and, with the exception of the sucralose/acetaminophen samples, processed the same day according to the parameter of interest at the HBOI-FAU Harmful Algal Bloom Laboratory.

2.3.1. Dissolved Nutrients: Surface water samples were collected in triplicate just below the surface into acid-washed 250 mL high-density polyethylene (HDPE) bottles and covered with ice in a dark cooler until return to the laboratory for processing. The samples were filtered (0.7 mm GF/F filters) and frozen until analysis at the University of UGA-SIEL, Athens, GA. At UGA-SIEL, samples were thawed, homogenized, and subsampled for either persulfate digestion (TN/TP) or direct analyses (NOx, NH4, and SRP) as mentioned for groundwater in 2.2.31. The resulting data were used to characterize ambient dissolved inorganic and total N and P concentrations, DIN:SRP ratios, and TDN:TDP ratios at the 18 surface water collection sites. Calibrated YSI Model 1030 and ProODO hand-held meters were used to document salinity, temperature, dissolved oxygen (DO), and pH at the time each water sample was collected.

2.3.2. Aqueous N Isotopes: Water samples collected in triplicate just below the surface were pre-filtered through 200 µm nylon netting into acid-washed 1 L high-density polyethylene (HDPE) bottles and covered with ice in a dark cooler until return to the laboratory for processing. Pre-filtering removes macrodetritus and macrozooplankton from the samples (Savoye *et al*. 2003). In the laboratory, the pre-filtered samples were filtered again through 0.7 mm GF/F filters. The water was frozen until analyzed at UGA-CAIS for dissolved N isotopes. At UGA-SIEL, the water samples were analyzed for $\delta^{15}N$ -NH_4 and $\delta^{15}N$ -NO_3 using the same processes mentioned above for groundwater in 2.2.31. The GF/F filter was saved and used for phytoplankton isotope and elemental composition analysis (see below in 2.3.5).

2.3.3. Sucralose and Acetaminophen: Unique surface water samples were collected at four sites in OPC neighborhood and fours sites in GG neighborhood. Each of the samples were collected in 1 L amber bottles and kept on ice until overnight shipment to FDEP's Central Laboratory in Tallahassee, Florida. Processing of the surface water samples was identical to the groundwater samples described above in section 2.2.3.2.

2.3.4. Macroalgal Tissue $\delta^{13}C$, $\delta^{15}N$ and C:N:P: Triplicate samples of macroalgae were collected at eight submerged aquatic vegetation (SAV) sampling stations. Because of the inconsistent growth of macroalgae in some sections of the SLE, especially in the upper reaches, cages were deployed to hold replicates of the red alga, *Gracilaria tikviheae*, provided by the Marine Botany Laboratory at HBOI. Once the algae was either collected from the natural environment or the deployed cages, the samples were cleaned of epiphytes and debris, rinsed briefly (< 5 s) in deionized water to remove excess salt, and dried in a Fisher Scientific Isotemp® oven at 65°C for 48 h. The dried macroalgae was ground to a fine powder using a Thompson Scientific Wiley Mini-Mill®, and stored in plastic screw- top vials. Sub-samples were shipped to the UGA-SIEL for stable C and N isotope analysis and tissue %C, %N, and %P. At UGA-SIEL, samples were split into two. One half was analyzed for stable C and N isotopes and %C and %N on a Thermo Delta V Isotope Ratio Mass Spectrometer (IRMS) coupled to a Carlo Erba NA1500

CHN-Combustion Analyzer via a Thermo Conflo III Interface. The other half was analyzed for %P, where approximately 2 mg of dried tissue was weighed in crucibles, ashed at 500°C for four hours, and extracted with 0.2 mL of Aqua Regia acid. The acid extracts were then diluted 41:1 with pure water for total P (as PO_4-P) analysis by Alpkem 300 series. The resulting stable C and N isotope and C:N:P data of the macroalgae were used to characterize temporal and spatial variation in tissue nutrient status, to determine inferences regarding nutrient availability in relation to various natural and anthropogenic N sources (Atkinson and Smith, 1983; Lapointe, 1987; Costanzo *et al*., 2001; Lapointe *et al*., 2015).

2.3.5. Phytoplankton Tissue $\delta^{13}C$, $\delta^{15}N$ and C:N:P: One liter bottles of surface water collected at 18 fixed sites were pre-filtered in triplicate through 200 μm nylon netting in the field to remove macrodetritus and macrozooplankton from the samples (Savoye *et al*., 2003). In the HAB laboratory, the pre-filtered samples were filtered again through 47mm GF/F filters to capture the phytoplankton and sent to the UGA-SIEL for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes and C:N:P analysis (note: the water was use for aqueous isotope analysis above). At UGA-SIEL, the "phytoplankton filters" were frozen, freeze-dried, and split into two equal halves. One half was analyzed for stable C and N isotopes and total C and N using the same equipment mentioned above for macroalgal tissue. The other half was ashed for Aqua Regia extraction and diluted for TP analysis; the same process outlined above for macroalgae total P. The resulting stable isotope and C:N:P data are used to identify nutrient sources and characterize temporal and spatial variation in phytoplankton nutrient status, which allow for inferences regarding nutrient availability in relation to various natural and anthropogenic nutrient sources and climate-related phenomena. Results were compared to the Redfield ratio of 106:16:1 (Redfield, 1958) where C:N ratios > 6.6 indicate increasing N-limitation, C:P ratios > 106 indicate P-limitation, and N:P ratios > 16 indicate P-limitation.

3.0 Results

3.1 Freshwater Inputs and Enterococcus Bacteria Counts

3.1.1 Rainfall: All four sampling events were conducted on clear days with no precipitation. While there was little rainfall days prior to the two Dry season events, a significant and persistent amount of rain fell the weeks prior to the second Dry season sampling in May and both Wet season sampling events (Fig. 4). The average daily rainfall between January 1 and September 30, 2015 was 28.2 ± 5.0 mm, with a range between 0 and 665 mm falling on March 1. During the Dry season (January 1 to May 31), rainfall ranged between 0 and 665 mm and averaged 26.2 ± 6.9 mm. The Wet season (typically June 1 to October 30) had an average rainfall of 31.3 ± 6.9 mm and daily precipitation ranged from 0 to 371 mm falling on August 31.

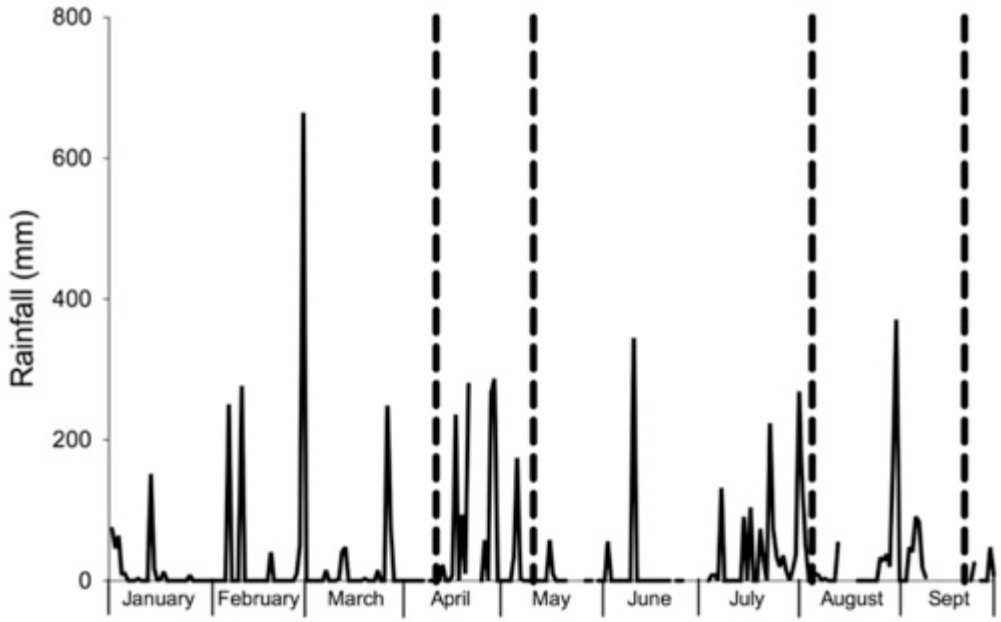

Fig. 4. Daily precipitation (mm) at a centrally located weather station north of the Stuart airport. Dashed bars represent sampling events.

3.1.2 Canal Discharges: Discharges from the S-80 structure along the C-44 ranged from 0 to 3,497 cfs on September 18 with a mean (± S.E.) of 312.5 ± 27.4. Between January 1 and September 30, 2015 water flowed from the S-80 water control structure to the South Fork of the SLR 50.6% of the time. During the Dry season, the mean flow rate increased to 394.0 ± 33.2 cfs, with rates ranging from 0 to 1,284 cfs (March 15). During the Dry season (January 1 to May 31, 2015), water from the C-44 canal flowed over the S-80 structure 65.6% of the time. During the Wet Season, the mean flow rate decreased to 209.7 ± 44.0, with rates ranging from 0 to 3,497 cfs (September 18, 2015). Water was released at the S-80 structure 31.9% of the Wet season (June 1 to September 30, 2015). No water was being released from the S-80 structure during the Dry 1 (April 2015) and Wet 1 (August 2015) sampling events. Little to no water was released for one month prior to the Dry 2 sampling event in May 2015, but the first day of sampling the water managers started releasing at a rate of 650 to 1250 cfs that lasted for the entire duration of the Dry 2 collection period. The Wet 2 (September 2015) samples were collected during releases ranging from 450 to 850 cfs, with releases ranging from 1,500 to 3,500 cfs the week prior to sample collection (Fig. 5a).

At the S-48 structure along the C-23 the flow rate ranged from 0 to 2,667 cfs on September 18, with a mean of 472.7 ± 14.3 cfs between January 1 and September 30, 2015. Water was released from the S-48 to the confluence of the North and South Forks of the SLR 57.9% of this time. During the Dry season, the mean flow rate was 27.5 ± 5.9 cfs with rates ranging from 0 to 491 cfs (April 28). Water was released from the S-48 structure 53.0% of the Dry season. During the Wet season, the mean flow rate increased to 136.4 ± 30.2 cfs, with rates ranging from 0 to 2,667

cfs (September 18, 2015). Water was released at the S-48 structure 63.9% of the Wet season. There were no releases from the S-48 structure during the first Dry sampling event (April 2015). Although there was low-level releases (< 215 cfs) the days prior, there was also no water released during first Wet season sampling event (August 2015). Water was being released in low volumes during Dry 2 (< 130 cfs) event in May 2015 and Wet 2 (< 900 cfs) event in September 2015 (Fig. 5b), with heavy releases (between 1,000 and 2,700 cfs) for eight consecutive days prior to sampling.

Along the C-24 in the North Fork, flow rates ranging from 0 to 2,442 cfs on September 18 were recorded at the S-49 with a mean rate of 155.2 ± 18.2 cfs between January 1 and September 30, 2015. Based on data from DBHYDRO, water was flowing from the S-49 to the North Fork of the SLR 48.4% of the time. During the Dry season, the mean flow rate was reduced to 43.0 ± 7.5 cfs, with rates ranging from 0 to 486 cfs on February 15. Water was flowing over the S-49 structure 33.1% of the time during the Dry season. During the Wet Season, the mean flow rate increased to 396.5 ± 35.9, with rates ranging from 0 to 2,442 cfs on September 18, 2015. Water was being released at the S-49 structure 67.2% of the Wet season. No water was being released from the S-49 during the Dry 1 (April 2015) sampling event. The releases during the Dry 2 (May 2015; < 215 cfs) and Wet 1 (August 2015; < 300 cfs) sampling events were significantly less than the releases taking place during the Wet 2 (September 2015). During the Dry 2 sampling event flow rates ranged from 560 to 1,040 cfs, but spiked to > 2,440 cfs only two days before the samples were collected (Fig. 5c).

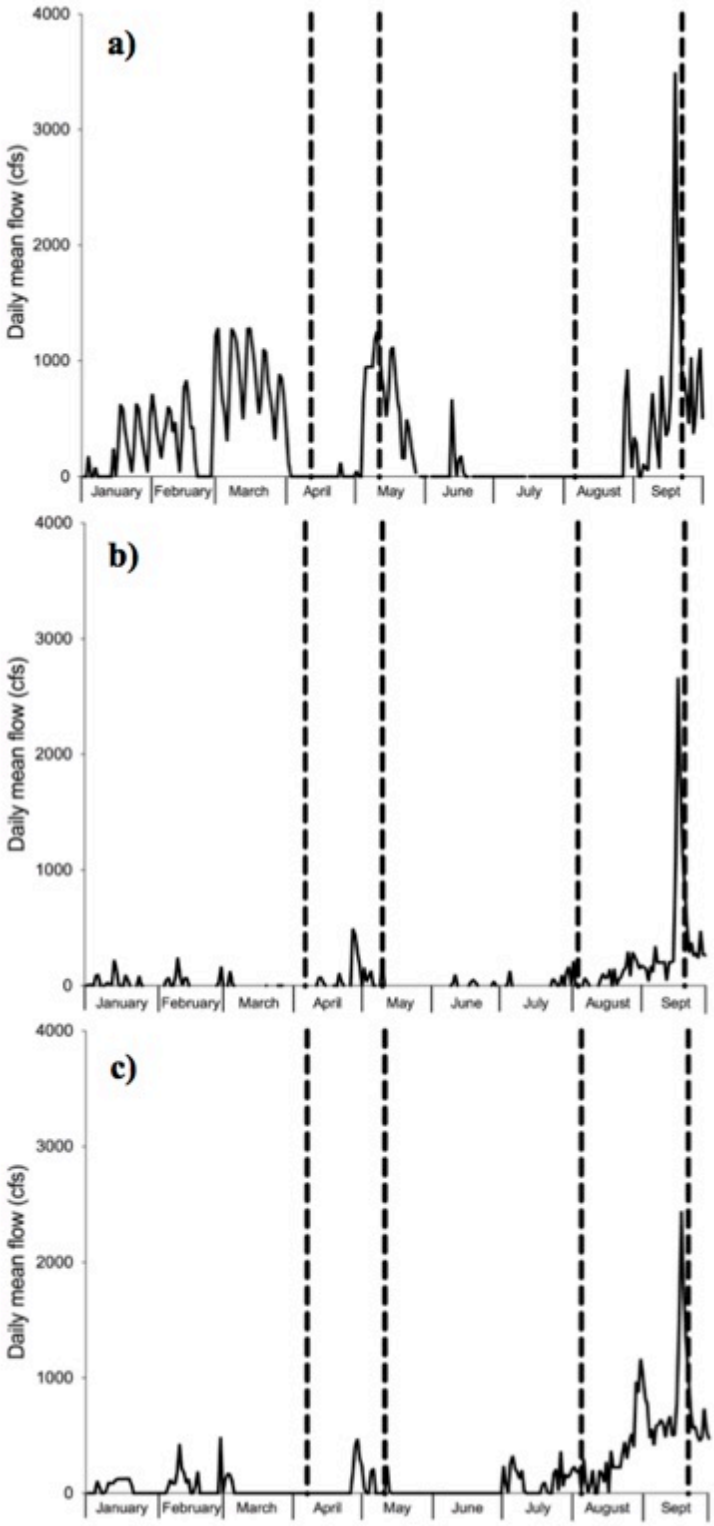

Fig. 5. Daily mean flows (cfs) from the a) S-80 water control structure along the C-44 canal, b) S-48 structure along the C-23 canal, and c) S-49 structure along the C-24 canal between January 1 and September 30, 2015. Black dashed bars represent sampling events.

3.1.3. Bacterial Health: Throughout the study, mean *Enterococcus* counts (cfu/100 mL ± SE) followed and upstream to downstream gradient where they were highest at Leighton Park (59.1 ± 9.8), followed by Roosevelt Bridge (31.2 ± 7.3), Sandsprit Park (14.4 ± 2.4), and the Stuart Sandbar (7.2 ± 1.2). At Leighton Park, the site just downstream of the Old Palm City sampling complex, the counts ranged from 4 to 390 cfu/100 mL throughout the study. Concentrations ranged from 15 to 390 cfu/100 mL during the Dry and 4 to 84 cfu/100 mL during the Wet seasons. As suggested by the ranges, the mean counts (cfu/100 mL ± SE) were higher in the Dry season (77.8 ± 16.2) than in the Wet season (35.3 ± 5.2). The concentrations were in the moderate to poor range (≥36 cfu/100 mL) 56.1% of the time at this site between January 1 and September 30, 2015 (Fig. 6a). At the Roosevelt Bridge, the counts ranged from 2 to 281 cfu/100 mL throughout the study. Counts ranged from 2 to 281 cfu/100 mL during the Dry season and 3 to 97 cfu/100 mL during the Wet season. Again, the mean concentrations (cfu/100 mL ± SE) were higher in the Dry season (37.05 ± 12.79) than in the Wet season (24.4 ± 5.5). At the Roosevelt Bridge, the counts were in the moderate to poor range19.5% of the time between January 1 and September 30, 2015 (Fig. 6b). At Sandsprit Park, just downstream from Golden Gates Estates sampling complex, the counts ranged from 0 to 80 cfu/100 mL throughout the study. Concentrations ranged from 0 to 41 cfu/mL during the Dry season and 2 to 80 during the Wet season. The mean counts (cfu/100 mL ± SE) were lower in the Dry season (12.3 ± 2.3) than in the Wet season (16.7 ± 4.4). The concentrations were in the moderate to poor range 7.3% of the time between January 1 and September 30, 2015 (Fig. 6c). The counts at the Stuart Sandbar in the St. Lucie Inlet, the site closest to the Crossroads (CR) site, ranged from 0 to 33 cfu/100 mL throughout the study. The ranges at this site did not vary much between the Dry (0 to 33 cfu/100mL) and Wet (1 to 28 cfu/100mL) seasons. The mean counts (cfu/100 mL ± SE) in the Dry season were slightly lower (6.6 ± 1.6) than in the Wet season (7.9 ± 1.7). The counts at this site were not documented to extend into the moderate to poor range between January 1 and September 30, 2015 (Fig. 6d). The highest *Enterococcus* counts at the Stuart Sandbar, Leighton Park, and Roosevelt Bridge, collected on January 5, March 2, and March 2, respectively, were documented during the Dry season. Conversely, the high counts at Sandsprit Park were collected on September 21, 2015, overlapping with our last sampling event.

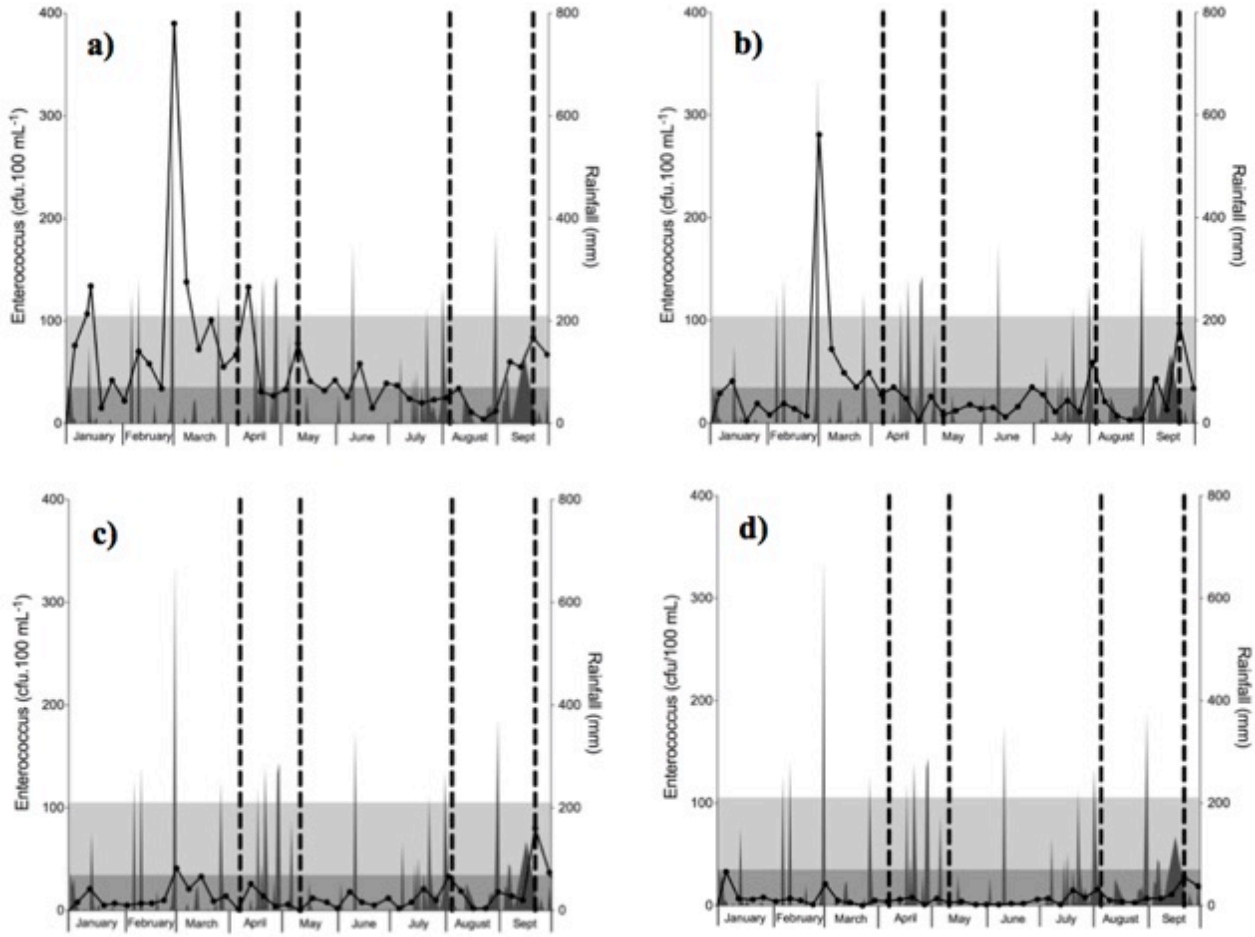

Fig. 6. Enterococcus counts (cfu/100 mL; black line with data points) versus daily rainfall (mm; solid gray line) between January 1 and September 30, 2015 at a) Leighton Park, b) Rosevelt Bridge, c) Sandsprit Park, and d) the Stuart Sandbar. Data supplied by Martin County Department of Health. The water quality scale (based on count data set by Florida Department of Health) includes Good (0 to 35 cfu/100 mL; dark gray), Moderate (36 to 104 cfu/100 mL; light gray), and Poor (>105 cfu/100 mL; white). The vertical dashed lines represent the four project sampling events.

3.2. Groundwater

3.2.1. Dissolved nutrients: Overall, dissolved nutrients documented at residential groundwater monitoring sites located at Old Palm City and Golden Gates Estates were significantly higher than those recorded at the non-residential reference sites during both the Dry and Wet seasons (Table 3). This includes both the biologically available reactive (DIN and SRP; Fig. 7a-c) and total (TDN and TDP; Fig. 7d-f) forms of N and P. Overall project mean DIN and TDN concentrations (± S.E.) were exceptionally high (215.6 ± 53.1 μM and 257.1 ± 54.4 μM, respectively; Table 3). With the exception of Old Palm City during the Wet season, the DIN at the Old Palm City and Golden Gates Estates well sites was comprised primarily of NO_3 in both the Dry (61.0% and 95.1%, respectively) and Wet (48.4% and 92.6%, respectively) seasons. Conversely, the majority of the DIN at the two reference sites was comprised of NH_4 during both the Dry (93.0%) and Wet (98.9%) seasons. The mean NO_3 concentrations (± S.E.) at the Old Palm City well sites ranged from 0.1 ± 0.0 μM during the Dry season to 969.3 ± 2.0 μM during the Wet season and at Golden Gates Estates from 0.02 ± 0.0 μM to 3589.1 ± 18.8 μM, both extremes occurring during the Dry season. The highest mean NO_3 concentration recorded at the reference sites was 3.25 ± 0.1 μM during the Dry season (Pendarvis Park).

Overall mean (± S.E.) groundwater phosphorus (both SRP and TDP) concentrations were high (18.2 ± 2.7 μM and 20.6 ± 2.9 μM, respectively) throughout the study (Table 3). When compared to the reference wells, SRP and TDP concentrations were elevated in both neighborhoods, but especially Old Palm City, during the Wet season (Table 3, Fig. 7b,e). The majority of the P documented in the groundwater at Old Palm City and Golden Gates Estates was in the reactive (PO_4) form, 89.4% and 91.9%, respectively during the Dry season and 88.9% and 87.5%, respectively during the Wet season. Conversely, the majority of the P in the groundwater at the reference sites was in the organic form, 63.4% during the Dry season and 73.1% during the Wet season. The mean SRP concentrations (± S.E.) at the Old Palm City well sites ranged from 0.4 ± 0.1 during the Dry season to 106.9 ± 7.9 during the Wet season. At Golden Gates Estates, values ranged from 0.6 ± 0.0 during the Dry season to 99.4 ± 2.4 during the Wet season. The highest mean PO_4 concentration (± S.E.) measured at the reference sites was 1.3 ± 0.0 (along Jensen Beach Boulevard).

The overall mean groundwater DIN:SRP and TDN:TDP were both ~26 (Table 3). The groundwater DIN:SRP ratios in both the Old Palm City and Golden Gates Estates residential areas were significantly lower (~12 and ~17, respectively) than the overall project mean. The highest N:P ratios for both inorganic (reactive) and total forms of N and P in groundwater were recorded at the reference sites (Table 3, Fig. 7c,f). At the reference sites, the mean DIN:SRP ratio (± S.E.) ranged from 11.1 ± 0.5 (Dry season) to 168.5 ± 27.9 (Wet season). At the Old Palm City and Golden Gates Estates well sites these ratios ranged from 0.8 ± 0.0 to 53.0 ± 7.2 and 0.2 ± 0.0 to 49.4 ± 0.4, respectively; where all extremes were recorded during the Dry season except the low ratio at Golden Gates Estates.

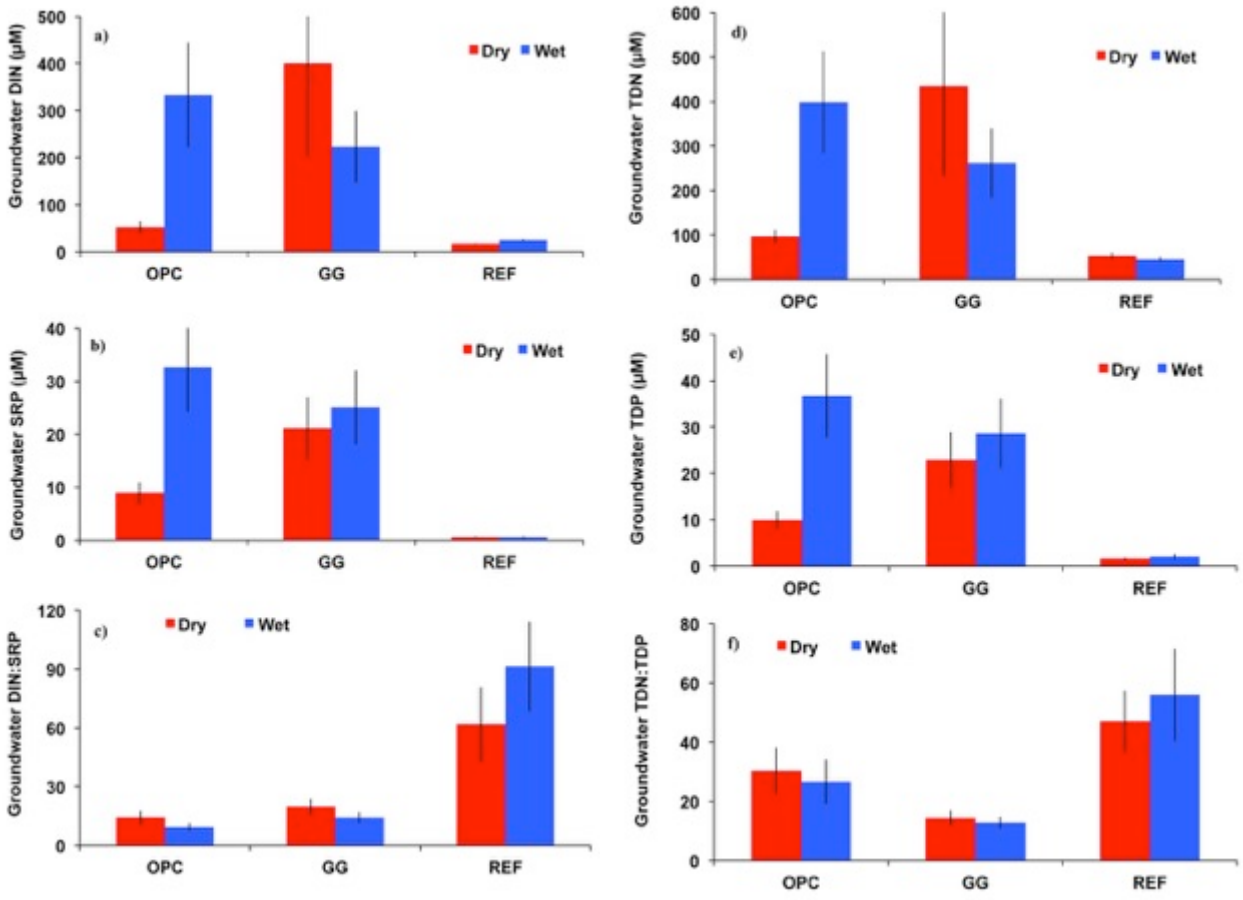

Fig. 7. Dissolved nutrient concentrations and ratios of inorganic N and P (a-c) and total N and P (d-f) recorded in groundwater at the Old Palm City and Golden Gates Estates well clusters and the reference sites.

Table 3. A comparison of mean (± S.E.) groundwater dissolved nutrient concentrations in two residential neighborhood and two reference sites broken down by the four sampling events and overall project means. "Overall Neighborhood Means" combine the four samplings in each of the two individual neighborhoods separately. "Overall Residential Means" combine the four samplings in the two neighborhoods combined. "Overall Reference Means" combine the four sampling events and the two reference sites. The "Overall Project Mean" combines all groundwater data.

Sampling Date	Location	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)
Dry 1 (April 2015)	Old Palm City	22.0±3.7	4.9±1.4	26.9±4.4	8.8±2.7	16.6±6.6	61.1±6.4	9.3±2.7	31.3±12.6	34.2±3.2	0.6±0.1
	Golden Gates	19.4±3.7	718.0±383.7	737.4±381.2	19.0±7.4	22.9±5.8	776.3±389.2	20.5±7.5	19.2±4.3	38.9±9.0	1.5±0.4
	Reference	14.1±0.3	2.0±0.6	16.1±0.8	0.9±0.2	22.9±5.3	63.5±15.5	1.5±0.0	42.1±9.5	47.4±16.2	0.6±0.1
Dry 2 (May 2015)	Old Palm City	18.8±1.5	58.8±22.2	77.5±22.0	9.0±3.1	12.1±1.1	132.5±24.1	10.6±2.9	29.2±10.2	55.0±8.8	1.5±0.5
	Golden Gates	19.8±3.3	44.0±22.6	63.8±21.1	23.2±9.5	16.6±5.8	93.0±24.9	25.5±9.5	9.5±2.3	29.2±3.9	2.2±0.6
	Reference	17.9±0.9	0.4±0.2	18.3±0.8	0.3±0.1	100.6±30.9	41.6±6.1	1.8±0.5	52.0±19.2	23.3±5.4	1.5±0.5
Dry Residential Mean		**20.0±1.6**	**225.8±112.4**	**245.8±111.7**	**15.7±3.4**	**17.4±2.7**	**284.5±113.8**	**17.2±3.5**	**21.4±3.9**	**28.7±3.4**	**1.5±0.2**
Dry Reference Mean		**16.0±0.7**	**1.2±0.4**	**17.2±0.6**	**0.6±0.1**	**61.7±19.0**	**52.6±8.6**	**1.6±0.3**	**47.0±10.3**	**35.3±8.9**	**1.0±0.3**
Overall Dry Season Mean		**19.2±1.3**	**187.7±93.8**	**204.2±91.9**	**12.9±2.9**	**25.4±4.5**	**242.3±93.6**	**14.4±3.0**	**26.1±3.4**	**26.9±3.3**	**1.4±0.2**
Wet 1 (August 2015)	Old Palm City	93.6±41.0	247.0±125.8	340.6±166.6	25.2±10.6	11.5±3.3	406.9±171.9	31.0±12.5	33.8±13.0	66.4±14.8	5.8±2.8
	Golden Gates	16.1±3.4	214.5±114.6	230.6±113.8	25.8±10.1	13.6±3.6	263.1±114.6	29.4±10.7	12.6±3.2	32.5±2.7	3.6±1.1
	Reference	26.7±2.8	0.0±0.0	26.7±2.8	0.6±0.3	97.7±34.2	46.8±7.6	2.8±0.9	52.0±20.7	20.1±4.9	2.1±0.8
Wet 2 (September 2015)	Old Palm City	249.8±127.1	75.7±27.8	325.4±153.3	40.1±13.1	7.3±1.7	390.0±157.8	42.4±13.2	19.3±7.5	64.6±12.4	2.4±0.4
	Golden Gates	16.9±3.5	198.8±106.6	215.7±105.2	24.4±9.9	14.9±4.4	259.4±109.5	27.9±10.9	12.9±2.3	43.7±5.2	3.6±1.0
	Reference	22.8±3.8	0.5±0.3	23.4±3.9	0.5±0.2	85.1±33.2	44.4±7.3	1.4±0.3	59.9±25.2	21.0±3.9	0.9±0.3
Wet Residential Mean		**85.5±31.4**	**186.5±51.3**	**272.0±64.7**	**28.4±5.4**	**12.1±1.8**	**322.2±66.7**	**32.3±5.7**	**18.9±3.6**	**50.3±4.9**	**3.8±0.8**
Wet Reference Mean		**24.8±2.3**	**0.3±0.2**	**25.0±2.4**	**0.6±0.2**	**91.4±22.8**	**45.6±5.0**	**2.1±0.5**	**56.0±15.6**	**20.6±3.0**	**1.5±0.4**
Overall Wet Season Mean		**74.4±25.8**	**152.6±42.9**	**227.1±54.1**	**23.4±4.6**	**26.5±5.7**	**272.0±56.0**	**26.8±4.9**	**25.6±4.4**	**44.9±4.2**	**3.4±0.6**
Overall Neighborhood Mean	**Old Palm City**	**96.0±35.1**	**96.6±34.3**	**192.6±58.8**	**20.8±4.6**	**11.9±1.9**	**247.6±61.0**	**23.3±4.9**	**28.4±5.4**	**55.0±5.5**	**2.6±0.8**
	Golden Gates	**18.1±1.7**	**293.8±106.3**	**311.9±105.7**	**23.1±4.5**	**17.0±2.5**	**348.0±107.8**	**25.8±4.8**	**13.5±1.6**	**36.1±2.9**	**2.7±0.4**
Overall Residential Mean	**Old Palm City + Golden Gates**	**52.7±16.0**	**206.1±61.5**	**258.9±64.3**	**22.1±3.2**	**14.7±1.6**	**303.4±65.7**	**24.7±3.4**	**20.1±2.6**	**44.5±3.1**	**2.7±0.4**
Overall Reference Mean	**Reference**	**20.4±1.5**	**0.8±0.2**	**21.1±1.4**	**0.6±0.1**	**76.6±14.9**	**49.1±4.9**	**1.9±0.3**	**51.5±9.2**	**28.0±4.9**	**1.3±0.3**
Overall Project Mean		**46.8±13.1**	**168.8±50.8**	**215.6±53.1**	**18.2±2.7**	**26.0±3.6**	**257.1±54.4**	**20.6±2.9**	**25.8±2.9**	**41.5±2.7**	**2.4±0.3**

3.2.2. Aqueous N Sources: The project-wide mean (± S.E.) for groundwater aqueous stable isotope values for ammonium (NH_4; 7.8 ± 0.4 $^{o}/_{oo}$) and nitrate (NO_3; 8.8 ±1.5 $^{o}/_{oo}$) were within the ranges of wastewater N (> +3 $^{o}/_{oo}$; Table 4). The seasonal mean $\delta^{15}N$ - NH_4 isotopic signature was also within the wastewater N range during the Dry and Wet seasons at both the residential and reference sites (Fig. 8, Table 4). For $\delta^{15}N$ - NO_3, the seasonal residential means and the reference mean during the Dry season were within the wastewater range, but the mean values at the reference sites during the Wet season reflected an atmospheric N signature (-1.8 ±1.3 $^{o}/_{oo}$; Fig. 8, Table 4).

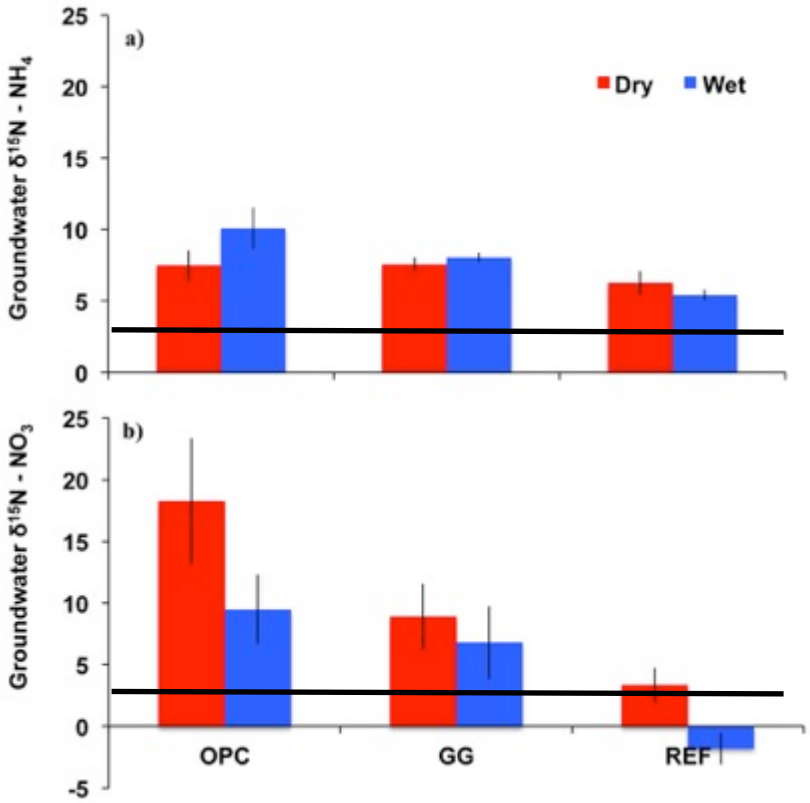

Fig. 8. Stable isotopic ratios of a) $\delta^{15}N$ - NH_4 and b) $\delta^{15}N$ - NO_3 in groundwater at the Old Palm City and Golden Gates Estates residential well clusters as well as the reference wells during the Dry and Wet seasons in 2015.

Table 4. A comparison of mean (± S.E.) groundwater aqueous isotope ($\delta^{15}N\text{-}NH_4$ and $\delta^{15}N\text{-}NO_3$) values in two residential neighborhood and two reference sites broken down by the four sampling events and overall project means. "Neighborhood Means" combine the four samplings in the two individual neighborhoods. "Residential Means" combine the four samplings in the two neighborhoods combined. "Reference Means" combines the four sampling events and the two reference sites. The "Project Mean" combines all groundwater data.

Sampling Event	Location	$\delta^{15}N\text{-}NH_4$ (‰)	$\delta^{15}N\text{-}NO_3$ (‰)
Dry 1 (April 2015)	Old Palm City	6.9±0.7	2.8±1.3
	Golden Gates	5.9±0.7	17.0±8.8
	Reference	7.5±1.5	5.5±2.6
Dry 2 (May 2015)	Old Palm City	8.2±0.6	15.0±4.7
	Golden Gates	9.2±2.0	19.5±5.4
	Reference	5.1±0.3	1.3±0.3
Dry Residential Mean		**7.5±0.5**	**13.1±2.7**
Dry Reference Mean		**6.3±0.8**	**3.4±1.4**
Overall Dry Season Mean		**7.3±0.5**	**11.3±2.3**
Wet 1 (August 2015)	Old Palm City	15.1±2.0	9.7±5.5
	Golden Gates	8.3±0.5	-0.2±2.5
	Reference	5.7±0.5	-5.2±1.6
Wet 2 (September 2015)	Old Palm City	5.1±0.4	6.4±2.4
	Golden Gates	7.8±0.4	13.8±4.7
	Reference	5.1±0.5	1.6±0.3
Wet Residential Mean		**9.0±0.7**	**8.1±2.0**
Wet Reference Mean		**5.4±0.4**	**-1.8±1.3**
Overall Wet Season Mean		**8.3±0.6**	**6.1±1.7**
Overall Neighborhood Mean	**Old Palm City**	**8.8±0.9**	**14.0±3.0**
	Golden Gates	**7.8±0.3**	**7.9±2.0**
Overall Residential Mean	**Old Palm City + Golden Gates**	**8.3±0.4**	**10.7±1.7**
Overall Reference Mean	**Reference**	**5.8±0.5**	**0.8±1.1**
Overall Project Mean		**7.8±0.4**	**8.8±1.5**

3.2.3. Sucralose and Acetaminophen: Groundwater sucralose concentrations were significantly higher during both the Dry and Wet seasons in Old Palm City and Golden Gates Estates than in the two reference wells, where sucralose was not detected (Fig. 9). At the two residential sites, sucralose was only detected in the shallow wells. In the Old Palm City shallow wells, the sucralose concentrations ranged from 1.8 to 30.0 µg/L in the Dry season and from 2.7 to 8.8 µg/L in the Wet season. Similar ranges of 0.11 to 32.0 µg/L in the Dry season and undetectable to 18 µg/L during the Wet season were documented in the Golden Gates Estates shallow wells.

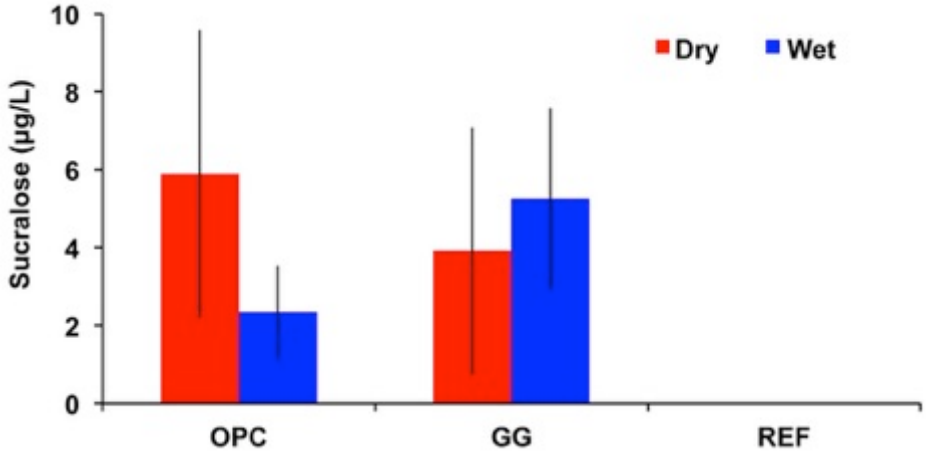

Fig. 9. Mean sucralose concentrations (± S.E.) during the Dry and Wet seasons in the two residential well clusters at Old Palm City and Golden Gates Estates and the two reference wells.

A trace amount of acetaminophen (0.004 µg/L) was detected in one residential groundwater sample throughout the study. The sample was collected during the Dry season at one of the three Golden Gates Estates shallow wells. No acetaminophen was detected in any of the other wells in these residential clusters or at the two references sites.

3.3 Surface Water and Tissue

3.3.1. Field Measurements: Environmental measurements for salinity, temperature, dissolved oxygen (DO), and pH varied by location and sampling event (Table 5). Project-wide, the mean (± S.E.) salinity for the primary canals (C23W and C44W) were lower (0.3 ± 0.1) than the Old Palm City complex (OPC1-5; 1.3 ± 0.6), SLE (C23E, C24W, DP, BR; 5.3 ± 1.1), Golden Gates Estates complex (GG1-5; 8.7 ± 2.6), the crossroads with the IRL (22.8 ± 5.4), and the nearshore reefs (BTR, SLR-N, SLR-S; 32.2 ± 0.4). Overall, the salinity was significantly higher during the Dry season (10.8 ± 2.1) than the Wet season (8.3 ± 1.9; Table 5). Looking at the four sampling events, the salinity was higher during the Wet 1 and Dry 1 collections, the two events that were not associated with freshwater releases (Table 5).

The project-wide mean (± S.E.) temperature for the primary canals (29.0 ± 0.7) and SLE (29.5 ± 0.4) were higher than the Golden Gates Estates complex (27.6 ± 0.4), Old Palm City complex (27.5 ± 0.6), the nearshore reefs (27.4 ± 0.4), and the crossroads with the IRL (27.1 ± 0.6). Overall, the temperature was significantly lower during the Dry (26.6 ± 0.3) than the Wet (29.4 ± 0.2) season (Table 5). Looking at the four sampling events, the temperature gradually increased from the Dry 1 to the Wet 2 collections (Table 5).

Project-wide, the Old Palm City sampling complex had the lowest (3.6 ± 0.5) mean (± S.E.) DO concentration (mg/L; Table 5). This was followed by slightly higher concentration in the Golden Gates Estates complex (4.2 ± 0.5), the primary canals (5.3 ± 0.8), the crossroads with the IRL (6.1 ± 0.3), the nearshore reefs (7.6 ± 0.1), and SLE (7.7 ± 0.4). Overall, the DO concentration did not vary between the Dry (5.5 ± 0.4) and Wet season (5.4 ± 0.5; Table 5). Looking at the four sampling events, the DO concentrations remained around 5.5 mg/L throughout the study (Table 5). A fish kill was observed during the Wet 2 (September 2015) sampling upstream of the S-48 water control structure in the C-23 canal where the DO concentration decreased to 1.2 mg/L.

The project-wide mean (± S.E.) pH followed a downstream to upstream gradient. pH was highest along the nearshore reefs (8.1 ± 0.0) and crossroads with the IRL (8.0 ± 0.1) followed by the Golden Gates Estates complex (7.7 ± 0.1), the Old Palm City complex (7.0 ± 0.1), and the lowest pH was observed in the SLE and primary canals (7.6 ± 0.1 for each). Overall, the pH did not vary between the Dry season (7.6 ± 0.1) and the Wet (7.5 ± 0.1) seasons (Table 5). Looking at the four sampling events, the pH was the same during both Dry season sampling events, but varied between the Wet 1 and Wet 2 collections (Table 5).

Table 5. Field measurements for the St. Lucie Estuary and nearshore reefs separated by four (Dry 1, Dry 2, Wet 1, and Wet 2) sampling events in 2015.

Site	Dry 1 (April 2015)				Dry 2 (May 2015)				Wet 1 (August 2015)				Wet 2 (September 2015)			
	Salinity (‰)	Temp. (˚C)	DO (mg/L)	pH	Salinity (‰)	Temp. (˚C)	DO (mg/l)	pH	Salinity (‰)	Temp. (˚C)	DO (mg/l)	pH	Salinity (‰)	Temp. (˚C)	DO (mg/l)	pH
C23W	0.6	26.6	6.3	7.4	0.2	28.8	6.4	7.4	0.6	30.9	5.4	7.6	0.2	29.0	1.2	7.6
C44W	0.2	26.0	5.8	7.2	0.0	30.2	7.0	7.9	0.4	31.6	8.0	8.0	0.2	29.0	2.2	7.5
C23E	10.2	28.1	7.9	8.0	6.8	29.5	7.4	7.8	6.9	29.8	8.2	8.0	0.3	31.1	6.2	7.1
C44E	1.8	27.1	8.2	7.8	0.2	28.3	6.9	7.5	3.4	31.3	5.3	7.8	0.2	29.8	5.8	7.0
OPC1	0.0	21.6	2.5	6.3	0.1	27.0	5.2	6.5	0.2	30.2	2.0	6.4	0.1	29.2	5.8	6.4
OPC2	0.0	23.5	2.4	6.6	0.0	25.9	1.3	6.6	0.3	28.1	1.3	7.0	0.1	30.7	4.2	6.7
OPC3	0.0	22.7	1.2	6.7	0.0	24.8	0.4	6.6	0.0	27.0	0.2	6.6	0.3	27.0	0.8	6.9
OPC4	3.3	26.7	6.1	7.7	0.8	27.9	5.8	7.5	8.6	30.3	6.8	7.5	0.2	30.9	4.3	7.1
OPC5	3.4	26.4	6.1	7.9	0.4	27.8	5.8	7.9	7.8	31.2	4.3	7.6	0.2	30.2	4.6	7.0
DP	10.8	27.7	7.1	7.7	10.6	29.1	6.7	7.6	9.9	30.1	9.3	7.9	0.6	31.2	7.5	7.2
BR	7.8	27.3	7.5	7.7	9.2	29.0	7.0	7.6	5.4	30.6	10.0	7.4	0.2	32.1	12.6	7.5
GG1	0.2	24.8	1.8	7.2	0.2	27.5	2.1	7.4	0.0	29.2	2.7	7.4	0.2	29.0	1.8	6.7
GG2	0.3	24.1	3.6	8.0	0.3	27.1	3.9	8.3	0.3	30.5	4.2	8.0	0.3	30.8	8.9	7.3
GG3	0.0	24.0	1.7	7.5	0.0	27.3	2.1	7.1	8.8	27.7	0.8	7.6	0.6	28.3	2.2	7.4
GG4	27.8	26.7	5.8	8.0	26.0	27.9	5.0	8.0	23.7	28.2	6.4	8.0	5.1	28.9	5.1	7.6
GG5	28.6	25.8	6.6	8.1	26.6	27.0	6.6	8.1	21.1	27.7	6.7	8.1	4.4	28.8	5.1	7.6
CR	29.7	26.0	6.5	8.0	28.9	26.8	6.4	8.0	25.8	26.8	6.6	8.1	6.8	28.8	5.1	7.8
BTR	33.3	24.9	7.6	8.2	33.0	26.5	7.5	8.1	32.9	26.9	8.9	8.1	32.1	28.9	7.6	8.1
SLR-N	33.4	25.7	7.4	8.1	30.8	28.5	7.5	8.2	31.7	27.8	7.6	8.2	29.3	29.0	6.9	8.1
SLR-S	33.4	25.7	7.5	8.1	33.3	27.1	7.5	8.2	32.7	28.0	7.9	8.3	31.0	29.3	7.3	8.1
Event Mean	**11.2±3.1**	**25.6±0.4**	**5.5±0.5**	**7.6±0.1**	**10.4±3.0**	**27.7±0.3**	**5.4±0.5**	**7.6±0.1**	**11.0±2.7**	**29.2±0.4**	**5.6±0.7**	**7.7±0.1**	**5.6±2.5**	**29.6±0.3**	**5.3±0.6**	**7.3±0.1**
Seasonal Mean	**10.8±2.1**	**26.6±0.3**	**5.5±0.4**	**7.6±0.1**					**8.3±1.9**	**29.4±0.2**	**5.4±0.5**	**7.5±0.1**				
Project Mean	**9.6±1.4**	**28.0±0.2**	**5.5±0.3**	**7.6±0.1**												

3.3.2. Dissolved Nutrients: Regardless of the analyte (Tables 6A,B) or location (Figs. 10-11), mean project-wide surface water dissolved nutrient concentrations were significantly higher (some greater than three-fold) in the Wet season than the Dry season. With few exceptions, the highest regional mean nutrient concentrations in the Dry and Wet seasons were primarily documented at one or both of the residential sampling networks (Old Palm City, Golden Gates Estates) followed by lower concentrations in the primary canals, Indian River Lagoon, and nearshore reefs (Tables 6A,B). The same regional trends were seen for comprehensive means that include all four sampling events (Table 6C).

Overall reactive DIN concentrations were relatively high (11.5 ± 0.9 μM) and increased nearly three fold between the Dry and Wet seasons. With the primary canals, Crossroads with the IRL, and the nearshore reef sites removed, the overall mean DIN concentration in the SLE increased to 14.1 ± 1.2 μM. With the exception of Old Palm City in the Dry season, reactive DIN concentrations were consistently highest at Old Palm City and Golden Gate Estates when compared to the primary canals (Tables 6A,B,C). In the Old Palm City network, the mean DIN concentrations (± S.E.) ranged from 1.0 ± 0.4 μM to 9.9 ± 4.1 μM in the Dry season and 7.8 ± 2.3 μM to 33.2 ± 6.7 μM in the Wet season. In Golden Gates Estates network the concentrations ranged from 1.8 ± 0.3 μM to 32.2 ± 2.5 μM in the Dry season and 12.2 ± 4.6 μM to 36.1 ± 2.2 μM in the Wet season. Conversely, the primary canals ranged from 4.5 ± 1.6 μM to 16.4 ± 1.2 μM in the Dry season and 9.4 ± 2.3 μM to 16.1 ± 6.8 μM in the Wet season (Fig. 10a). The

surface water DIN at the five Old Palm City sites was primarily comprised of NO_3 (67.1 ± 13.1%) during the Dry season and NH_4 (56.3 ± 10.9%) during the Wet season. The majority of the DIN in the surface water at the five Golden Gate Estates sites was NH_4 during both the Dry (65.8 ± 7.0%) and Wet (57.9 ± 7.8%) seasons. Like Old Palm City, the DIN in the two primary canals was comprised primarily of NO_3 (87.1 ± 9.3%) during the Dry season and NH_4 (69.5 ± 14.3%) during the Wet season.

Similar to DIN, soluble reactive phosphorus (SRP) concentrations were high project-wide (3.0 ± 0.3 μM) and increased three-fold between the Dry and Wet seasons. In the SLE proper, the overall mean concentration (3.3 ± 0.3 μM) was not significantly different than the overall project mean. Surface water SRP concentrations were highest at Old Palm City throughout the study (Table 6A,B,C), but especially during the Wet season when the mean concentration increased to 19.3 ± 0.4 μM (Fig. 10b). Although elevated in both primary canals, the Wet season SRP was especially high (~10 μM) just upstream of the S-48 water control structure in the C-23 canal. Cumulative seasonal SRP mean concentrations (± S.E.) for the primary canals (1.8 ± 0.3 μM) were lower than those documented in Old Palm City (2.8 ± 0.5 μM) during the Dry season, but the same (7.5 μM) during the Wet season (Tables 5A,B), despite the Wet season spike at OPC3. In the Old Palm City network, the mean surface water SRP concentrations (± S.E.) ranged from 0.3 ± 0.1 μM to 7.3 ± 0.2 μM in the Dry season and 0.4 ± 0.1 μM to 19.3 ± 0.4 μM in the Wet season. The SRP concentrations were considerably lower in the Golden Gates Estates network where the concentrations ranged from 0.2 ± 0.0 μM to 2.2 ± 0.5 μM in the Dry season and 0.5 ± 0.1 μM to 3.7 ± 0.9 μM in the Wet season. The primary canals ranged from 1.2 ± 0.2 μM to 2.4 ± 0.4 μM in the Dry season and 5.2 ± 1.5 μM to 9.9 ± 2.7 μM in the Wet season (Table 6A,B; Fig. 10b).

The ratio of DIN:SRP averaged 15.5 ± 3.3 project-wide and decreased over two-fold (from 20 to 8) between the Dry and Wet seasons (Tables 6A,B). This overall value increased to 22.4 ± 4.7 when looking only at the SLE proper. With the exception of OPC1, relatively low mean seasonal ratios (~6) were seen at the five Old Palm City network sites during both seasons (Tables 6A,B,C). The mean DIN:SRP ratio (± S.E.) at Old Palm City ranged from 0.2 ± 0.1 to 20.3 ± 11.4 in the Dry season and 1.2 ± 0.1 to 20.0 ± 0.2 in the Wet season. The highest ratios were documented in Golden Gates Estates, especially at GG1 and GG2 (Fig.10c). The seasonal and overall study means at these five sites were consistently >46 (Tables 6A,B,C). The mean DIN:SRP ratio (± S.E.) at Golden Gates Estates ranged from 1.8 ± 0.5 to 166.0 ± 32.2 in the Dry season and 2.5 ± 0.6 to 135.7 ± 64.6 in the Wet season. With the exception of the Dry season in the C-44 canal, the DIN:SRP ratios were low in the primary canals with a collective mean ratio was 8.8 ± 2.0 in the Dry season and 1.6 ± 0.4 in the Wet season (Fig. 10c, Tables 5A,B).

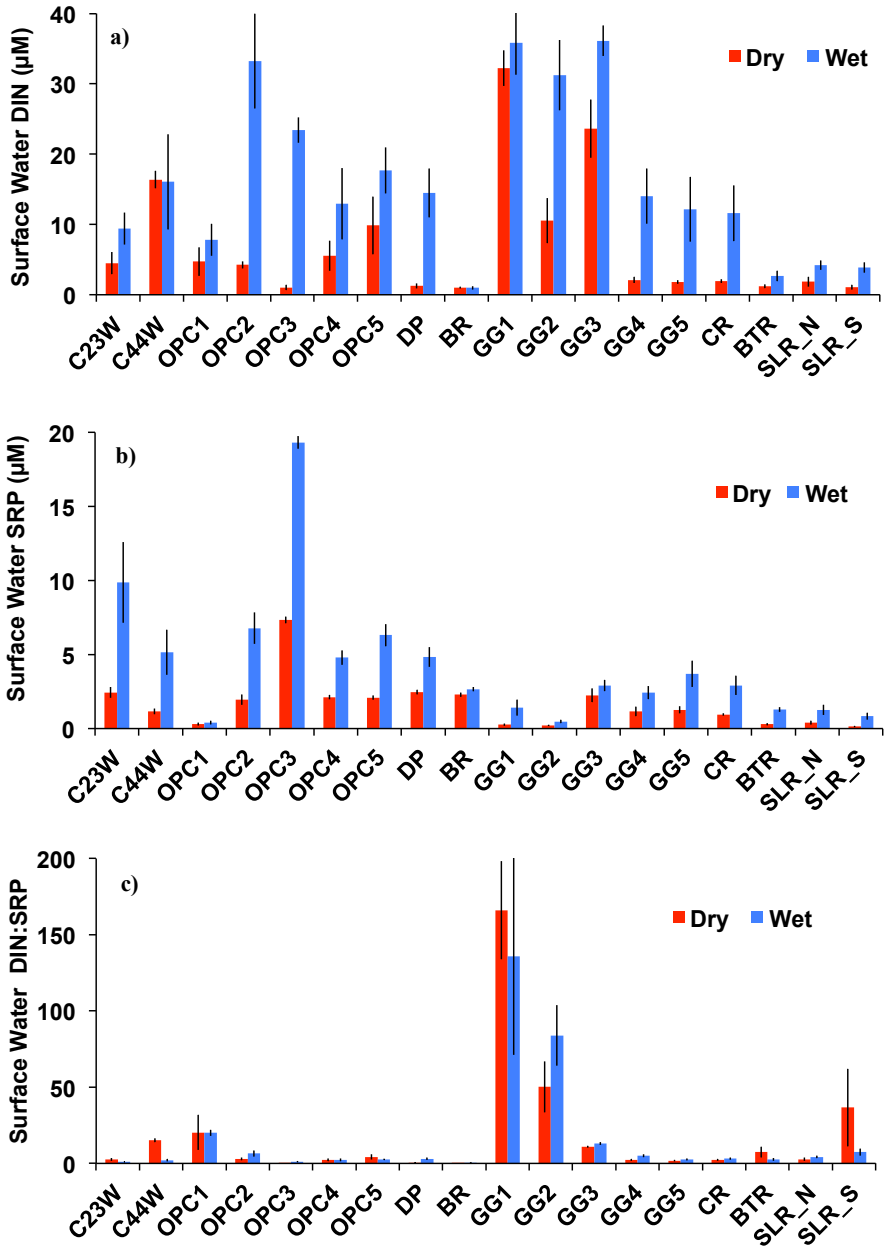

Fig. 10. Dissolved inorganic nutrient concentrations and ratios of a) dissolved inorganic N, soluble reactive P, and c) DIN:SRP ratios recorded in surface water at 18 sites located in the primary canals (C23W, C44W), St. Lucie Estuary (OPC1-5, DP, BR, GG1-5), crossroads with the Indian River Lagoon (CR), and nearshore reefs (BTR, SLR-N, SLR-S).

For TDN, regional means were all higher than the overall project-wide mean (± S.E.) of 64.4 ± 2.2 μM with the exception of Beau Rivage, the crossroads with the Indian River Lagoon, and the nearshore reefs (Table 6C). When combining the regions in the SLE proper, the mean TDN concentration was also higher (73.0 ± 2.5 μM) than the overall project-wide mean. These concentrations were especially high in Old Palm City's All American Ditch (OPC2, OPC3) and the Golden Gates Estates sites in the community retention pond (GG1, GG2) and where the connecting drainage ditch empties into an upstream tributary of Willougby Creek (GG3; Fig. 11a). During both the Dry and Wet seasons at Golden Gates Estates, the mean TDN concentrations (± S.E.) show a dilution gradient from upstream (community retention pond) to downstream (Lower SLE). The gradient within this network spanned 79.1 ± 3.4 μM in the retention pond down to 27.1 ± 2.4 μM in the Lower Estuary during the Dry season and 107.2 ± 7.2 μM in the retention pond down to 61.6 ± 9.8 μM in the Lower Estuary during the Wet season (Tables 6A,B; Fig. 11a). Comparatively, the mean TDN (± S.E.) in the primary canals increased ~20 μM between the Dry (68.2 ± 1.1 μM) and Wet (88.1 ± 8.7 μM) seasons. The mean concentrations (± S.E.) in the canals ranged from 66.9 ± 1.4 μM to 69.5 ± 1.8 μM in the in the Dry season and 79.4 ± 12.3 μM to 96.7 ± 12.2 μM in the Wet season (Tables 6A,B; Fig. 11a). The surface water TDN at the five Old Palm City sites was comprised of 8.6 ± 2.4% reactive DIN during the Dry season and 19.1 ± 3.4% during the Wet season. The TDN in the surface water at the five Golden Gate Estates sites was comprised of more DIN in both the Dry (21.3 ± 7.6%) and Wet (28.6 ± 3.6%) seasons. The TDN in two primary canals was comprised of 15.5 ± 9.0% DIN during the Dry season and 15.0 ± 5.2% DIN during the Wet season.

The overall mean (± S.E.) concentration of TDP was also relatively high throughout the study (4.2 ± 0.3 μM); exceeded only at Old Palm City, the primary canals, and the Lighthouse community on Dyer Point (Table 6C). Overall, the seasonal means (± S.E.) increased nearly two-fold between the Dry and Wet seasons (Table 4A,B). In the SLE proper, they were only slightly higher (4.6 ± 0.4 μM) than the overall project mean. In the Old Palm City network, the mean TDP concentrations (± S.E.) ranged from 1.0 ± 0.2 μM to 10.2 ± 0.2 μM in the Dry and 1.2 ± 0.1 μM to 3.0 ± 0.4 μM in the Wet seasons. The TDP concentrations were lower in the Golden Gates Estates network where the concentrations ranged from 1.1 ± 0.1 μM to 3.0 ± 0.4 μM in the Dry and 1.6 ± 0.3 μM to 4.3 ± 0.9 μM in the Wet seasons. The primary canals ranged from 2.9 ± 0.2 μM to 3.2 ± 0.6 μM in the Dry season and 7.3 ± 1.8 μM to 11.3 ± 2.9 μM in the Wet season (Table 6A,B; Fig. 11b). Concentrations at the Lighthouse community at Dyer Point increased from a Dry season mean of 3.7 ± 0.2 μM to a Wet season mean of 6.1 ± 0.8 μM (Fig. 11b).

The overall mean surface water TDN:TDP ratio was ~26, a value exceeded only by overall project means documented for Old Palm City and Golden Gates Estates community (Table 6C). A slightly higher ratio (28.9 ± 2.5) was documented in the SLE proper. Seasonal comparisons show that mean Dry season ratios (± S.E.) were lower during the Dry season (23.2 ± 2.0) than during the Wet season (28.2 ± 3.0; Tables 6A,B). Regardless of season, Golden Gates Estates consistently had the highest TDN:TDP ratios throughout the study. Furthermore, the nearshore reefs had exceptionally high mean ratios during the Wet season, second only to Golden Gates Estates. The lowest mean ratios (± S.E.) were seen at the Crossroads site in the Indian River Lagoon (9.1 ± 1.2) and the nearshore reefs (11.4 ± 0.7) in the Dry season and primary canals (11.8 ± 1.1) and the Lighthouse community on Dyer Point (14.7 ± 0.9) in the Wet season (Tables 6A,B,C; Fig.11c).

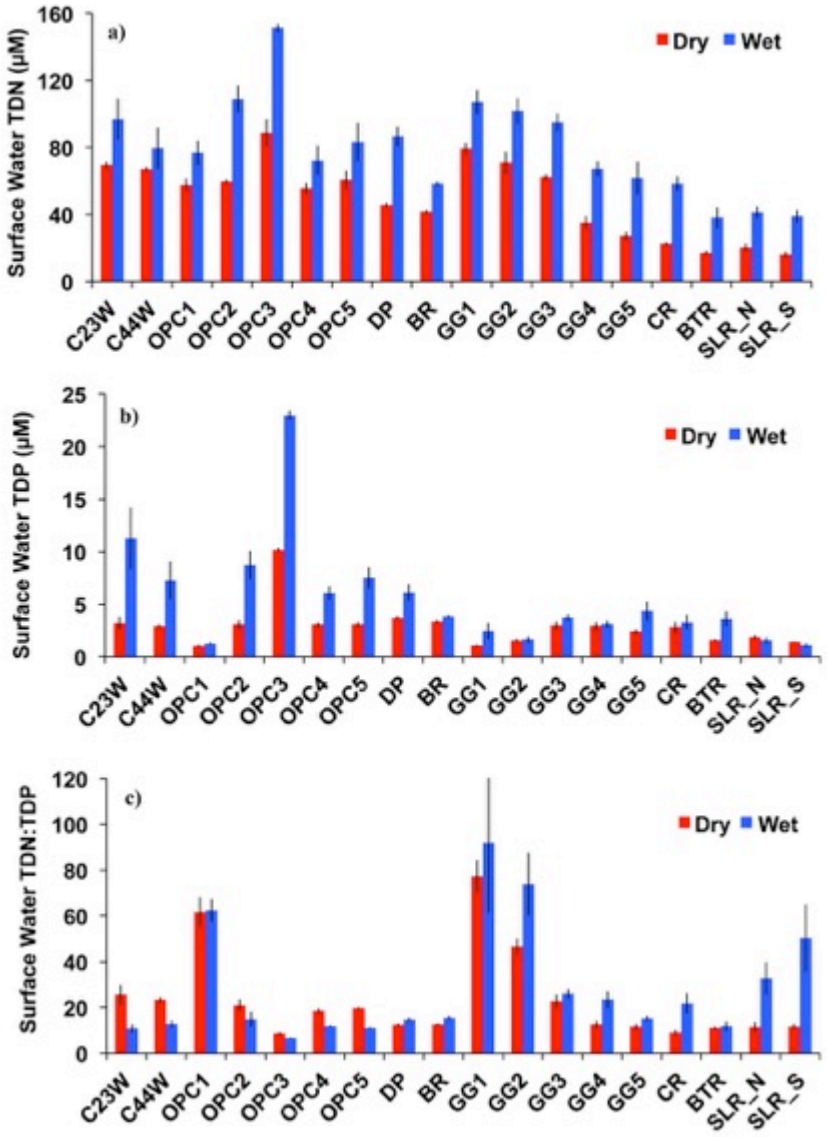

Fig 11. Dissolved nutrient concentrations and ratios of a) total N, b) total P, and c) TDN:TDP ratios recorded in surface water at 18 sites located in the primary canals (C23W, C44W), St. Lucie Estuary (OPC1-5, DP, BR, GG1-5), crossroads with the Indian River Lagoon (CR), and nearshore reefs (BTR, SLR-N, SLR-S).

Table 6A. A comparison of mean (± S.E.) surface water dissolved nutrient concentrations throughout the St. Lucie Estuary broken down by the two Dry season sampling events and region. "Overall Dry Season Mean" combines the two samplings for all 18 sampling sites.

Table 6A. A comparison of mean (± S.E.) surface water dissolved nutrient concentrations throughout the St. Lucie Estuary broken down by the two Dry season sampling events and region. "Dry Season Project-Wide Mean" combines the two samplings for all 18 sampling sites.

Sampling Event	Region	Site	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)
Dry 1 (April 2015)	Primary Canals	C23W	1.9±0.1	6.0±0.1	8.0±0.0	1.7±0.1	4.7±0.3	65.6±0.2	1.9±0.1	34.8±1.8	57.7±0.2	0.2±0.0
		C44W	1.0±0.0	12.7±0.0	13.6±0.1	0.8±0.0	17.2±0.1	64.4±0.2	2.6±0.1	24.9±0.5	50.7±0.2	1.8±0.1
	Old Palm City	OPC1	6.5±0.3	2.8±0.3	9.3±0.1	0.4±0.2	39.8±16.6	49.1±1.3	0.8±0.2	64.1±10.3	39.9±1.3	0.4±0.0
		OPC2	3.8±0.2	1.5±0.2	5.3±0.4	1.2±0.1	4.6±0.5	56.3±0.3	2.1±0.0	26.3±0.3	51.1±0.6	1.0±0.1
		OPC3	0.9±0.2	0.9±0.1	1.8±0.3	6.9±0.3	0.3±0.0	70.8±0.2	9.9±0.3	7.1±0.2	68.9±0.4	3.0±0.1
		OPC4	0.3±0.1	0.5±0.0	0.8±0.2	1.9±0.2	0.4±0.1	48.3±0.2	2.6±0.3	18.9±2.1	47.5±0.3	0.7±0.1
		OPC5	0.2±0.1	0.5±0.1	0.7±0.1	1.8±0.0	0.4±0.1	48.0±0.2	2.5±0.1	18.9±0.4	47.3±0.3	0.7±0.1
	North Fork	DP	0.4±0.2	1.5±0.2	1.9±0.3	2.4±0.3	0.5±0.1	39.9±0.1	3.2±0.3	13.3±0.9	45.5±0.9	1.3±0.1
		BR	0.6±0.1	0.6±0.2	1.2±0.3	0.9±0.2	2.8±1.1	21.1±0.9	2.2±0.3	12.5±1.0	38.7±0.1	0.8±0.1
	Golden Gates	GG1	17.0±0.9	9.6±0.2	26.7±1.0	0.3±0.1	114.8±49.2	71.8±1.9	1.1±0.2	67.8±9.9	45.1±0.9	0.8±0.0
		GG2	2.0±0.1	1.6±0.3	3.5±0.4	0.2±0.0	18.2±3.8	56.6±0.4	1.4±0.1	40.8±2.6	53.0±0.8	1.2±0.1
		GG3	31.3±1.0	1.6±0.3	32.9±1.1	3.3±0.1	10.1±0.1	64.2±0.6	3.8±0.1	16.9±0.3	31.4±1.0	0.5±0.0
		GG4	0.6±0.6	0.9±0.1	1.5±0.6	0.5±0.1	3.2±1.1	26.3±0.6	2.4±0.4	11.4±1.7	24.9±0.8	2.0±0.3
		GG5	0.6±0.4	0.8±0.0	1.4±0.4	0.8±0.3	2.3±1.0	22.0±0.8	2.4±0.5	9.9±1.8	20.7±0.4	1.6±0.2
	Indian River Lagoon	CR	0.6±0.4	1.6±0.3	2.1±0.5	2.4±0.3	0.8±0.1	47.4±1.0	3.6±0.3	9.8±1.0	19.0±1.5	1.3±0.1
	Reefs	BTR	0.5±0.5	1.0±0.1	1.5±0.5	0.2±0.1	12.3±6.2	14.4±0.5	1.4±0.2	10.6±1.1	12.9±0.6	1.2±0.3
		SLR_N	2.0±1.2	1.0±0.0	2.9±1.2	0.3±0.3	6.0±0.0	17.3±4.4	1.5±0.1	12.2±4.2	14.4±3.4	1.2±0.4
		SLR_S	0.9±0.3	0.9±0.0	1.8±0.3	0.1±0.0	71.5±44.8	17.7±3.3	1.4±0.1	12.3±2.0	15.9±3.4	1.4±0.1
	Dry 1 Event Mean		**3.9±1.1**	**2.5±0.5**	**6.5±1.2**	**1.4±0.2**	**17.6±5.3**	**44.5±2.7**	**2.6±0.3**	**22.9±2.5**	**38.0± 2.2**	**1.2±0.1**
Dry 2 (May 2015)	Primary Canals	C23W	0.1±0.1	1.0±0.1	1.0±0.1	3.2±0.3	0.3±0.0	73.3±38.8	4.5±0.3	16.5±1.1	72.3±0.8	1.3±0.6
		C44W	0.2±0.2	18.9±0.2	19.1±0.4	1.5±0.2	13.1±2.0	69.5±10.6	3.2±0.3	21.7±1.8	50.4±1.8	1.7±0.1
	Old Palm City	OPC1	0.0±0.0	0.2±0.1	0.2±0.1	0.2±0.0	0.8±0.2	66.2±10.6	1.2±0.2	59.0±10.1	66.0±0.6	1.0±0.2
		OPC2	0.1±0.1	3.2±0.3	3.2±0.3	2.7±0.1	1.2±0.1	62.7±10.6	4.0±0.0	15.8±0.1	59.5±0.3	1.2±0.1
		OPC3	0.0±0.0	0.3±0.0	0.3±0.0	7.7±0.1	0.0±0.0	106.7±10.6	10.4±0.2	10.3±0.2	106.4±0.4	2.7±0.2
		OPC4	0.0±0.0	10.2±0.8	10.2±0.8	2.4±0.1	4.4±0.3	62.6±10.6	3.5±0.1	18.2±1.1	52.4±1.5	1.1±0.2
		OPC5	0.3±0.2	18.7±0.0	19.0±0.2	2.4±0.1	8.1±0.4	73.2±10.6	3.6±0.0	20.5±0.2	54.2±0.4	1.2±0.1
	North Fork	DP	0.3±0.2	0.3±0.1	0.6±0.3	2.2±0.1	0.4±0.0	43.4±10.6	3.5±0.1	11.5±0.4	43.0±2.1	1.2±0.1
		BR	0.1±0.1	0.8±0.1	0.9±0.1	1.0±0.0	1.8±0.2	24.1±10.6	3.4±1.0	12.5±0.6	42.5±1.4	1.3±0.1
	Golden Gates	GG1	20.4±0.2	17.4±0.4	37.8±0.4	0.2±0.0	217.3±11.1	86.4±10.6	1.0±0.1	86.7±8.1	48.6±1.5	0.8±0.1
		GG2	13.0±2.0	4.5±0.8	17.6±1.3	0.2±0.1	82.1±18.7	84.9±10.6	1.7±0.3	52.3±4.7	67.4±5.7	1.4±0.2
		GG3	11.6±0.3	2.8±0.2	14.4±0.2	1.2±0.1	11.9±1.1	60.0±10.6	2.2±0.3	28.4±2.7	45.6±2.9	0.9±0.3
		GG4	1.8±0.2	1.0±0.3	2.7±0.4	1.8±0.1	1.5±0.2	43.7±10.6	3.4±0.6	13.8±2.8	41.0±1.5	1.6±0.5
		GG5	1.3±0.1	0.9±0.0	2.3±0.1	1.7±0.0	1.3±0.1	32.1±10.6	2.4±0.1	13.4±0.5	29.8±1.9	0.7±0.0.1
	Indian River Lagoon	CR	1.0±0.3	0.8±0.0	1.8±0.3	2.6±0.0	0.2±0.1	43.6±10.6	3.8±0.1	8.4±2.3	22.3±1.6	2.4±1.0
	Reefs	BTR	0.1±0.1	0.9±0.3	1.0±0.3	0.4±0.1	2.6±0.1	19.5±10.6	1.7±0.1	11.6±0.4	18.5±1.0	1.3±0.1
		SLR_N	0.1±0.1	0.7±0.2	0.8±0.2	0.5±0.0	1.6±0.3	23.2±10.6	2.2±0.3	10.8±1.1	22.4±0.9	1.7±0.3
		SLR_S	0.0±0.0	0.4±0.1	0.4±0.1	0.2±0.0	1.7±0.4	14.5±24.9	1.3±0.1	11.1±1.2	14.2±0.5	1.1±0.1
	Dry 2 Event Mean		**2.8±0.8**	**4.6±0.9**	**7.4±1.4**	**1.8±0.2**	**19.5±7.1**	**55.0±3.5**	**3.2±0.3**	**23.5±2.9**	**47.6± 3.0**	**1.4±0.1**
Overall Dry Season Mean - All Sites			**3.4±0.7**	**3.6±0.5**	**6.9±0.9**	**1.6±0.2**	**18.6±4.5**	**49.7±2.2**	**2.9±0.2**	**23.2±1.9**	**42.8± 1.9**	**1.3±0.1**
Dry Season Means by Region	**Primary Canals**	C23W, C24W	**0.8±0.2**	**9.6±2.0**	**10.4±2.0**	**1.8±0.3**	**8.8±2.0**	**68.2±1.1**	**3.1±0.3**	**24.5±2.1**	**57.8±2.7**	**1.3±0.2**
	Old Palm City	OPC1-5	**1.2±0.4**	**3.9±1.1**	**5.1±1.1**	**2.8±0.5**	**6.0±2.5**	**64.4±3.1**	**4.1±0.6**	**25.9±3.7**	**59.3±3.3**	**1.3±0.2**
	Sewered North Fork	DP	**0.3±0.1**	**0.9±0.3**	**1.2±0.3**	**2.5±0.2**	**0.5±0.1**	**45.5±1.2**	**3.7±0.2**	**12.4±0.6**	**44.2±1.2**	**1.2±0.0**
	Septic North Fork	BR	**0.3±0.1**	**0.7±0.1**	**1.0±0.1**	**2.3±0.1**	**0.5±0.1**	**41.6±1.0**	**3.4±0.1**	**12.5±0.5**	**40.6±1.0**	**1.1±0.1**
	Golden Gates	GG1-5	**10.0±1.9**	**4.1±1.0**	**14.1±2.5**	**1.0±0.2**	**46.3±13.4**	**54.8±4.1**	**2.2±0.2**	**34.1±4.9**	**40.7±2.6**	**1.1±0.1**
	Indian River Lagoon	CR	**0.8±0.2**	**1.2±0.2**	**2.0±0.3**	**0.9±0.1**	**2.3±0.6**	**22.6±1.0**	**2.8±0.5**	**9.1±1.2**	**20.6±1.2**	**1.8±0.5**
	Reefs	BTR, SLRN/S	**0.6±0.2**	**0.8±0.1**	**1.4±0.3**	**0.3±0.1**	**17.2±10.0**	**17.8±1.1**	**1.6±0.1**	**11.4±0.7**	**16.4±1.1**	**1.3±0.1**

Table 6B. A comparison of mean (± S.E.) surface water dissolved nutrient concentrations throughout the St. Lucie Estuary broken down by the two Wet season sampling events and region. "Overall Wet Season Mean" combines the two samplings for all 18 sampling sites.

Table 6B. A comparison of mean (± S.E.) surface water dissolved nutrient concentrations throughout the St. Lucie Estuary broken down by the two Wet season sampling events and region. "Wet Season Project-Wide Mean" combines the two samplings for all 18 sampling sites.

Sampling Event	Region	Site	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)
Wet 1 (August 2015)	Primary Canals	C23W	1.7±0.5	2.8±0.6	4.4±1.1	3.8±0.1	1.2±0.3	69.4±1.0	4.8±0.0	14.6±0.3	65.0±0.2	1.0±0.1
		C44W	0.3±0.3	0.7±0.3	1.0±0.5	1.7±0.1	0.5±0.3	52.0±0.6	3.3±0.2	16.0±0.5	51.1±0.1	1.5±0.1
	Old Palm City	OPC1	0.0±0.0	2.9±0.6	2.9±0.6	0.1±0.0	20.6±4.3	61.6±3.8	1.2±0.1	53.5±0.6	58.7±4.4	1.0±0.1
		OPC2	16.3±0.4	1.9±0.2	18.2±0.2	9.1±0.2	2.0±0.0	91.0±1.2	11.7±0.1	7.8±0.1	72.8±1.4	2.6±0.3
		OPC3	19.1±0.8	0.8±0.2	19.9±0.7	19.3±0.3	1.0±0.0	146.9±1.8	23.4±0.2	6.3±0.1	127.0±2.5	4.1±0.2
		OPC4	0.7±0.5	0.9±0.3	1.6±0.8	3.7±0.1	0.4±0.2	52.9±1.9	4.7±0.0	11.4±0.3	51.3±2.5	0.9±0.1
		OPC5	6.1±0.2	4.3±0.1	10.4±0.3	4.6±0.0	2.2±0.1	58.9±0.3	5.3±0.0	11.2±0.1	48.51±0.4	0.6±0.0
	North Fork	DP	0.5±0.3	8.9±5.6	9.4±5.9	3.3±0.2	2.8±1.7	74.0±2.8	4.4±0.2	16.8±0.2	64.6±4.7	1.1±0.1
		BR	0.4±0.2	0.8±0.3	1.2±0.5	3.0±0.1	0.4±0.2	55.7±1.1	4.1±0.2	13.6±0.3	54.5±1.4	1.2±0.2
	Golden Gates	GG1	22.84±2.9	22.8±0.9	45.6±2.0	0.2±0.1	261.4±71.1	114.5±13.2	0.7±0.1	159.7±5.4	68.8±14.9	0.5±0.1
		GG2	16.4±2.7	7.0±0.8	23.4±2.9	0.3±0.1	112.5±32.9	97.0±5.4	1.0±0.1	101.6±11.4	73.7±8.2	0.7±0.1
		GG3	36.4±3.4	1.9±0.5	38.3±2.9	3.6±0.4	10.8±0.5	92.2±5.1	4.2±0.4	22.2±1.1	53.9±2.4	0.6±0.0
		GG4	4.0±1.7	1.9±1.5	5.9±3.1	1.5±0.4	3.3±1.1	63.7±9.2	2.5±0.4	27.9±7.0	57.9±11.3	0.9±0.2
		GG5	0.7±0.4	1.2±0.3	1.9±0.1	1.7±0.0	1.1±0.1	40.3±3.6	2.3±0.1	17.3±1.8	38.4±3.5	0.6±0.1
	Indian River Lagoon	CR	1.3±1.1	1.7±0.8	3.0±1.8	1.5±0.3	1.7±0.8	49.2±1.6	1.8±0.4	29.3±5.9	46.3±3.4	0.4±0.1
	Reefs	BTR	1.5±0.1	2.5±0.5	4.0±0.5	0.9±0.1	4.3±0.8	48.6±8.9	4.9±0.8	10.8±3.2	44.6±8.4	4.0±0.7
		SLR_N	0.6±0.3	2.3±0.4	2.84±0.4	0.5±0.1	5.8±1.3	42.6±4.2	0.9±0.0	47.6±4.4	39.7±4.1	0.4±0.1
		SLR_S	1.2±0.3	2.8±1.3	4.0±1.1	0.3±0.0	11.9±2.4	45.4±5.0	0.6±0.1	79.7±14.7	41.4±5.5	0.3±0.1
	Wet 1 Event Mean		**7.2±1.4**	**3.8±0.8**	**11.0±1.8**	**3.3±0.6**	**24.7±9.3**	**69.8±3.9**	**4.5±0.7**	**36.0±5.5**	**58.8± 2.9**	**1.2±0.2**
Wet 2 (September 2015)	Primary Canals	C23W	14.1±0.3	0.3±0.2	14.4±0.3	15.9±0.5	0.9±0.0	124.1±0.4	17.8±0.3	7.0±0.1	109.7±0.6	1.9±0.2
		C44W	17.5±0.1	13.7±0.0	31.2±0.2	8.6±0.2	3.6±0.1	106.8±0.8	11.3±0.1	9.5±0.2	75.6±0.7	2.7±0.1
	Old Palm City	OPC1	6.8±0.4	5.9±0.8	12.7±1.1	0.7±0.1	19.4±0.4	92.0±4.3	1.3±0.2	71.3±6.1	79.3±3.2	0.7±0.1
		OPC2	24.9±0.1	23.3±0.2	48.3±0.2	4.4±0.2	10.9±0.4	126.8±0.5	5.8±0.2	22.0±0.7	78.5±0.3	1.4±0.1
		OPC3	26.1±2.0	0.9±0.1	27.0±1.9	19.3±0.9	1.4±0.1	155.7±2.4	22.5±0.9	6.9±0.2	128.7±1.5	3.2±0.3
		OPC4	9.9±0.3	14.3±0.6	24.3±0.9	5.9±0.1	4.1±0.2	91.6±2.5	7.5±0.4	12.3±0.6	67.4±1.6	1.6±0.5
		OPC5	7.5±0.2	17.5±0.2	25.0±0.4	8.0±0.1	3.1±0.0	107.5±7.5	9.8±0.2	11.0±0.6	82.6±7.1	1.8±0.1
	North Fork	DP	1.9±0.3	17.7±0.3	19.5±0.4	6.3±0.0	3.1±0.1	99.3±0.7	7.8±0.2	12.7±0.2	79.7±0.7	1.5±0.2
		BR	0.5±0.3	0.3±0.2	0.8±0.3	2.4±0.1	0.3±0.1	60.9±1.1	3.5±0.1	17.4±0.5	60.1±1.1	1.1±0.2
	Golden Gates	GG1	18.7±0.7	7.4±1.3	26.0±1.8	2.6±0.1	10.0±1.0	99.8±5.4	4.2±0.1	24.0±1.1	73.8±4.1	1.5±0.1
		GG2	16.9±2.2	22.2±5.3	39.1±7.3	0.7±0.0	55.5±8.7	106.3±15.4	2.3±0.2	46.1±6.1	67.2±8.3	1.6±0.2
		GG3	24.1±1.9	9.8±1.3	33.9±3.1	2.2±0.2	15.3±0.4	97.9±9.9	3.3±0.2	29.9±2.5	64.0±7.0	1.1±0.2
		GG4	12.6±0.3	9.6±0.2	22.2±0.3	3.3±0.1	6.7±0.1	70.7±0.1	3.8±0.1	18.9±0.7	48.5±0.2	0.4±0.1
		GG5	7.9±0.5	14.6±0.7	22.4±1.2	5.7±0.2	4.0±0.0	82.9±3.4	6.3±0.3	13.1±0.2	60.5±2.3	0.7±0.1
	Indian River Lagoon	CR	9.2±0.3	11.1±0.3	20.2±0.4	4.3±0.1	4.7±0.1	67.4±1.0	4.7±0.2	14.3±0.8	47.1±0.6	0.4±0.3
	Reefs	BTR	1.4±0.9	0.0±0.0	1.4±0.9	1.6±0.1	0.9±0.6	27.5±1.2	2.3±0.5	12.9±2.4	26.2±1.9	0.7±0.5
		SLR_N	5.2±0.5	0.35±0.1	5.5±0.5	2.0±0.1	2.8±0.2	40.0±6.5	2.2±0.1	17.7±1.9	34.5±6.8	0.2±0.2
		SLR_S	2.7±1.0	1.2±0.7	3.8±1.3	1.3±0.1	3.0±1.2	32.2±1.5	1.6±0.1	21.0±2.2	28.5±0.5	0.2±0.1
	Wet 2 Event Mean		**11.5±1.1**	**9.4±1.1**	**21.0±1.8**	**5.3±0.7**	**8.3±1.8**	**88.3±4.6**	**6.5±0.8**	**20.4±2.2**	**67.3± 3.5**	**1.3±0.1**
Overall Wet Season Mean - All Sites			**9.4±0.9**	**6.6±0.7**	**16.0±1.4**	**4.3±0.5**	**16.5±4.8**	**79.0±3.2**	**5.5±0.5**	**28.2±3.0**	**63.1± 2.3**	**1.2±0.1**
Wet Season Means by Region	**Primary Canals**	C23W, C44W	**8.4±2.3**	**4.4±1.7**	**12.7±3.5**	**7.5±1.7**	**1.6±0.4**	**88.1±8.7**	**9.3±1.7**	**11.8±1.1**	**75.3±6.5**	**1.8±0.2**
	Old Palm City	OPC1-5	**11.7±1.7**	**7.3±1.4**	**19.0±2.4**	**7.5±1.2**	**6.5±1.4**	**98.5±35.0**	**9.3±1.4**	**21.4±4.0**	**79.5±5.0**	**1.8±0.2**
	Sewered North Fork	DP	**1.2±0.4**	**13.3±3.2**	**14.5±3.5**	**4.8±0.7**	**2.9±0.8**	**86.6±5.8**	**6.1±0.8**	**14.7±0.9**	**72.2±4.0**	**1.3±0.1**
	Septic North Fork	BR	**0.4±0.2**	**0.5±0.2**	**1.0±0.3**	**2.7±0.1**	**0.4±0.1**	**58.3±1.3**	**3.8±0.2**	**15.5±0.9**	**57.3±1.5**	**1.1±0.1**
	Golden Gates	GG1-5	**16.0±1.9**	**9.8±1.5**	**25.9±2.6**	**2.2±0.3**	**48.0±16.0**	**86.5±4.5**	**3.0±0.3**	**46.1±8.5**	**60.7±2.8**	**0.9±0.1**
	Indian River Lagoon	CR	**5.2±1.8**	**6.4±2.1**	**11.6±4.0**	**2.9±0.7**	**3.2±0.7**	**58.3±4.1**	**3.3±0.7**	**21.8±4.3**	**46.7±1.6**	**0.4±0.1**
	Reefs	BTR, SLRN/S	**2.1±0.4**	**1.5±0.3**	**3.6±0.4**	**1.1±0.1**	**4.8±1.0**	**39.4±2.5**	**2.1±0.4**	**31.6±6.4**	**35.8±2.4**	**1.0±0.4**

Table 6C. A comparison of comprehensive means (± S.E.) for combined Wet and Dry season surface water dissolved nutrient concentrations at 18 sites throughout the St. Lucie Estuary and nearshore reefs broken sampling site and region.

Sampling Event	Region	Site	Ammonium (µM)	Nitrate (µM)	DIN (µM)	SRP (µM)	DIN:SRP Ratio	TDN (µM)	TDP (µM)	TDN:TDP Ratio	DON (µM)	DOP (µM)
Comprehensive Project Means by Site	Primary Canals	C23W	4.45+1.7	2.5+0.7	7.0+1.5	6.2+1.7	1.8+0.5	83.1+7.2	7.2+1.9	18.2+3.1	76.2+6.0	1.1+0.2
		C44W	4.7+2.2	11.5+2.0	16.2+3.3	3.2+1.0	8.6+2.1	73.2+6.2	5.1+1.1	18.0+1.8	57.0+3.3	1.9+0.1
	Old Palm City	OPC1	3.3+1.0	2.9+0.7	6.3+1.5	0.4+0.1	20.1+5.5	67.2+4.9	1.1+0.1	62.0+3.4	61.0+4.5	0.8+0.1
		OPC2	11.3+3.0	7.5+2.8	18.7+5.4	4.4+0.9	4.7+1.2	84.2+8.4	5.9+1.1	18.0+2.1	65.5+3.3	1.5+0.2
		OPC3	11.5+3.5	0.7+0.1	12.2+3.5	13.3+1.8	0.7+0.2	120.0+10.3	16.6+1.9	7.6+0.5	107.8+7.3	3.2+0.2
		OPC4	2.7+1.3	6.5+1.8	9.2+2.9	3.5+0.5	2.3+0.6	63.9+5.1	4.6+0.6	15.2+1.1	54.6+2.4	1.1+0.2
		OPC5	3.5+1.0	10.3+2.4	13.8+2.8	4.2+0.7	3.5+0.9	71.9+7.0	5.3+0.8	15.4+1.3	58.1+4.6	1.1+0.1
	North Fork	DP	0.8+0.2	7.1+2.4	7.9+2.6	3.7+0.5	1.7+0.5	66.1+6.8	4.9+0.5	13.6+0.6	58.2+4.7	1.3+0.1
		BR	0.4+0.1	0.6+0.1	1.0+0.1	2.5+0.1	0.4+0.1	50.0+2.6	3.6+0.1	14.0+0.7	49.0+2.7	1.1+0.1
	Golden Gates	GG1	19.7+0.9	14.3+1.9	34.0+2.5	0.8+0.3	150.9+34.7	93.1+5.7	1.8+0.4	84.5+15.1	59.1+5.0	0.9+0.1
		GG2	12.1+2.0	8.8+2.7	20.9+4.2	0.4+0.1	67.1+13.4	86.2+6.7	1.6+0.2	60.2+7.9	65.3+3.6	1.2+0.1
		GG3	25.8+2.9	4.0+1.1	29.9+2.9	2.6+0.3	12.0+0.7	78.6+5.6	3.4+0.3	24.4+1.8	48.7+4.0	0.8+0.1
		GG4	4.7+1.5	3.3+1.2	8.1+2.6	1.8+0.3	3.7+0.7	51.1+5.6	3.0+0.3	18.0+2.5	43.1+4.4	1.2+0.2
		GG5	2.6+0.9	4.4+1.8	7.0+2.7	2.5+0.6	2.2+0.4	44.3+7.1	3.4+0.5	13.4+1.0	37.3+5.0	0.9+0.1
	Indian River Lagoon	CR	3.0+1.1	3.8+1.3	6.8+2.4	1.9+0.4	2.7+0.5	40.4+5.8	3.0+0.4	15.5+2.9	33.7+4.0	1.1+0.3
	Reefs	BTR	0.9+0.3	1.1+0.3	2.0+0.4	0.8+0.2	5.0+1.9	27.5+4.4	2.6+0.5	11.5+0.9	25.6+4.1	1.8+0.4
		SLR_N	2.0+0.7	1.1+0.2	3.0+0.6	0.8+0.2	3.6+0.7	30.8+3.8	1.7+0.2	22.1+4.7	27.7+3.5	0.9+0.2
		SLR_S	1.2+0.4	1.3+0.4	2.5+0.6	0.5+0.2	22.0+12.9	27.5+3.9	1.2+0.1	31.0+9.1	25.0+3.6	0.8+0.2
Comprehensive Project Means by Region	**Primary Canals**	C23W, C44W	**4.6+1.4**	**7.0+1.4**	**11.6+2.0**	**4.7+1.0**	**5.2+1.3**	**78.1+4.8**	**6.2+1.1**	**18.1+1.8**	**66.6+3.9**	**1.5+0.2**
	Old Palm City	OPC1-5	**6.5+1.1**	**5.6+0.9**	**12.1+1.6**	**5.1+0.7**	**6.3+1.4**	**81.4+4.2**	**6.7+0.8**	**23.6+2.7**	**69.4+3.3**	**1.5+0.1**
	Sewered North Fork	DP	**0.8+0.2**	**7.1+2.4**	**7.9+2.6**	**3.7+0.5**	**1.7+0.5**	**66.1+6.8**	**4.9+0.5**	**13.6+0.6**	**58.2+4.7**	**1.3+0.1**
	Septic North Fork	BR	**0.4+0.1**	**0.6+0.1**	**1.0+0.1**	**2.5+0.1**	**0.4+0.1**	**50.0+2.6**	**3.6+0.1**	**14.0+0.7**	**49.0+2.7**	**1.1+0.1**
	Golden Gates	GG1-5	**13.0+1.4**	**7.0+1.0**	**20.0+2.0**	**1.6+0.2**	**47.2+10.3**	**70.7+3.7**	**2.6+0.2**	**40.1+4.9**	**50.7+2.3**	**1.0+0.1**
	Indian River Lagoon	CR	**3.0+1.1**	**3.8+1.3**	**6.8+2.4**	**1.9+0.4**	**2.7+0.5**	**40.4+5.8**	**3.0+0.4**	**15.5+2.9**	**33.7+4.0**	**1.1+0.3**
	Reefs	BTR, SLRN/S	**1.3+0.3**	**1.2+0.2**	**2.5+0.3**	**0.7+0.1**	**10.6+4.7**	**28.6+2.3**	**1.8+0.2**	**21.5+3.6**	**26.1+2.1**	**1.1+0.2**
Overall Project-Wide Means			**6.4+0.6**	**5.1+0.4**	**11.5+0.9**	**3.0+0.3**	**17.5+3.3**	**64.4+2.2**	**4.2+0.3**	**25.7+1.8**	**52.9+1.7**	**1.3+0.1**

3.3.3. Aqueous N Isotopes: The overall means (± S.E.) for surface water aqueous stable isotope values were more enriched for ammonium (δ^{15}N-NH_4; 7.5 ± 0.4 $^{o}/_{oo}$) than nitrate (δ^{15}N-NO_3; 2.5 ±0.4 $^{o}/_{oo}$; Table 7); a trend that remained consistent throughout the study, regardless of season or location (Fig. 12, Table 7). The overall means for the SLE proper were also more enriched for NH_4 (8.3 ± 0.5 $^{o}/_{oo}$) than NO_3 (3.7 ± 0.5 $^{o}/_{oo}$), but both forms had an isotopic signature that fell in the range for wastewater N (> +3 $^{o}/_{oo}$). In the SLE, both ammonium and nitrate were more depleted during the Dry season (6.7 ± 0.7 and 3.2 ± 0.7 $^{o}/_{oo}$, respectively) than in the Wet season (10.0 ± 0.7 and 4.1 ± 0.6 $^{o}/_{oo}$, respectively), but regardless of season, these mean signatures also suggest a predominantly sewage N source. Overall, mean NH_4 and NO_3 values were most enriched at the Golden Gates Estates and Old Palm City sampling complexes where both forms of inorganic N had a strong sewage N signal (Table 7). Although both residential neighborhoods were enriched, Golden Gates Estates retention pond had a stronger wastewater N signal than Old Palm City's All American Ditch during both the Dry and Wet seasons (Fig. 12, Table 7). The most depleted values were generally found in the lower regions (crossroads with the IRL and nearshore reefs), especially for δ^{15}N-NO_3 (Fig. 12). When looking at individual sites, the isotopic signature for NH_4 consistently pointed towards a wastewater N source, whereas the overall signatures for NO_3 varied by site (Table 7). Nitrate had a predominantly wastewater signature (> +3 $^{o}/_{oo}$) in the C-44 canal (C44W), at one site in Old Palm City's All American Ditch (OPC2), the Golden Gates community retention pond (GG1-2), and the Lower Estuary (GG5). A predominantly fertilizer signature was documented for nitrate in the C-23 canal (C23W), all other Old Palm City sites (OPC1, OPC3-5), the Lighthouse community at Dyer Point (DP), Beau Rivage West (BR), and the drainage ditch linking the Golden Gates Estates community retention pond to Willoughby Creek (GG3-4). The crossroads with the Indian River Lagoon and nearshore reefs had a signature that suggested a predominantly atmospheric or fertilizer N source (Table 7).

Table 7. A comparison of mean (± S.E.) surface water aqueous isotope ($\delta^{15}N\text{-}NH_4$ and $\delta^{15}N\text{-}NO_3$) throughout the St. Lucie Estuary broken down by site, region, event, season, and overall project means.

			DRY 1 (April 2015)		DRY 2 (May 2015)		WET 1 (August 2015)		WET 2 (September 2015)	
	Region	Site	$\delta^{15}N\text{-}NH_4$ (‰)	$\delta^{15}N\text{-}NO_3$ (‰)	$\delta^{15}N\text{-}NH_4$ (‰)	$\delta^{15}N\text{-}NO_3$ (‰)	$\delta^{15}N\text{-}NH_4$ (‰)	$\delta^{15}N\text{-}NO_3$ (‰)	$\delta^{15}N\text{-}NH_4$ (‰)	$\delta^{15}N\text{-}NO_3$ (‰)
Event Means	Primary Canals	C23W	10.4±1.3	0.6±1.0	2.9±0.3	1.4±0.1	3.4±0.1	0.1±0.2	7.3±0.3	1.5±0.3
		C44W	8.6±0.8	2.8±0.6	4.6±0.1	4.6±0.1	3.6±0.3	1.3±0.5	11.0±0.3	5.7±0.1
	Old Palm City	OPC1	15.3±0.6	0.8±0.5	-0.5±0.1	2.7±0.7	3.7±0.2	0.0±0.4	4.2±0.1	3.9±0.2
		OPC2	6.5±0.3	11.7±7.3	6.4±0.1	4.4±1.1	15.5±0.0	1.7±0.3	10.6±0.4	11.4±0.2
		OPC3	3.5±0.1	3.1±0.8	2.5±0.1	2.7±0.2	10.8±0.1	1.8±0.4	12.8±0.1	2.2±0.2
		OPC4	2.6±0.1	1.0±0.4	2.1±0.2	1.9±0.6	1.0±1.4	-0.5±1.9	13.5±0.1	6.8±0.2
		OPC5	2.2±0.0	2.5±0.5	4.4±0.2	3.9±0.4	10.7±0.2	-0.1±1.1	11.9±0.1	4.2±0.1
	North Fork	DP	7.5±0.7	1.4±0.7	-1.9±1.8	-0.2±0.3	0.8±1.0	1.9±1.0	5.1±0.8	5.1±0.1
		BR	16.9±1.3	2.6±8.0	0.5±0.1	0.2±0.4	1.7±0.3	-0.2±0.3	1.2±0.1	0.9±0.1
	Golden Gates	GG1	12.9±0.2	12.1±0.2	16.2±0.3	6.5±0.3	18.3±0.1	5.7±3.2	16.8±0.1	14.4±2.5
		GG2	9.9±0.1	5.0±0.0	12.4±0.1	1.8±1.6	10.1±0.2	0.1±0.5	22.2±0.1	12.6±0.1
		GG3	7.9±0.1	-0.9±1.2	10.0±0.1	-2.3±0.2	9.5±0.5	-0.5±0.1	14.6±0.2	5.0±0.1
		GG4	2.2±0.7	-1.0±0.1	2.0±0.0	-1.6±0.4	3.6±1.6	2.3±3.4	13.6±0.1	2.6±0.3
		GG5	5.0±0.4	-1.4±0.4	16.1±0.1	17.7±0.3	15.3±0.1	16.9±0.1	13.7±0.2	-1.0±0.2
	Indian River Lagoon	CR	11.0±15.1	-2.6±0.7	2.7±0.3	-3.6±0.8	6.4±0.5	-3.0±0.8	9.1±0.2	-0.7±0.3
	Reefs	BTR	-	-2.1±1.2	-3.1±0.7	-5.7±0.7	4.5±1.6	2.2±2.7	12.3±1.1	-
		SLR_N	-	-2.0±1.2	-1.3±0.5	-4.5±1.2	6.5±1.7	-0.9±1.4	5.9±0.3	-
		SLR_S	-	2.3±4.9	0.1±1.3	-3.5±0.0	3.6±1.3	3.5±1.6	5.3±0.0	-
	Event Means		**8.1±0.9**	**2.0±0.8**	**4.1±0.8**	**1.6±0.8**	**7.2±0.7**	**1.8±0.6**	**10.7±0.7**	**5.0±0.7**
Regional Means by Season	**Primary Canals**	C23W, C44W	**6.6±1.0**	**2.4±0.6**			**6.3±0.9**	**2.1±0.7**		
	Old Palm City	OPC1-5	**4.5±0.8**	**3.5±0.8**			**9.3±0.9**	**3.1±0.7**		
	Sewered North Fork	DP	**2.8±2.3**	**0.6±0.5**			**3.0±1.1**	**3.5±0.8**		
	Septic North Fork	BR	**8.7±3.7**	**1.7±4.5**			**1.4±4.2**	**0.3±0.3**		
	Golden Gates	GG1-5	**9.4±0.9**	**3.8±1.3**			**13.8±0.9**	**5.8±1.2**		
	Indian River Lagoon	CR	**6.1±5.2**	**-3.1±0.5**			**7.8±0.7**	**-1.9±0.6**		
	Reefs	BTR, SLRN/S	**-1.4±0.6**	**-2.5±1.0**			**6.7±0.8**	**1.6±1.2**		
Overall Mean by Site	Primary Canals	C23W	**6.0±1.0**	**0.9±0.3**						
		C44W	**6.9±0.9**	**3.6±0.5**						
	Old Palm City	OPC1	**5.7±1.8**	**1.9±0.5**						
		OPC2	**9.8±1.1**	**7.3±2.1**						
		OPC3	**7.4±1.4**	**2.5±0.2**						
		OPC4	**4.0±1.5**	**2.3±0.9**						
		OPC5	**7.3±1.2**	**2.6±0.6**						
	North Fork	DP	**2.9±1.2**	**2.1±0.6**						
		BR	**5.1±2.1**	**0.9±1.9**						
	Golden Gates	GG1	**16.0±0.6**	**9.7±1.4**						
		GG2	**13.7±1.7**	**5.1±1.6**						
		GG3	**10.5±0.8**	**0.3±0.9**						
		GG4	**5.4±1.5**	**0.7±1.0**						
		GG5	**12.5±1.3**	**8.1±2.8**						
	Indian River Lagoon	CR	**7.0±2.3**	**-2.5±0.4**						
	Reefs	BTR	**4.6±2.3**	**-1.9±1.4**						
		SLR_N	**3.7±1.4**	**-2.5±0.8**						
		SLR_S	**3.6±1.2**	**1.3±2.0**						
Overall Means by Region	**Primary Canals**	C23W, C44W	**6.5±0.7**	**2.3±0.4**						
	Old Palm City	OPC1-5	**6.9±0.7**	**3.3±0.5**						
	Sewered North Fork	DP	**2.9±1.2**	**2.1±0.6**						
	Septic North Fork	BR	**5.1±2.1**	**0.9±1.9**						
	Golden Gates	GG1-5	**11.6±0.7**	**4.9±0.9**						
	Indian River Lagoon	CR	**7.0±2.3**	**-2.5±0.4**						
	Reefs	BTR, SLRN/S	**4.0±0.9**	**-1.1±0.9**						
Overall Project Mean			**7.5±0.4**	**2.5±0.4**						

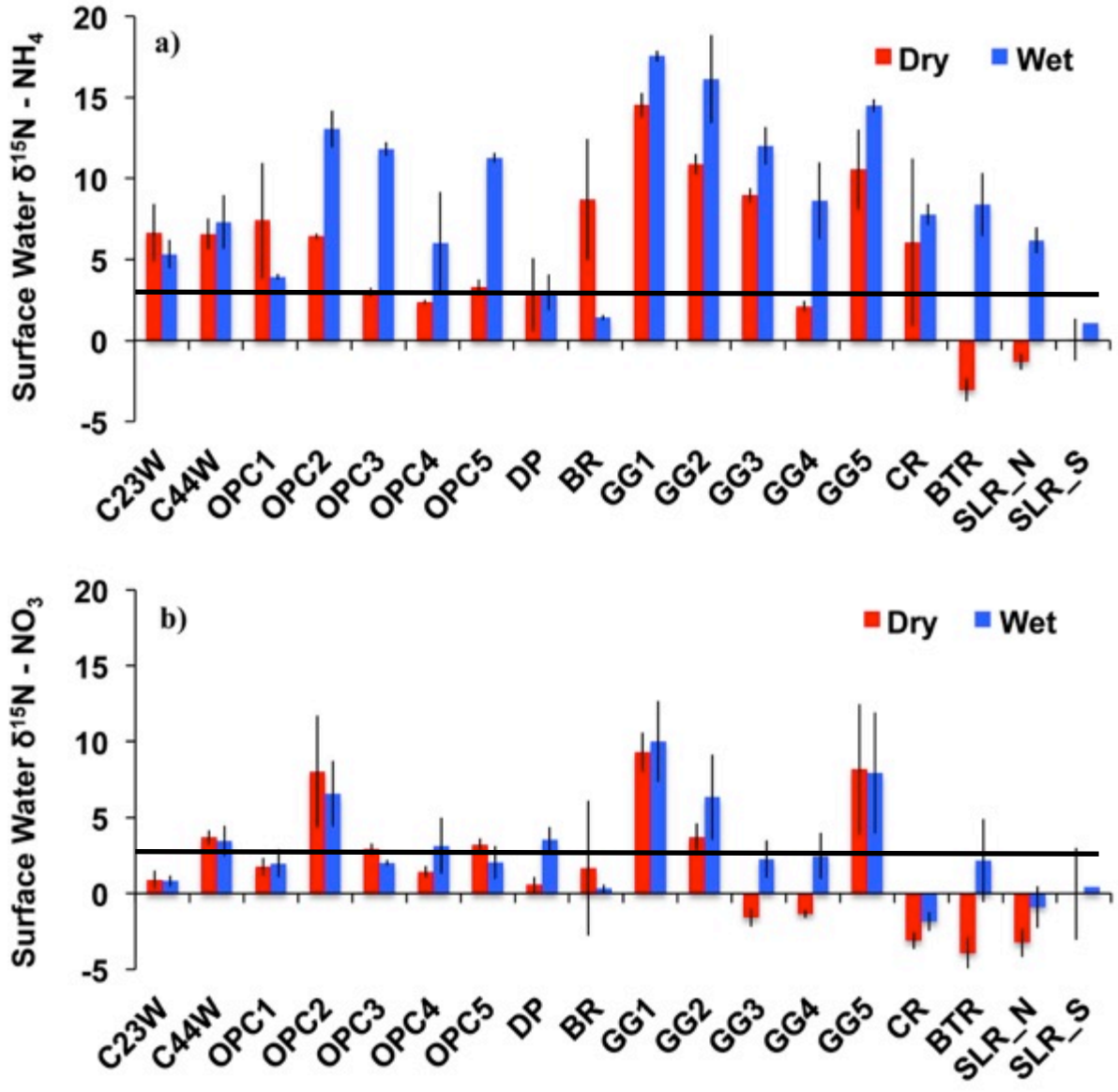

Fig 12. Stable isotopic ratios ($^{o}/_{oo}$ ± S.E.) for a) δ^{15}N-NH_4 and b) δ^{15}N-NO_3 in in surface water at 18 sites located in the primary canals (C23W, C44W), St. Lucie Estuary (OPC1-5, DP, BR, GG1-5), crossroads with the Indian River Lagoon (CR), and nearshore reefs (BTR, SLR-N, SLR-S) during the 2015 Dry and Wet seasons.

3.3.4. Sucralose and Acetaminophen: Sucralose was detected at each of the five surface water sites in Old Palm City and Golden Gates Estates during the Dry and Wet seasons. The highest concentrations were documented in Old Palm City's All American Ditch (OPC2) and just downstream of the Golden Gates Estates retention pond (GG2 and GG3; Fig. 13). The lowest concentrations were seen in the furthest downstream sites, OPC5 in the South Fork and GG5 in the Lower Estuary. This dilution effect was especially noticeable within the Golden Gates Estates sampling complex (Fig. 13). During the Dry season the sucralose concentrations ranged from 0.1 to 1.3 µg/L at Old Palm City and from 0.1 to 5.5 µg/L at Golden Gates Estates. In the Wet season, concentrations ranged from 0.1 to 4.9 µg/L at Old Palm City and from just above detection (0.03 µg/L) to 5.4 µg/L.

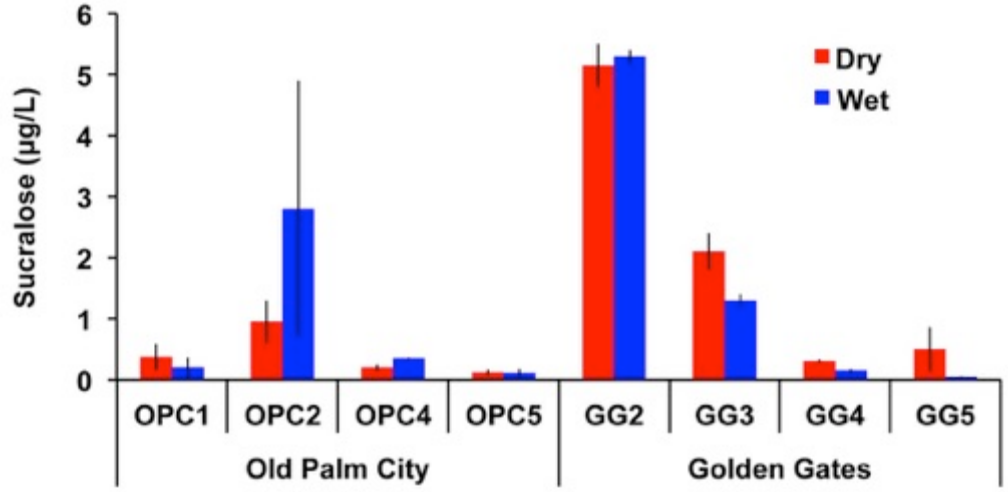

Fig.13. Mean sucralose concentrations (± S.E.) during the Dry and Wet seasons at surface water sites in Old Palm City and Golden Gates Estates.

Acetaminophen was detected in low concentrations at five of the eight surface water sites; three in Old Palm City and two in Golden Gates Estates. During the Dry season trace amounts (0.003 µg/L) were detected at both OPC1 and OPC2 in the All American Ditch and OPC5 (0.005 µg/L) in the South Fork. In the Wet season, acetaminophen was only detectable at OPC5 (0.003 µg/L) and GG2 and GG3 (both 0.003 µg/L) in and just downstream of the Golden Gates Estates retention pond.

3.3.5. Macroalgal Tissue $\delta^{13}C$, $\delta^{15}N$ and C:N:P: The overall project mean (± S.E.) for carbon stable isotopes ($\delta^{13}C$), including all sites from the confluence with the primary canals to the nearshore reefs, was -19.0 ± 0.4 $^{o}/_{oo}$ (Table 8). With the nearshore reefs and IRL removed, the overall project mean (± S.E.) in the SLE was -23.5 ± 0.4 $^{o}/_{oo}$. The seasonal SLE mean was lighter during the Dry season (-24.3 ± 0.6 $^{o}/_{oo}$) than during the Wet season (-22.7 ± 0.5 $^{o}/_{oo}$), which reflected slight differences in carbon sources between the seasonal samplings. An upstream to downstream gradient exists with lighter values near the primary canals, followed by gradually heavier values between the residential sites (Old Palm City and Golden Gates Estates), the crossroads with the IRL, and the nearshore reefs (Fig. 14a; Table 8), with the macroalgae on the

nearshore reefs being the most enriched. This carbon isotope gradient reflects the lighter, more depleted terrestrial dissolved inorganic carbon (DIC) source coming from the lower salinity primary canals to the more enriched marine DIC sources in the coastal waters. Project-wide, the $\delta^{13}C$ values ranged from -28.0 ± 0.3 $^{o}/_{oo}$ (C23E) to -12.1 ± 1.3 $^{o}/_{oo}$ (SLR-N) during the Dry season and -25.8 ± 0.1 $^{o}/_{oo}$ (C23E) to -14.7 ± 0.9 $^{o}/_{oo}$ (BTR) during the Wet season (Table 8).

The overall project mean (± S.E.) for nitrogen stable isotopes ($\delta^{15}N$), including all sites from the confluence with the primary canals to the nearshore reefs, was +4.4 ± 0.1 $^{o}/_{oo}$ (Table 8). With the nearshore reefs and IRL removed, the overall project mean (± S.E.) in the SLE was +5.7 ± 0.2 $^{o}/_{oo}$. The seasonal SLE mean was more depleted during the Dry season (+5.1 ± 0.2 $^{o}/_{oo}$) than during the Wet season (+6.3 ± 0.2 $^{o}/_{oo}$). The overall project mean for macroalgae collected on the nearshore reefs was more depleted (+3.5 ± 0.1 $^{o}/_{oo}$) than the SLE concentrations, but the signature on the nearshore reefs was still consistently > +3 $^{o}/_{oo}$, the lower threshold for wastewater N. With the exception of the confluence of the C-23 and the North Fork (C23E), the concentrations were significantly more enriched during the Wet season than the Dry season (Fig. 14b, Table 8). When comparing areas just downstream of the primary canals, residential areas, the crossroads with the IRL, and nearshore reefs, the $\delta^{15}N$ values were most enriched at the residential sites, followed by the outflow of the primary canals, IRL, and nearshore reefs, respectively (Table 8). Project-wide the $\delta^{15}N$ values ranged from +2.2 ± 0.1 $^{o}/_{oo}$ (SLR-S) to +6.5 ± 0.4 $^{o}/_{oo}$ (OPC5) during the Dry season and +3.7 ± 0.2 $^{o}/_{oo}$ (SLR-N) to +8.0 ± 1.0 $^{o}/_{oo}$ (GG5) during the Wet season.

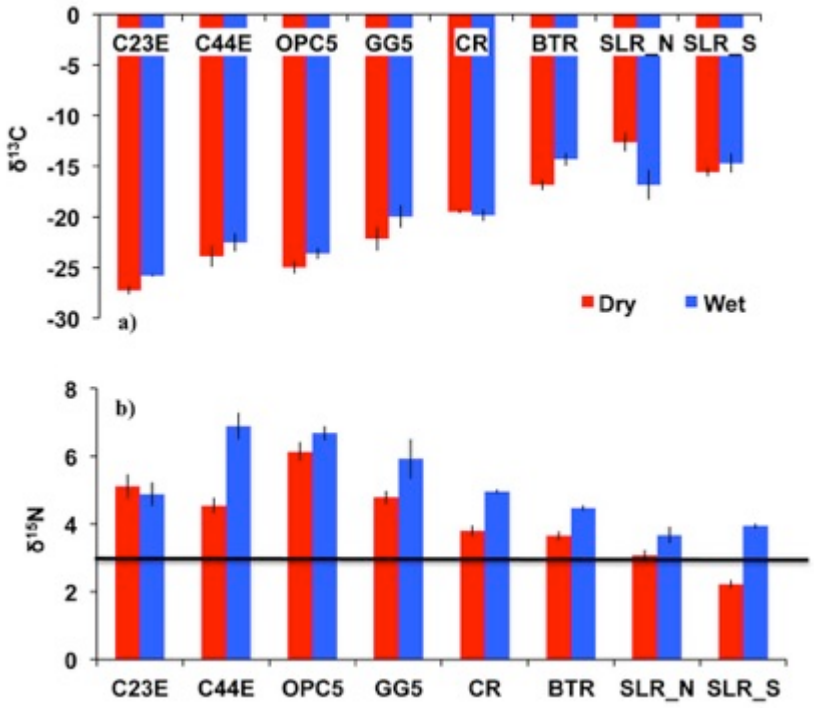

Fig. 14. A comparison of mean stable isotopic ratios ($^{o}/_{oo}$ ± S.E.) in macroalgae collected throughout the St. Lucie Estuary and nearshore reefs broken down by sampling season. The black line represents the lower $\delta^{15}N$ threshold (+3 $^{o}/_{oo}$) for wastewater N.

The overall mean (± S.E.) C:N ratio for all sites and all sampling events was 15.2 ± 0.4 (Table 8). The ratios were generally the same in the SLE and crossroads with the IRL (~12) and both were significantly lower there than the ratios documented on the nearshore reefs (~18; Fig. 15a, Table 8). The overall mean (± S.E.) in the SLE (no IRL or nearshore reef sites) decreased to 11.8 ± 0.5. The overall seasonal SLE mean for C:N did not vary between the Dry (11.9 ± 0.4) and Wet (11.8 ± 0.8) seasons. However, at the site level, lower C:N ratios (higher N inputs) in the Wet season were documented in the lower system (Golden Gates Estates, crossroads with the IRL, and the nearshore reefs; Fig. 15a).

The mean (± S.E.) project-wide C:P ratio for all sites and all sampling events was 420 ± 17 (Table 8). The mean C:P ratios were lowest (highest concentrations of P) in the SLE, slightly higher in the crossroads with the IRL, and highest (lowest concentrations of P) on the nearshore reefs (Fig. 15b, Table 8). Unlike C:N ratios, C:P ratios gradually increased in an upstream (near canals) to downstream (nearshore reefs) gradient, again suggesting exposure to higher P concentrations near the canals and in the SLE than on the reefs (Fig. 15b, Table 8). The overall mean (± S.E.) in the SLE (no IRL or nearshore reef sites) was 233 ± 17; a value significantly lower than the overall project-wide mean. The overall seasonal SLE mean for C:P was slightly lower during the Dry (224 ± 24) season than during the Wet (243 ± 23) season. However, when looking at individual sites, most sites showed lower C:P ratios (higher P concentrations) during the Wet season, especially near the C-44 canal, crossroads with the IRL, and the southern nearshore reefs in St. Lucie Inlet Preserve State Park (Fig. 15b).

The project-wide N:P ratio remained at ~27 throughout the study, with lower N:P ratios in the SLE (suggesting N-limitation) than in the crossroads with the IRL and nearshore reefs (suggesting P-limitation; Table 8). In the SLE, the mean overall project N:P ratio was 20.0, with lower mean ratios during the Dry season (19.0) than during the Wet season (21.2). The lowest N:P ratios were recorded near the confluence of the C-23 canal and the North Fork at C-23E (~10 in both the Dry and Wet seasons), suggesting N-limiting conditions or higher P inputs from this canal (Fig. 15c, Table 8). All other sites in the study had comprehensive N:P ratios >22, indicating P-limitation (Table 8). An increasing trend in the ratio between the areas near the canals, residential sites, and crossroads with the IRL, respectively was seen during both sampling seasons. The mean N:P ratios at the nearshore reef sites were consistently just below those recorded at the crossroads (CR) in the IRL near St. Lucie Inlet.

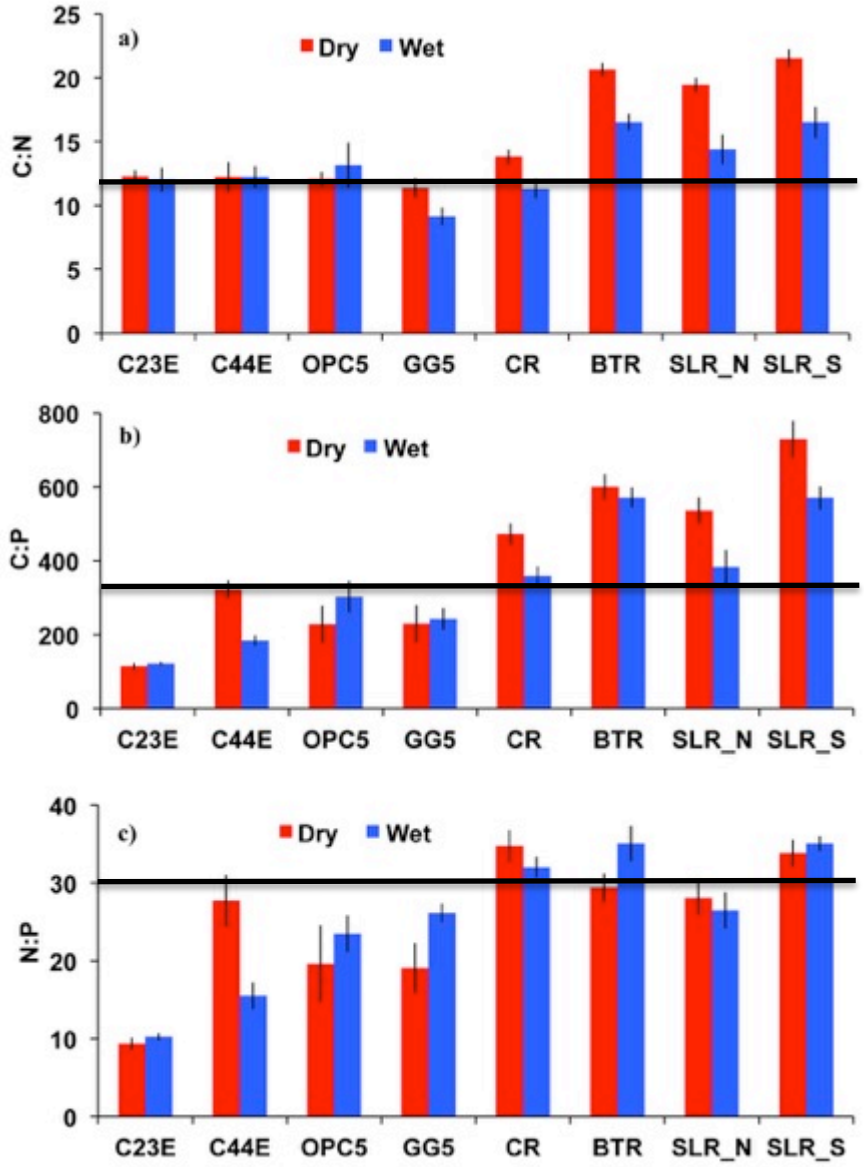

Fig. 15. A comparison of mean (± S.E.) C:N:P ratios in macroalgae collected throughout the St. Lucie Estuary and nearshore reefs broken down by sampling season. The black lines represent difference between N and P limitation where C:N > 12 represents N-limitation, C:P >360 represents P-limitation, N:P > 30 represents P-limitation, and N:P < 30 represents N-limitation.

Table 8. Mean (± S.E.) stable isotope concentrations and elemental C, N, and P ratios for macroalgae collected in the St. Lucie Estuary and nearshore reefs. Near canal means include locations just downstream of the water control structures in the primary canals. Residential means include the downstream end of the Old Palm City and Golden Gates Estates sampling networks. Indian River represents the crossroads between the Indian River Lagoon and St. Lucie Estuary. Nearshore reef means are for the three reef sites.

Sampling Event	Region	Site	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	%C	%N	%P	C:N Ratio	C:P Ratio	N:P Ratio
Dry 1 (April 2015)	Near Canals	C23E	-28.0±0.3	5.8±0.3	29.2±0.6	2.8±0.2	0.6±0.0	12.3±1.0	132±3	10.9±1.6
		C44E	-21.6±0.4	4.4±0.5	16.6±4.6	1.3±0.2	0.1±0.0	14.3±1.6	307±42	21.9±2.0
	Residential	OPC5	-24.3±0.6	6.5±0.4	29.4±2.1	3.0±0.4	0.2±0.0	11.7±1.0	340±20	29.9±2.0
		GG5	-26.8±0.2	5.1±0.0	21.6±0.7	2.3±0.1	0.5±0.0	11.3±0.5	114±8	10.1±2.0
	Indian River	CR	-19.3±0.2	3.8±0.3	22.2±0.4	2.0±0.1	0.1±0.0	13.1±0.9	520±42	39.8±2.0
	Reefs	BTR	-18.8±1.0	3.4±0.3	28.8±1.6	1.7±0.0	0.1±0.0	19.6±1.3	586±74	30.7±2.0
		SLR_N	-12.1±1.3	2.9±0.3	23.1±0.9	1.5±0.1	0.1±0.0	18.2±0.4	593±32	32.8±2.0
		SLR_S	-15.5±0.8	2.3±0.3	27.5±2.2	1.5±0.1	0.1±0.0	22.0±0.5	860±59	39.2±4.7
	Dry 1 Event Mean		**-19.7±0.9**	**4.0±0.2**	**24.8±0.9**	**1.9±0.1**	**0.2±0.0**	**15.8±0.7**	**468±41**	**29.0±2.0**
Dry 2 (May 2015)	Near Canals	C23E	-26.5±0.4	4.4±0.3	26.7±0.5	2.6±0.1	0.7±0.0	12.3±0.4	96±3	7.9±0.1
		C44E	-26.2±0.3	4.7±0.1	30.8±2.1	3.5±0.2	0.2±0.0	10.2±0.2	340±24	33.6±3.0
	Residential	OPC5	-25.7±1.1	5.7±0.3	27.2±1.2	2.6±0.0	0.6±0.0	12.5±0.7	117±5	9.4±0.7
		GG5	-19.98±0.2	4.6±0.3	23.9±1.1	2.5±0.2	0.3±0.0	11.4±1.1	287±65	23.5±3.6
	Indian River	CR	-19.6±0.3	3.8±0.2	24.1±1.1	2.0±0.2	0.2±0.0	14.4±0.7	432±36	30.4±2.6
	Reefs	BTR	-15.9±0.3	3.8±0.1	31.2±1.6	1.7±0.1	0.1±0.0	21.1±0.4	607±38	28.8±1.8
		SLR_N	-13.1±1.4	3.2±0.2	23.5±2.0	1.3±0.1	0.1±0.0	20.5±0.8	489±56	24.0±2.8
		SLR_S	-15.6±0.6	2.2±0.1	23.5±1.1	1.3±0.1	0.1±0.0	21.3±1.1	665±51	31.2±1.5
	Dry 2 Event Mean		**-18.4±0.7**	**3.8±0.1**	**26.5±0.7**	**2.0±0.1**	**0.2±0.0**	**17.0±0.7**	**449±31**	**25.7±1.4**
Dry Season Means by Region	**Near Canals**	**C23E, C44E**	**-25.6±0.7**	**4.8±0.2**	**25.8±2.0**	**2.6±0.3**	**0.4±0.1**	**12.3±0.6**	**219±34**	**18.5±3.2**
	Residential	**OPC5, GG5**	**-23.3±0.8**	**5.3±0.2**	**25.2±0.9**	**2.6±0.1**	**0.4±0.1**	**11.7±0.5**	**229±36**	**19.3±2.6**
	Indian River	**CR**	**-19.5±0.2**	**3.8±0.2**	**23.2±0.7**	**2.0±0.1**	**0.1±0.0**	**13.8±0.6**	**472±29**	**34.7±2.1**
	Reefs	**BTR, SLRN/S**	**-15.2±0.5**	**3.1±0.1**	**26.9±0.9**	**1.5±0.1**	**0.1±0.0**	**20.5±0.4**	**609±24**	**30.0±1.1**
Project-Wide Dry Season Mean			**-19.0±0.6**	**3.9±0.1**	**25.8±0.6**	**2.0±0.1**	**0.2±0.0**	**16.5±0.5**	**457±25**	**27.0±1.2**
Wet 1 (August 2015)	Near Canals	C23E	-25.8±0.1	4.9±0.4	27.2±0.1	2.7±0.2	0.6±0.0	12.0±0.9	122±5	10.3±0.5
		C44E	-22.8±1.3	6.8±0.5	28.3±0.4	2.6±0.1	0.4±0.1	12.9±0.7	186±18	14.7±1.9
	Residential	OPC5	-23.6±0.7	6.3±0.2	31.6±0.8	2.8±0.4	0.4±0.1	15.1±1.8	318±56	19.9±2.1
		GG5	-18.4±0.5	5.1±0.2	17.7±1.8	2.3±0.1	0.2±0.0	9.2±1.0	251±41	26.7±1.5
	Indian River	CR	-19.0±0.6	4.9±0.4	22.1±1.4	2.1±0.0	0.2±0.0	12.3±0.8	375±36	30.5±1.6
	Reefs	BTR	-14.7±0.9	3.9±0.1	22.2±1.7	1.7±0.2	0.1±0.0	16.5±1.2	570±32	35.1±1.0
		SLR_N	-16.8±1.5	3.7±0.2	19.9±1.9	1.8±0.2	0.1±0.0	14.4±1.2	383±46	26.5±2.3
		SLR_S	-14.3±0.6	4.5±0.1	26.6±1.4	1.9±0.1	0.1±0.0	16.5±0.7	571±27	35.1±2.3
	Wet 1 Event Mean		**-18.3±0.6**	**4.8±0.2**	**24.1±0.8**	**2.1±0.1**	**0.2±0.0**	**14.3±0.5**	**393±24**	**26.8±1.4**
Wet 2 (September 2015)	Near Canals	C44E	-21.4±0.0	7.2±0.0	14.2±0.0	1.8±0.0	0.2±0.0	9.5±0.0	178±0	18.9±0.0
	Residential	OPC5	-23.5±0.1	7.7±0.1	29.3±0.4	4.3±0.1	0.3±0.0	8.0±0.1	262±5	33.0±0.6
		GG5	-23.8±1.8	8.0±1.0	25.4±1.4	3.4±0.2	0.3±0.0	8.9±1.1	221±13	24.9±1.6
	Indian River	CR	-21.5±0.2	5.1±0.2	24.8±0.9	3.1±0.2	0.2±0.0	9.3±0.2	326±8	35.2±0.3
	Wet 2 Event Mean		**-22.7±0.5**	**6.8±0.5**	**25.2±1.6**	**3.4±0.3**	**0.3±0.0**	**8.8±0.3**	**265±18**	**30.4±2.0**
Wet Season Means by Region	**Near Canals**	**C23E, C44E**	**-23.7±0.8**	**6.1±0.5**	**26.1±1.7**	**2.5±0.1**	**0.5±0.1**	**12.1±0.6**	**161±14.2**	**13.6±1.4**
	Residential	**OPC5, GG5**	**-22.2±0.7**	**6.4±0.3**	**26.7±1.5**	**3.0±0.3**	**0.3±0.0**	**11.6±1.2**	**279±29**	**24.5±1.5**
	Indian River	**CR**	**-19.9±0.6**	**5.0±0.1**	**23.0±1.0**	**2.5±0.2**	**0.2±0.0**	**11.3±0.7**	**359±25**	**32.1±1.3**
	Reefs	**BTR, SLRN/S**	**-15.4±0.7**	**4.0±0.1**	**22.6±1.1**	**1.8±0.1**	**0.1±0.0**	**15.7±0.6**	**496±28**	**31.6±1.4**
Project-Wide Wet Season Mean			**-18.9±0.6**	**5.1±0.2**	**24.2±0.7**	**2.3±0.1**	**0.2±0.0**	**13.5±0.5**	**376±22**	**27.5±1.1**
Comprehensive Project Means by Site	**Near Canals**	**C23E**	**-26.8±0.4**	**5.0±0.3**	**27.7±0.4**	**2.7±0.1**	**0.6±0.0**	**12.2±0.4**	**117±6**	**9.7±0.5**
		C44E	**-23.3±0.7**	**5.6±0.4**	**24.5±2.4**	**2.4±0.3**	**0.3±0.0**	**12.2±0.7**	**260±26**	**22.2±2.7**
	Residential	**OPC5**	**-24.1±0.4**	**6.5±0.2**	**30.0±0.6**	**3.1±0.3**	**0.4±0.1**	**12.8±1.1**	**276±33**	**22.1±2.3**
		GG5	**-21.2±0.8**	**5.3±0.3**	**21.7±1.0**	**2.5±0.1**	**0.3±0.0**	**10.4±0.6**	**235±30.6**	**22.2±2.0**
	Indian River	**CR**	**-19.62±0.3**	**4.3±0.2**	**23.1±0.6**	**2.2±0.1**	**0.2±0.0**	**12.8±0.5**	**426±23**	**33.6±1.3**
	Reefs	**BTR**	**-16.0±0.5**	**3.9±0.1**	**29.1±1.0**	**1.8±0.1**	**0.1±0.0**	**19.3±0.6**	**591±24**	**31.3±1.5**
		SLR_N	**-14.6±0.9**	**3.4±0.2**	**21.7±1.1**	**1.6±0.1**	**0.1±0.0**	**17.0±0.8**	**463±32**	**27.3±1.5**
		SLR_S	**-15.1±0.5**	**3.1±0.2**	**23.5±1.0**	**1.5±0.1**	**0.1±0.0**	**19.0±0.9**	**650±34**	**34.5±1.0**
Comprehensive Project Means By Region	**Near Canals**	**C23E, C44E**	**-24.8±0.6**	**5.4±0.3**	**26.0±1.3**	**2.5±0.2**	**0.4±0.1**	**12.2±0.4**	**196±22**	**16.5±2.1**
	Residential	**OPC5, GG5**	**-22.7±0.5**	**5.9±0.2**	**26.0±0.9**	**2.8±0.2**	**0.3±0.0**	**11.6±0.7**	**256±23**	**22.2±1.5**
	Indian River	**CR**	**-19.62±0.3**	**4.3±0.2**	**23.1±0.6**	**2.2±0.1**	**0.2±0.0**	**12.8±0.5**	**426±23**	**33.6±1.3**
	Reefs	**BTR, SLRN/S**	**-15.3±0.4**	**3.5±0.1**	**25.0±0.7**	**1.7±0.1**	**0.1±0.0**	**18.4±0.4**	**560±19**	**30.7±0.9**
Overall Project-Wide Means			**-19.0±0.4**	**4.4±0.1**	**25.1±0.5**	**2.1±0.1**	**0.2±0.0**	**15.2±0.4**	**420±17**	**27.2±0.8**

3.3.6. Phytoplankton Tissue $\delta^{13}C$, $\delta^{15}N$ and C:N:P: The overall project mean (± S.E.) for carbon stable isotopes ($\delta^{13}C$) at all 18 collection sites was -26.9 ± 0.3 $^{o}/_{oo}$ (Table 9C). With the canals, IRL, and nearshore reefs removed, the overall project mean (± S.E.) in the SLE was -28.2 ± 0.3 $^{o}/_{oo}$. When broken down by season, the SLE mean (± S.E.) was heavier during the Dry (-27.6 ± 0.4 $^{o}/_{oo}$) than during the Wet (-28.8 ± 0.4 $^{o}/_{oo}$) season, reflecting the more enriched marine DIC in the Dry season with less rainfall. Overall, the lightest values were recorded in the Beau Rivage (BR) West, Lighthouse at Dyer Point (DP) and Old Palm City communities and the heaviest values were recorded at the crossroads with the IRL and the nearshore reefs (Table 9C). At the Golden Gates Estates sampling complex (GG1-GG5) an upstream to downstream gradient of lighter values in the community retention pond to heavier values in the Lower Estuary was consistently seen throughout the study. Again, this reflects the lighter terrestrial DIC in upstream, lower salinity areas and more enriched downstream values associated with higher salinity and enriched marine DIC sources (Fig. 16a).

The overall project mean (± S.E.) for nitrogen stable isotopes ($\delta^{15}N$) at all 18 collection sites was +5.0 ± 0.3 $^{o}/_{oo}$ (Table 9C). When broken down by season, the project-wide mean (± S.E.) did not change between the Dry (+5.0 ± 0.4 $^{o}/_{oo}$) and Wet (+5.0 ± 0.4 $^{o}/_{oo}$) seasons. With the canals, IRL, and nearshore reefs removed, the overall project mean (± S.E.) in the SLE was +5.2 ± 0.4 $^{o}/_{oo}$. The seasonal SLE means were similar in the Dry (+5.3 ± 0.5 $^{o}/_{oo}$) and Wet (+5.1 ± 0.5 $^{o}/_{oo}$) seasons. The overall project mean isotopic signature for phytoplankton collected on the nearshore reefs was more depleted (4.8 ± 1.2 $^{o}/_{oo}$) than the SLE values, but still consistently > +3 $^{o}/_{oo}$, the lower threshold for wastewater pollution. With the exception of one collection at OPC4, the signature was consistently enriched (+7 to +11 $^{o}/_{oo}$) at GG2 in the Golden Gates community retention pond during both the Dry and Wet seasons (Fig. 16b Table 9A,B,C). Project-wide, the means were > +3 $^{o}/_{oo}$ at each site and ranged from +3.1 $^{o}/_{oo}$ at C-23W, OPC3, and GG3 to +9.6 $^{o}/_{oo}$ at GG2 (Table 9C). When comparing sites in the primary canals, residential areas, the IRL, and nearshore reefs, the $\delta^{15}N$ values were consistently more enriched near the residential sites and nearshore reefs than in the primary canals and IRL (Tables 9A,B,C). Seasonally, the $\delta^{15}N$ values ranged from +1.3 ± 1.3 $^{o}/_{oo}$ (CR) to +12.4 ± 1.1 $^{o}/_{oo}$ (OPC5) during the Dry season and +0.6 ± 0.8 $^{o}/_{oo}$ (OPC5) to +11.1 ± 1.4 $^{o}/_{oo}$ (GG2) during the Wet season.

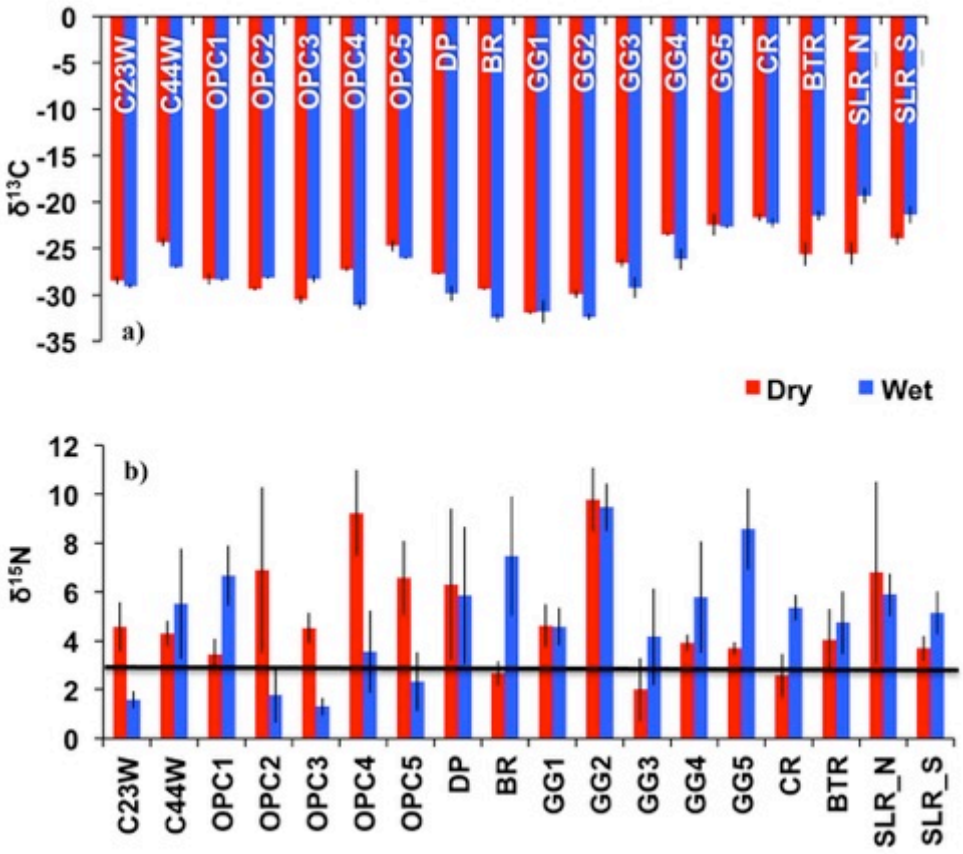

Fig. 16. A comparison of mean stable isotopic ratios ($^{o}/_{oo}$ ± S.E.) in phytoplankton collected in the primary canals, the St. Lucie Estuary, IRL, and nearshore reefs broken down by sampling season. The black line represents the lower $\delta^{15}N$ threshold (+3 $^{o}/_{oo}$) for wastewater N.

The mean (± S.E.) project-wide C:N ratio for all sites and all sampling events was 8.7 ± 0.3 (Table 9C). With the canals, IRL, and nearshore reefs removed, the overall C:N mean (± S.E.) in the SLE was nearly the same (8.9 ± 0.3). Both project-wide and SLE means suggest N-limiting conditions as C:N ratios were > 6.6. Project-wide, the mean C:N ratios were consistently lower, indicating more dissolved N, in Wet season (6.8 ± 0.1) than in the Dry season (10.5 ± 0.4; Fig. 17a; Tables 9A,B). The lowest C:N ratios (highest N concentrations) were in the primary canals during the Dry season and the lower reaches of the study area (Golden Gates, IRL, and nearshore reefs) during the Wet season (Table 8A,B).

The mean (± S.E.) project-wide C:P ratios for all sites and all sampling events was 55 ± 2 (Table 9C). With the canals, IRL, and nearshore reefs removed, the overall C:P mean (± S.E.) in the SLE decreased to 50 ± 2. Neither project-wide nor SLE means suggest P-limiting conditions as C:P ratios were <106. Project-wide, the mean C:P ratios were consistent throughout the Dry (54 ± 3) and Wet (55 ± 2) seasons and the lowest mean C:P ratios (highest P inputs) were consistently seen in the primary canals, at the east end of the All American Ditch (OPC3), and in the Golden Gates Estates community retention pond (GG2; Fig. 17b, Tables 9A,B,C).

The mean (± S.E.) project-wide N:P ratio was 7.1 ± 0.3, with lower N:P ratios, or higher P concentrations, in the Dry (5.5 ± 0.3) than in the Wet (8.6 ± 0.5) season (Fig. 17c, Tables 9A,B,C). In the SLE proper, the overall N:P ratio decreased to 5.9 ± 0.2. All ratios, with the exception of the nearshore reefs during both Wet season samplings have ratios < 16, suggesting predominantly N-limiting conditions in the SLE and fluctuation between N and P limitation just outside of St. Lucie Inlet (Fig. 17c, Table 9B). N:P ratios ranged from 1.4 ± 0.4 in the C-44 canal to 15.6 ± 1.2 at the SLR-S site during the Dry season and from 3.2 ± 0.1 at OPC2 (where the All American Ditch drains to the South Fork tidal creek) to 28.8 ± 3.6 at BTR in the Wet season (Tables 9A,B). The mean (± S.E.) N:P ratios were similar for Old Palm City and Golden Gates Estates in both the Dry (~5) and Wet (~6.5) seasons. Project-wide, there was no difference between the mean N:P ratios at these two residential sites (5.6 at each) and this ratio was similar to that of the primary canals (5.5; Table 9C).

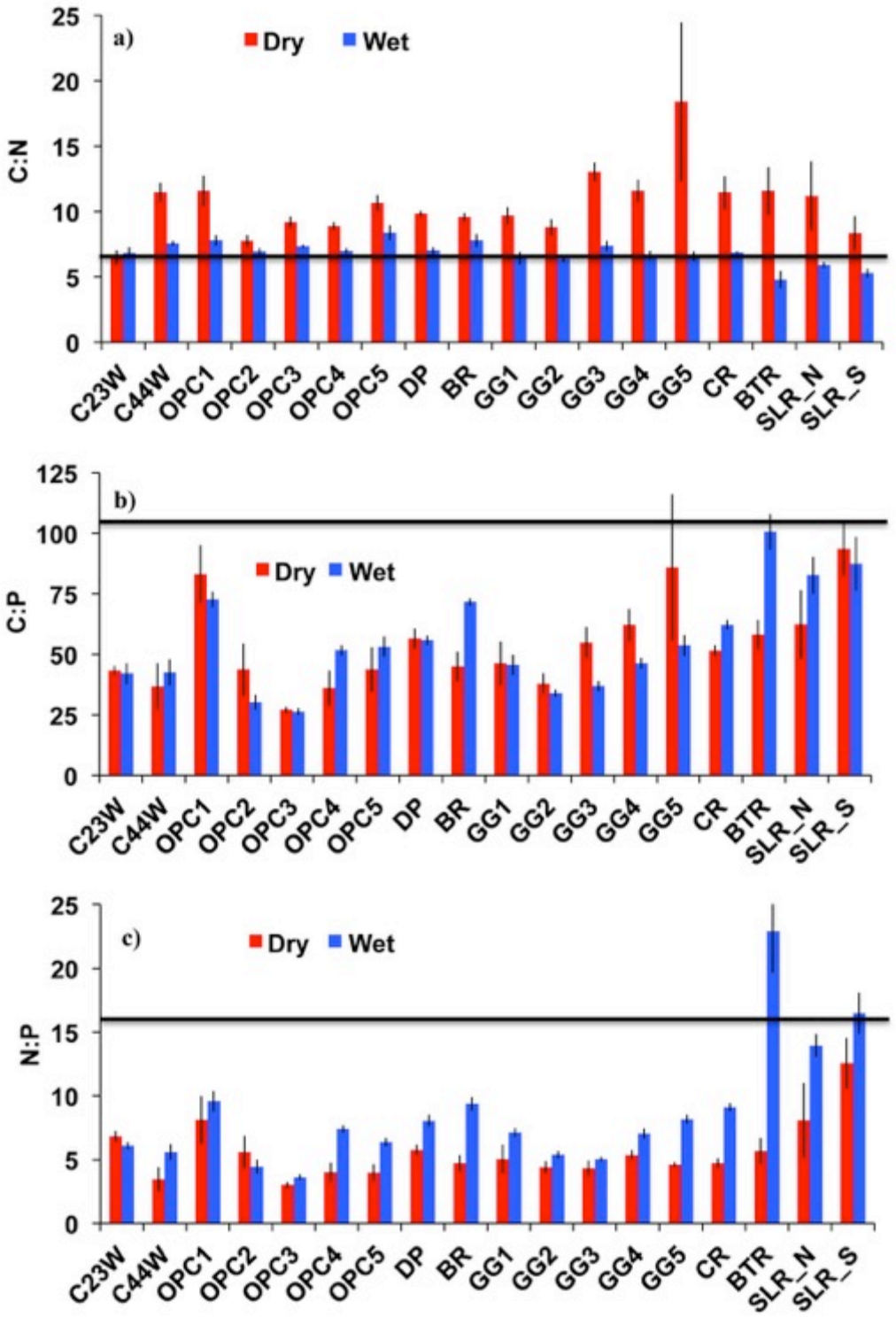

Fig. 17. A comparison of mean (± S.E.) C:N:P ratios in phytoplankton collected in the primary canals, the St. Lucie Estuary, IRL, and nearshore reefs broken down by sampling season. The black lines represent difference between N and P limitation where C:N > 6.6 represents N-limitation, C:P >106 represents P-limitation, N:P > 16 represents P-limitation, and N:P < 16 represents N-limitation.

Table 9A. A comparison of mean (± S.E.) stable isotopes and C:N:P ratios in phytoplankton throughout the St. Lucie Estuary and nearshore reefs broken down by the two Dry season sampling events and region. "Overall Dry Season Mean" combines the two samplings for all 18 sampling sites.

Sampling Event	Region	Site	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C (µg/L)	N (µg/L)	P (µg/L)	C:N Ratio	C:P Ratio	N:P Ratio
Dry 1 (April 2015)	Primary Canals	C23W	-27.5+0.2	4.1+1.4	97.1+5.2	21.1+0.2	6.2+0.4	5.4+0.5	41+1	7.6+0.5
		C44W	-25.1+0.6	4.1+0.1	368.6+43.8	34.9+3.3	70.0+21.5	12.5+2.2	16+4	1.4+0.4
	Old Palm City	OPC1	-27.0+0.1	3.3+0.2	1032.7+94.9	85.4+6.9	47.2+6.6	14.1+0.3	59+9	4.1+0.6
		OPC2	-28.9+0.0	10.3+6.4	176.7+8.8	28.6+1.8	24.8+6.2	7.3+1.3	20+4	2.9+0.7
		OPC3	-29.6+0.2	4.7+0.7	569.0+35.0	66.3+2.0	58.4+5.2	10.0+0.6	25+1	2.5+0.2
		OPC4	-27.7+0.2	12.4+1.1	301.5+18.9	41.6+1.1	39.4+3.3	8.5+0.6	20+3	2.4+0.2
		OPC5	-26.0+0.2	7.1+3.3	333.5+22.9	41.7+4.2	35.9+0.9	9.4+1.1	24+1	2.6+0.3
	North Fork	DP	-27.5+0.1	3.8+1.6	274.2+19.4	31.4+2.1	13.8+0.9	10.2+0.2	52+7	5.1+0.2
		BR	-29.3+0.3	3.2+0.8	325.2+69.2	39.6+7.1	24.0+1.0	9.6+1.1	35+9	3.7+0.7
	Golden Gates	GG1	-31.7+0.1	4.9+0.3	332.5+16.5	37.1+1.8	31.9+1.9	10.5+0.2	27+1	2.6+0.7
		GG2	-29.8+0.0	11.5+2.3	445.0+37.7	55.0+2.4	34.8+2.5	9.4+0.7	33+0	3.5+0.1
		GG3	-27.2+0.5	2.5+0.5	554.0+34.1	47.6+5.7	34.6+3.3	13.8+1.3	42+2	3.0+0.1
		GG4	-23.5+0.1	3.9+0.5	312.0+33.7	33.2+0.9	15.2+1.0	10.9+1.5	53+5	4.9+0.3
		GG5	-20.8+0.5	3.6+0.2	204.0+14.5	21.3+0.9	10.7+0.3	11.2+0.5	49+3	4.4+0.2
	Indian River Lagoon	CR	-21.9+0.5	3.9+0.7	186.1+10.7	21.3+2.4	9.0+0.9	10.4+1.7	54+4	5.2+0.2
	Reefs	BTR	-28.3+0.7	2.6+2.3	193.9+9.2	18.5+5.3	10.0+1.1	14.1+5.4	51+3	4.2+1.3
		SLR_N	-27.2+1.1	8.8+6.4	161.1+10.8	32.5+18.1	10.8+1.4	10.8+8.3	40+4	8.0+4.3
		SLR_S	-23.3+1.4	3.1+0.5	129.9+27.0	17.9+2.0	4.9+1.1	9.1+4.8	70+3	9.5+3.0
	Dry 1 Event Mean		**-26.8+0.4**	**5.5+0.7**	**333.2+30.2**	**37.5+2.6**	**26.7+2.8**	**10.4+0.4**	**40+2**	**4.3+0.4**
Dry 2 (May 2015)	Primary Canals	C23W	-29.3+0.1	5.1+1.7	389.4+28.6	59.8+4.5	21.8+0.4	7.6+0.2	46+3	6.1+0.4
		C44W	-23.5+0.1	4.5+1.1	648.1+32.3	72.4+4.1	29.2+0.6	10.5+0.1	57+3	5.5+0.3
	Old Palm City	OPC1	-29.6+0.1	3.6+1.4	294.0+28.3	38.4+4.4	7.1+0.8	9.0+0.4	108+3	12.1+1.0
		OPC2	-29.8+0.1	3.5+2.3	340.2+67.2	48.3+6.9	12.9+1.9	8.2+0.5	67+4	8.3+0.5
		OPC3	-31.4+0.2	4.4+1.2	913.0+187.2	127.3+25.0	81.8+15.1	8.4+0.2	29+2	3.5+0.3
		OPC4	-26.7+0.2	6.1+2.1	524.6+19.1	66.6+5.1	26.2+2.0	9.3+0.4	52+3	5.6+0.1
		OPC5	-23.3+0.1	6.1+0.4	679.6+25.9	67.0+2.0	27.8+1.7	11.9+0.1	64+5	5.4+0.4
	North Fork	DP	-27.8+0.0	7.9+5.3	370.3+22.9	45.7+2.1	15.8+0.8	9.5+0.2	61+4	6.4+0.3
		BR	-29.4+0.1	2.2+0.6	370.4+12.1	45.3+2.7	17.7+1.1	9.6+0.3	55+5	5.8+0.6
	Golden Gates	GG1	-32.1+0.3	4.3+1.9	611.3+57.9	84.3+16.6	24.5+3.1	8.9+1.3	65+6	7.5+0.5
		GG2	-30.0+0.9	8.1+0.7	866.3+177.1	125.0+17.3	51.8+1.4	8.1+1.2	43+8	5.3+0.6
		GG3	-25.9+0.5	1.5+2.8	294.3+22.0	28.2+0.8	11.1+0.3	12.3+1.2	68+4	5.6+0.3
		GG4	-23.4+0.3	4.0+0.5	217.3+18.9	21.0+1.0	8.1+0.7	12.2+1.5	71+11	5.8+0.6
		GG5	-24.1+1.9	3.8+0.6	300.8+138.4	13.9+1.5	6.4+0.3	25.6+11.5	123+57	4.8+0.3
	Indian River Lagoon	CR	-21.4+0.7	1.3+1.3	206.7+34.3	19.5+0.5	10.8+1.7	12.5+2.4	49+2	4.2+0.7
	Reefs	BTR	-22.9+0.4	5.0+1.6	64.3+2.7	8.3+0.7	2.8+0.7	9.1+0.4	65+11	7.2+1.1
		SLR_N	-23.0+0.1	3.9+0.1	179.3+4.7	17.8+0.2	4.8+0.0	11.8+0.2	97+3	8.2+0.2
		SLR_S	-24.5+0.4	4.3+0.9	47.6+5.0	7.4+0.4	1.1+0.1	7.6+0.4	117+6	15.6+1.2
	Dry 2 Event Mean		**-26.6+0.5**	**4.4+0.4**	**410.8+37.0**	**50.4+5.2**	**20.4+2.8**	**10.6+0.8**	**68+5**	**6.8+0.4**
Overall Dry Season Mean - All Sites			**-26.7+0.3**	**5.0+0.4**	**371.6+24.0**	**43.9+2.9**	**23.6+2.0**	**10.5+0.4**	**54+3**	**5.5+0.3**
Dry Season Means by Region	**Primary Canals**	C23W, C44W	**-26.4+0.7**	**4.4+0.5**	**375.8+60.2**	**47.0+6.3**	**31.8+8.5**	**9.0+0.9**	**40+5**	**5.1+0.7**
	Old Palm City	OPC1-5	**-28.0+0.4**	**6.1+0.9**	**516.5+53.6**	**61.1+5.6**	**36.1+4.2**	**9.6+0.4**	**47+5**	**4.9+0.6**
	Sewered North Fork	DP	**-27.7+0.1**	**6.3+3.1**	**322.2+25.3**	**38.5+3.5**	**14.8+0.7**	**9.9+0.2**	**56+4**	**5.8+0.4**
	Septic North Fork	BR	**-29.4+0.1**	**2.7+0.5**	**347.8+33.0**	**42.5+3.6**	**20.8+1.6**	**9.6+0.3**	**45+6**	**4.7+0.6**
	Golden Gates	GG1-5	**-26.8+0.7**	**4.8+0.6**	**413.7+41.9**	**46.7+6.4**	**22.9+2.7**	**12.3+1.3**	**57+7**	**4.7+0.3**
	Indian River Lagoon	CR	**-21.6+0.4**	**2.6+0.9**	**196.4+16.7**	**20.4+1.2**	**9.9+1.0**	**11.4+1.2**	**52+2**	**4.7+0.4**
	Reefs	BTR, SLRN/S	**-25.0+0.6**	**4.8+1.2**	**126.4+14.7**	**17.0+3.5**	**5.8+1.0**	**10.3+1.1**	**72+7**	**8.8+1.3**

Table 9B. A comparison of mean (± S.E.) stable isotopes and C:N:P ratios in phytoplankton throughout the St. Lucie Estuary and nearshore reefs broken down by the two Wet season sampling events and region. "Overall Wet Season Mean" combines the two samplings for all 18 sampling sites.

Sampling Event	Region	Site	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C (µg/L)	N (µg/L)	P (µg/L)	C:N Ratio	C:P Ratio	N:P Ratio
Wet 1 (August 2015)	Primary Canals	C23W	-28.5±0.1	2.0±0.6	362.1±10.3	54.5±1.7	18.2±0.5	7.8±0.2	52±1	6.6±0.1
		C44W	-26.7±0.1	8.2±2.9	266.1±4.4	39.5±1.6	13.0±0.9	7.9±0.2	54±4	6.8±0.7
	Old Palm City	OPC1	-28.6±0.3	7.3±1.6	340.1±23.1	46.7±0.6	13.2±0.8	8.5±0.5	66±3	7.8±0.4
		OPC2	-28.4±0.1	1.3±0.5	252.4±14.5	39.9±1.6	27.5±0.6	7.4±0.2	24±1	3.2±0.1
		OPC3	-27.4±0.0	1.3±0.5	638.1±10.4	97.5±1.9	65.9±4.1	7.7±0.0	25±1	3.3±0.2
		OPC4	-30.3±0.1	4.6±2.1	333.9±31.4	57.9±3.2	16.7±1.1	6.7±0.3	52±2	7.7±0.1
		OPC5	-26.1±0.2	0.6±0.8	259.2±13.0	31.5±1.4	11.1±0.5	9.6±0.1	60±4	6.3±0.3
	North Fork	DP	-28.1±0.1	1.6±0.1	323.1±24.6	55.3±3.7	14.1±0.5	6.8±0.2	59±3	8.6±0.3
		BR	-31.6±0.4	8.2±5.4	792.9±183.2	122.3±30.0	27.7±6.8	7.8±1.1	74±2	9.8±1.1
	Golden Gates	GG1	-34.5±0.1	5.2±0.9	349.5±6.9	54.8±1.9	16.6±0.4	7.5±0.2	55±1	7.3±0.2
		GG2	-31.6±0.1	7.8±0.2	761.6±47.5	134.2±5.4	60.2±1.7	6.6±0.2	33±3	4.9±0.3
		GG3	-26.7±0.5	1.1±1.7	424.5±43.2	60.8±4.9	26.9±2.2	8.1±0.3	41±2	5.0±0.4
		GG4	-23.7±0.5	5.5±0.8	209.8±12.8	34.7±0.6	11.3±0.5	7.1±0.3	48±4	6.8±0.3
		GG5	-22.9±0.3	8.4±2.8	211.0±19.7	33.7±1.4	8.9±0.6	7.3±0.4	62±5	8.4±0.6
	Indian River Lagoon	CR	-21.3±0.3	4.3±0.3	206.5±5.1	35.0±1.0	8.8±0.2	6.9±0.1	61±2	8.8±0.3
	Reefs	BTR	-21.7±0.4	2.9±1.8	76.4±2.4	14.4±0.3	1.9±0.2	6.2±0.3	105±13	17.0±2.3
		SLR_N	-21.1±0.1	4.8±0.2	109.3±11.0	20.4±1.7	3.0±0.2	6.3±0.2	96±11	15.3±1.2
		SLR_S	-22.8±1.1	4.7±0.2	109.8±17.7	22.1±2.4	2.8±0.1	5.8±0.5	103±17	17.7±2.3
	Wet 1 Event Mean		**-26.8±0.5**	**4.6±0.5**	**334.8±29.1**	**53.1±4.7**	**19.3±2.4**	**7.3±0.1**	**59±3**	**8.4±0.6**
Wet 2 (September 2015)	Primary Canals	C23W	-29.5±0.2	1.2±0.4	255.5±15.8	50.8±2.9	20.3±0.6	5.9±0.1	33±1	5.5±0.2
		C44W	-27.2±0.1	1.8±1.2	221.3±6.0	35.9±1.4	18.2±0.6	7.2±0.2	32±2	4.4±0.2
	Old Palm City	OPC1	-28.0±0.1	6.0±2.2	164.1±7.2	27.3±0.5	5.4±0.2	7.0±0.3	79±2	11.3±0.2
		OPC2	-27.8±0.0	2.2±2.4	196.6±13.7	35.4±1.5	13.8±0.1	6.5±0.2	37±2	5.7±0.2
		OPC3	-29.1±0.1	1.3±0.6	434.0±18.9	72.3±2.9	41.1±2.1	7.0±0.1	28±2	3.9±0.3
		OPC4	-31.9±0.6	2.5±2.9	247.6±22.6	40.0±3.4	12.4±0.4	7.2±0.1	51±4	7.1±0.5
		OPC5	-25.9±0.1	4.0±1.9	198.0±34.1	32.9±7.1	11.6±2.9	7.2±0.4	46±5	6.5±0.6
	North Fork	DP	-31.6±0.4	10.1±3.4	324.6±13.7	53.5±5.3	15.9±0.6	7.2±0.5	53±2	7.5±0.8
		BR	-33.3±0.1	6.7±0.7	429.7±11.3	64.6±1.8	16.1±0.3	7.8±0.1	69±1	8.9±0.1
	Golden Gates	GG1	-29.0±0.1	3.6±1.4	501.7±34.0	109.6±9.4	35.3±1.3	5.4±0.1	37±3	6.9±0.8
		GG2	-33.1±0.2	11.1±1.4	1263.0±134.5	241.3±18.3	92.2±6.7	6.1±0.4	35±2	5.8±0.3
		GG3	-31.6±0.2	7.2±2.7	458.4±33.4	81.5±4.7	35.7±1.3	6.6±0.2	33±1	5.1±0.2
		GG4	-28.6±0.1	6.1±5.0	160.3±10.9	30.6±3.5	9.3±0.1	6.3±0.6	45±3	7.3±0.8
		GG5	-22.4±0.1	8.9±1.9	228.7±4.5	45.8±1.9	12.9±0.6	5.9±0.1	46±2	7.9±0.4
	Indian River Lagoon	CR	-23.2±0.0	6.4±0.4	278.5±14.9	47.7±2.0	11.3±0.4	6.8±0.2	64±3	9.4±0.6
	Reefs	BTR	-21.2±1.1	6.0±1.6	96.5±6.6	33.6±3.0	2.6±0.2	3.4±0.2	96±8	28.8±3.6
		SLR_N	-17.5±0.1	7.0±1.6	138.0±1.2	29.1±0.8	5.2±0.2	5.6±0.2	69±2	12.6±0.9
		SLR_S	-19.9±0.9	5.6±1.9	113.6±7.1	27.7±1.0	4.3±0.9	4.8±0.2	72±10	15.2±2.5
	Wet 2 Event Mean		**-27.3±0.6**	**5.4±0.6**	**317.2±36.2**	**58.9±6.8**	**20.2±2.8**	**6.3±0.2**	**51±3**	**8.9±0.8**
Overall Wet Season Mean - All Sites			**-27.0±0.4**	**5.0±0.4**	**326.0±23.1**	**56.0±4.1**	**19.8±1.9**	**6.8±0.1**	**55±2**	**8.6±0.5**
Wet Season Means by Region	**Primary Canals**	C23W, C44W	**-28.0±0.3**	**3.4±1.2**	**276.2±16.3**	**45.2±2.5**	**17.4±0.9**	**7.2±0.3**	**42±3**	**5.8±0.3**
	Old Palm City	OPC1-5	**-28.3±0.3**	**3.2±0.6**	**306.4±25.6**	**48.1±4.0**	**21.9±3.3**	**7.5±0.2**	**47±3**	**6.3±0.4**
	Sewered North Fork	DP	**-29.9±0.8**	**5.9±2.8**	**323.9±12.6**	**54.4±2.9**	**15.0±0.5**	**7.0±0.3**	**56±2**	**8.1±0.4**
	Septic North Fork	BR	**-32.5±0.4**	**7.5±2.4**	**611.3±115.5**	**93.5±18.6**	**21.9±4.0**	**7.8±0.5**	**72±2**	**9.4±0.5**
	Golden Gates	GG1-5	**-28.4±0.8**	**6.5±0.8**	**456.9±60.7**	**82.7±11.7**	**30.9±4.8**	**6.7±0.2**	**43±2**	**6.5±0.3**
	Indian River Lagoon	CR	**-22.2±0.4**	**5.4±0.5**	**242.5±17.6**	**41.4±3.0**	**10.0±0.6**	**6.9±0.1**	**62±2**	**9.1±0.3**
	Reefs	BTR, SLRN/S	**-20.7±0.5**	**5.3±0.6**	**107.3±5.6**	**24.5±1.7**	**3.3±0.3**	**5.3±0.3**	**90±5**	**17.8±1.5**

Table 9C. A comparison of comprehensive means (± S.E.) for combined Dry and Wet season phytoplankton stable isotope and C:N:P ratios at 18 sites throughout the St. Lucie Estuary and nearshore reefs broken sampling site and region.

Sampling Event	Region	Site	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C (µg/L)	N (µg/L)	P (µg/L)	C:N Ratio	C:P Ratio	N:P Ratio
Comprehensive Project Means by Site	Primary Canals	C23W	-28.7+0.3	3.1+0.7	276.0+35.4	46.6+4.7	16.6+1.9	6.7+0.3	43+2	6.5+0.3
		C44W	-25.6+0.5	4.9+1.0	376.0+51.4	45.7+4.8	32.6+8.2	9.5+0.7	40+5	4.5+0.6
	Old Palm City	OPC1	-28.3+0.3	5.1+0.8	457.7+104.3	49.4+6.8	18.2+5.3	9.7+0.8	78+6	8.8+1.0
		OPC2	-28.7+0.2	4.3+1.9	241.5+24.4	38.0+2.7	19.8+2.4	7.3+0.3	37+6	5.0+0.7
		OPC3	-29.4+0.4	3.1+0.6	638.5+66.7	90.8+9.0	61.8+5.7	8.3+0.4	27+1	3.3+0.2
		OPC4	-29.1+0.6	6.4+1.4	351.9+33.0	51.5+3.7	23.7+3.2	7.9+0.3	44+4	5.7+0.6
		OPC5	-25.3+0.4	4.5+1.1	367.6+57.2	43.3+4.7	21.6+3.3	9.5+0.5	49+5	5.2+0.5
	North Fork	DP	-28.8+0.5	6.1+2.0	323.0+13.5	46.5+3.2	14.9+0.4	8.4+0.5	56+2	6.9+0.5
		BR	-30.9+0.5	5.1+1.4	479.5+69.7	68.0+11.9	21.4+2.1	8.7+0.4	58+5	7.0+0.8
	Golden Gates	GG1	-31.8+0.6	4.6+0.6	448.7+37.6	71.4+9.3	27.0+2.3	8.1+0.6	46+5	6.1+0.6
		GG2	-31.1+0.5	9.6+0.8	834.0+100.9	138.9+20.8	59.8+6.5	7.6+0.5	36+2	4.9+0.3
		GG3	-27.9+0.7	3.1+1.2	432.8+31.6	54.5+6.2	27.1+3.1	10.2+0.9	46+4	4.7+0.3
		GG4	-24.8+0.7	4.9+1.1	224.9+18.8	29.9+1.8	11.0+0.9	9.1+0.9	54+4	6.2+0.4
		GG5	-22.5+0.6	5.9+1.1	236.1+32.1	28.7+3.7	9.7+0.8	12.5+3.4	70+15	6.4+0.6
	Indian River Lagoon	CR	-21.9+0.3	4.0+0.6	219.5+13.5	30.9+3.5	10.0+0.5	9.2+0.9	57+2	6.9+0.7
	Reefs	BTR	-23.5+0.9	4.4+0.9	107.8+15.6	18.7+3.1	4.3+1.0	8.2+1.4	79+8	14.3+3.1
		SLR_N	-22.1+1.2	6.3+1.7	144.0+8.8	25.6+4.6	6.0+1.0	8.3+1.4	73+8	11.3+1.6
		SLR_S	-22.6+0.7	4.4+0.5	100.2+11.8	18.8+2.4	3.3+0.6	6.8+0.8	90+8	14.5+1.4
Comprehensive Project Means by Region	**Primary Canals**	C23W, C44W	**-27.2+0.4**	**4.0+0.6**	**326.0+32.2**	**46.1+3.3**	**24.6+4.4**	**8.1+0.5**	**41+3**	**5.5+0.4**
	Old Palm City	OPC1-5	**-28.2+0.3**	**4.7+0.6**	**411.4+32.5**	**54.6+3.5**	**29.0+2.8**	**8.6+0.3**	**47+3**	**5.6+0.4**
	Sewered North Fork	DP	**-28.8+0.5**	**6.1+2.0**	**323.0+13.5**	**46.5+3.2**	**14.9+0.4**	**8.4+0.5**	**56+2**	**6.9+0.5**
	Septic North Fork	BR	**-30.9+0.5**	**5.1+1.4**	**479.5+69.7**	**68.0+11.9**	**21.4+2.1**	**8.7+0.4**	**58+5**	**7.0+0.8**
	Golden Gates	GG1-5	**-27.6+0.5**	**5.6+0.5**	**435.3+36.7**	**64.7+7.0**	**26.9+2.8**	**9.5+0.8**	**50+4**	**5.6+0.2**
	Indian River Lagoon	CR	**-21.9+0.3**	**4.0+0.6**	**219.5+13.5**	**30.9+3.5**	**10.0+0.5**	**9.2+0.9**	**57+2**	**6.9+0.7**
	Reefs	BTR, SLRN/S	**-22.8+0.5**	**5.0+0.6**	**116.6+7.8**	**20.9+2.0**	**4.5+0.5**	**7.8+0.7**	**81+5**	**13.4+1.2**
Overall Project-Wide Means			**-26.9+0.3**	**5.0+0.3**	**348.7+16.7**	**50.0+2.6**	**21.7+1.4**	**8.7+0.3**	**55+2**	**7.1+0.3**

4.0 Discussion

4.1. Dissolved Nutrients

Inorganic, or reactive, forms of nitrogen (N) and phosphorus (P) from septic tank effluent are most readily available for assimilation by macroalgae and phytoplankton and are important in assessing ecosystem health and potential for harmful algal bloom production in a system. Two forms of inorganic nitrogen, ammonium (NH_4) and nitrate (NO_3), collectively contribute to dissolved inorganic nitrogen (DIN) concentrations in the surface and groundwater. The inorganic form of phosphorus is soluble reactive phosphorus (SRP; PO_4).

Analysis of groundwater N and P in residential areas and reference sites indicate a high degree of OSTDS contamination. The residential groundwater in Old Palm City and, especially, Golden Gates Estates was comprised primarily of NO_3, a sign of nitrification (or conversion from NH_4 to NO_3) through initial treatment in septic systems. Conversely, most of the DIN at the reference sites during both seasons was in the form of NH_4, indicating little conversion from ammonium to nitrate in these areas. Like reactive N, SRP concentrations were also significantly higher in the residential areas than at the reference sites. In natural areas, like the reference sites, P typically binds to soils and can be effectively stripped from the groundwater. This process results in

significantly lower relative concentrations of P than N and subsequent high N:P ratios in relatively undeveloped areas. When the soils are routinely exposed to high concentrations of groundwater P for prolonged periods, as in the residential areas relying on OSTDS, they eventually become saturated, ultimately decreasing the soils ability to strip P from the groundwater. This results in high P concentrations in the groundwater and consequently lower N:P ratios. Although evident within both residential well clusters, this phenomenon was especially pronounced during the Wet season at Old Palm City where the mean SRP concentration spiked to ~107 μM in the intermediate well. This was significantly higher than the highest mean concentration of 1.3 μM measured at the reference sites (along Jensen Beach Boulevard). These low SRP concentrations combined with the high N:P ratios at the reference sites indicate that groundwater P was actively binding to these soils (Fig. 7b,e). The ratio of N:P is used to gauge the relative degree of N versus P limitation in a system. The lower N:P ratios at the residential well complexes indicate N-limitation whereas the higher N:P ratios at the reference sites suggest P-limitation.

Similar to the groundwater results, surface water dissolved nutrient analyses suggest OSTDS contamination and a general dilution effect from upstream to downstream sampling sites within the residential sampling complex in Golden Gates Estates. As with groundwater, the surface water DIN in Old Palm City and the primary canals was predominantly comprised of NO_3 during the Dry season and NH_4 during the Wet season. The lower water table during the Dry season likely provided additional time for NH_4 to be converted to NO_3 through the nitrification process, whereas higher Wet season water tables facilitated the direct release of NH_4 to groundwater without nitrification to NO_3 (Bicki and Brown, 1990). Contrary to Old Palm City, most of the surface water DIN in Golden Gate Estates was comprised of NH_4 during both the Dry (66%) and Wet (58%) seasons whereas the groundwater DIN was comprised primarily (>90%) of NO_3. High surface water SRP concentrations in Old Palm City's All American Ditch during the Wet season reinforced the notion that P-saturated soils in this community facilitated the release of higher concentrations of P into surface waters. Elevated SRP concentrations were also observed just upstream of the S-48 structure in the C-23 canal. Possibly related, Lapointe *et al*. (2012) also documented higher P concentrations in the North Fork than other segments of the SLE during the prolonged releases in 2005-2006 following hurricanes Charley, Frances, and Jeanne in 2004 and Dennis, Katrina, Rita, and Wilma in 2005.

More so than the inorganic forms, TDN showed consistently strong dilution curves in both seasons between the upstream and downstream ends of the Golden Gates Estates sampling complex. Water quality targets for both TDN (< 51.4 μM) and TDP (< 2.6 μM) were established for the SLE in the IRL-South Project Implementation Report (USACE/SFWMD, 2004). The only SLE and IRL sites to meet the TDN criterion were Beau Rivage and the crossroads with the Indian River Lagoon while none of the SLE and IRL sites met the TDP criterion (Table 6C). Within Golden Gates Estates, high DIN and TDN concentrations coupled with relatively low reactive SRP and TDP concentrations in the community retention pond (GG1-2) resulted in exceptionally high N:P ratios (P-limitation) during both the Wet and Dry seasons; providing further evidence of OSTDS contamination to surrounding surface waters from this community. Conversely, Old Palm City (especially in the All American Ditch), the primary canals, and the Lighthouse community at Dyer Point (DP) all had relatively high SRP and TDP concentrations. This, again, is likely the result of P-saturated soils and possibly the application of P-rich

fertilizers, resulting in lower ratios (N-limitation). The Lighthouse Community at Dyer Point was converted to sewer in 2004. The original sampling design factored in grab samples at this conversion site to compare with Beau Rivage West (BR), a high priority community for future conversion. Because of the close proximity to the C-23 canal and sample collection occurring on an outgoing tide, the C-23 canal influenced the results at DP instead of capturing a clear signature from the Lighthouse Point community itself. Thus, a clear comparison between the sewered community and community relying on OSTDS could not be made.

The origin of excessive nutrients in the SLE has been widely debated for decades. Although releases from Lake Okeechobee are known to exacerbate poor water quality conditions in the SLE, the general consensus is the majority of the nutrient loading originates in the adjacent watershed (Zheng *et al*., *in prep*; Graham *et al*., 2015). The long-term annual average TN and TP loadings to the SLE by Lake Okeechobee between 1997 and 2015 are estimated to be 1,148,168 and 117,286 lbs, respectively (Zheng *et al.*, *in prep*). Because reactive forms of N and P are responsible for fueling eutrophication and community shifts, including harmful algal blooms that impair waterways, it is important to understand the approximate contributions of these readily assimilated forms. Based on data from SFWMD's database DBHYDRO, approximately 18% (206,670 lbs) of the total N coming out of the Lake Okeechobee through the S-308C water control structure near the Hoover Dike and 20% (229,634 lbs) of the total N coming out of the S-80 water control structure near the South Fork (included Lake Okeechobee plus C-44 basin; a value close to the 15% identified through grab samples in this study in section 3.3.2) is in the reactive form; ammonium and nitrate. Similarly, approximately 35% (41,050 lbs) of the total P coming out of Lake Okeechobee through the S-308C and 51% (59,816 lbs) of the total P coming out of the S-80 (includes Lake Okeechobee plus C-44 basin) is in the reactive form; orthophosphate. Thus, 22,963 lbs of reactive N and 18,766 lbs reactive P are coming in from the C-44 basin (the agricultural and urban lands between the S-308C at the Hoover Dike and S-80 structure).

In addition to N and P contributions from the C-44 basin, significant N and P inputs from the 17,687 known OSTDS in the Martin County portion of the watershed as calculated by Florida Department of Health. According to Bicki *et al.* (1984), the average person eliminates18 lbs of N per year. To be conservative in our estimations, we reduced the value in half to 9 lbs of N per year; a value similar to the 9.7 lbs/person/year used by FDEP (Polley, 2014). Multiplying 9 lbs N per person per year by an average of 2.5 people per 17,687 homes on OSTDS yields an estimated 397,958 lbs N per year originating from OSTDS in the Martin County portion of the SLE watershed. The total from the C-44 basin (22,963 lbs) added to the total from OSTDS in the watershed (397,958) yields a total of 420,921 lbs N from the watershed versus 206,670 from Lake Okeechobee releases. For OSTDS inputs of P, Bicki *et al.* (1984) estimates that the average person eliminates 1.66 lbs of P per year. Again, to be conservative in our estimations, we reduced the value in half to 0.83 lbs of P per year. Multiplying 0.83 lbs P per person per year by an average of 2.5 people per 17,687 homes on OSTDS yields an estimated 36,700 lbs P per year from OSTDS. Thus, the total contribution from the C-44 basin (18,766 lbs) added to the total contribution from OSTDS in the watershed (36,700) yields a total of 55,466 lbs P from the watershed versus 41,050 from Lake Okeechobee releases. Furthermore, if the total estimated number of OSTDS in Martin County (31,634) are used, then these N and P loading estimates

increase by a factor of 79%. These calculations illustrate why OSTDS are now considered the second largest nitrogen source to Florida's surface waters (Budruzzman *et al.*, 2012).

Thus, when this watershed-based contribution of reactive N and P is added to the conservatively estimated reactive N and P inputs from OSTDS, the direct nutrient inputs from the SLE watershed is the largest nutrient source and surpasses inputs from Lake Okeechobee. This is also supported by Zheng *et al*. (*in prep*) that identifies the long-term (1997 -2015) TN and TP contributions from Lake Okeechobee to be 30% and 17%, respectively, compared to 52% and 67%, respectively, from the watershed.

Ye and Sun (2013) estimated that conversion of 32,429 homes in the SLE watershed from OSTDS to centralized sewer would result in a reduction of 202,371 lbs sewage N being released to the SLE (~6.4 lbs/converted OSTDS). The Basin Management Action Plans (BMAP) associated with the impaired waters designation by FDEP requires a 236,000 lb reduction. Thus, conversion to centralized sewer would account for 86% of the BMAP requirement. Using the same calculation, conversion of the 1,078 homes in Old Palm City and 775 homes in Golden Gates Estates (based on parcels identified in Keene, 2015) would result in the elimination of ~11,859 lbs sewage N being released to the SLE (~5% of the BMAP requirement). The FDEP is currently issuing BMAP credit for elimination of TN loadings to the SLE associated with OSTDS to septic conversion and hookup to package treatment plants, but no credit for the elimination of TP loadings. The addition of credit for TP loadings would potentially provide additional motivation to local governments and municipalities to initiate large conversion projects.

4.2. Nutrient Limitation

Surface water, macroalgae, and phytoplankton C:N:P ratios all suggest relatively high levels of P, and subsequent N-limitation, throughout the SLE. Excluding the upstream sites within the residential sampling complexes, surface water N:P ratios suggest N-limitation throughout the estuary and P-limitation on the nearshore reefs south of the inlet in the Wet season (Figs. 10-11). Similar to the surface water N:P ratios, the N:P ratios documented in macroalgae and phytoplankton also indicate the predominance of N-limitation throughout the SLE and, as especially seen in macroalgae, P-limitation on the nearshore reefs (Figs. 15 and 17). N-limited environments are especially susceptible to input of N to the SLE will promote bottom-up phase shifts more so than inputs of P because the algae are already saturated with P. This information is most helpful to resource managers and local governments responsible for creating policy, such as fertilizer ordinances, on nutrient loadings. Fertilizer ordinances in the SLE watershed were first implemented in 2011 for Martin County, the Town of Sewall's Point, the City of Stuart, and the City of Port St. Lucie where the use of fertilizers are banned between June 1 and September 30 (the wet season) each year. These ordinances also have varying setbacks for application near waterbodies (e.g., 25 feet in Martin County and 10 feet in the Town of Sewall's Point) and criteria for applying P and use of slow release N.

4.3 Human Tracers of Nutrient Pollution:

Stable isotopes of $\delta^{15}N$ in surface water, macroalgae, and phytoplankton and the artificial sweetener sucralose all independently suggest wastewater contamination in the SLE that extends out to the nearshore reefs north and south of St. Lucie Inlet. Groundwater ammonium and nitrate were enriched in both residential areas compared to the reference sites, and were predominantly coming from a wastewater source ($\delta^{15}N$ values > +3 $^{o}/_{oo}$). Most surface water sites also showed nitrogen from a wastewater source, indicated by the enriched $\delta^{15}N$ values in phytoplankton collected at most surface water sites and benthic macroalgae growing throughout the sampling network. The mean phytoplankton $\delta^{15}N$ value in the SLE was +5.2 $^{o}/_{oo}$, a value similar to the SLE macroalgae signature of +5.7 $^{o}/_{oo}$. These values are slightly lower than the overall mean $\delta^{15}N$ (+6.3 $^{o}/_{oo}$) for macroalgae collected throughout the IRL in 2011-2012 (Lapointe *et al*., 2015b), possibly reflecting greater contributions from agricultural areas in the SLE watershed compared to the IRL-wide study. The most enriched $\delta^{15}N$ values were documented in macroalgae collected in the South Fork; +5.6 $^{o}/_{oo}$ immediately downstream of the S-80 structure (C44E) and +6.5 $^{o}/_{oo}$ in Old Palm City (OPC5; Table 8). Regardless of season, all mean $\delta^{15}N$ values in macroalgae were enriched (> +3 $^{o}/_{oo}$) with the exception of SLR-S in the Dry season (Fig. 14). Like the nitrogen isotopes in macroalgae, sucralose was detected in the surface wells in both the Old Palm City and Golden Gates Estates well clusters in both the Dry and Wet seasons. The highest mean sucralose concentration (~6 µg/L) was documented in the groundwater at Old Palm City during the Dry season. Although significantly lower than the 24 µg/L documented at an Indian River County wastewater treatment plant (Tarnowski, 2014), the sucralose was entering into the surface waters in both of these communities (Figs. 9 and 13). The mean concentration in the groundwater at Golden Gates Estates was similar to that documented in the surface water in the community retention pond adjacent to the well cluster (GG2), suggesting high levels of sewage contamination. The surface water concentrations documented at GG2-3 and OPC2 in Old Palm City's All American Ditch were significantly higher than those documented by Tarnowski (2014) in the Indian River County canals and St. Sebastian River (generally < 1 µg/L). However, the Indian River County canals and St. Sebastian River were larger and likely more diluted than the small drainage systems in Old Palm City and Golden Gates Estates. Like the dissolved nutrients results, there was evidence of an upstream to downstream gradient in sucralose concentrations between GG2 and GG5, which speaks to the dilution factor and helps to explain the differences in the two studies.

4.4 System Responses

Freshwater blooms of the potentially toxic *Microcystis aeruginosa* have been increasing worldwide as a result of growing N and P inputs from urban, agricultural, and industrial sources (Paerl and Otten, 2013). In the SLE, two unprecedented blooms of this species formed following massive releases of fresh water from Lake Okeechobee and the C-44 watershed in 2005 and again in 2013. *Microcystis aeruginosa* bloom potential is directly correlated to phosphorus inputs (Horst 2014a,b). The higher the P input into a system, the lower the associated N:P ratio. According to Parrish (2014), this species experiences optimal growth and abundance when the N:P ratio is < 44:1, as seen in the SLE during the 2005 bloom when N:P ratios decreased to ~9:1 (Lapointe *et al*., 2012). During this 2015 study, low mean N:P ratios were documented in the

water column nutrients (< 29:1), benthic macroalgae (~20:1), and phytoplankton (~6:1) and therefore, theoretically capable of supporting such blooms in the SLE had the organism been present. Therefore, once transported into the SLE with the freshwater discharges, blooms of *M. aeruginosa* are likely fueled by nutrient inputs from the surrounding watershed, especially high P inputs from the C-23 and C-24 canals and residential communities like Old Palm City.

In addition to P uptake, the enriched $\delta^{15}N$ value (+8.6 $^{o}/_{oo}$) of the *M. aeruginosa* collected in August 2013 also points to wastewater N as a primary N source fueling the bloom. Based on the ground and surface water aqueous isotope results from this 2015 study, this highly urbanized estuary is locally impacted by thousands of OSTDS that enrich tidal creeks and canals with high concentrations of ammonium, nitrate, and fecal bacteria (Fig. 18; Lapointe *et al.*, 2012). Interestingly, *M. aeruginosa* has a much higher affinity for ammonium than nitrate (Parrish, 2014), which suggests that NH_4 is likely the primary form of N fueling these blooms. However, Horst *et al.* (2014a,b) shows that while the other major form of inorganic N, nitrate, may not be as important in fueling the bloom, it is the primary factor determining the blooms toxicity.

This 2015 study documents high concentrations of both ammonium and nitrate entering into the systems especially around the residential neighborhoods. In both 2005 and 2013, toxic strains of *M. aeruginosa*, which produced the hepatotoxin microcystin, were documented in the SLE (Phlips *et al.*, 2012; FDEP, *unpublished data*), ultimately suggesting that there were high enough concentrations of nitrate in the SLE to promote toxin production. These results provide further evidence of the need for advanced wastewater treatment (N-removal) within the watershed.

Downstream of the SLE, multiple lines of evidence support nutrient enrichment of the nearshore reefs in Martin County. The biological composition of these reefs corroborates the environmental chemistry presented above. The reefs support multiple nutrient indicator species including at least three species of sponges in the genus *Clionia* (Herren and Monty, 2006). These indicators of wastewater N are commonly seen boring into the brain corals *Diploria clivosa* on the Martin County reefs (Ward-Paige *et al.*, 2005; see orange sponge on the cover). The Martin County reefs also support a diverse and abundant array of echinoderms, such as sea urchins (Herren and Monty, 2006) and the green alga *Codium intertextum* (see green alga on the cover). *Codium* spp. is a known indicator of wastewater pollution (Lapointe *et al*., 2005) and was collected on the SLR-N site just south of St. Lucie Inlet.

In addition to nutrient pollution, bacterial contamination of ground and surface waters has been associated with high densities of OSTDS in the watersheds of the IRL and its tributaries (Lapointe and Krupa, 1995a,b; Belanger *et al.*, 2007; Lapointe *et al.*, 2012) and is considered a primary source of bacterial contamination associated with immunologic perturbations in populations of bottlenose dolphins, *Tursiops truncatus* Montagu, in the IRL (Schaefer *et al.*, 2009; Bossart *et al.*, 2014). Interestingly, Schaefer *et al.* (2011) found a positive correlation between the number of IRL bottlenose dolphins colonized by *Escherichia coli* and the number of septic tanks in the area they lived. The Martin and St. Lucie County Health Departments are responsible for posting warnings along the SLE based on such bacterial counts. Because of the generally poor conditions in this system, several warnings are issued each year.

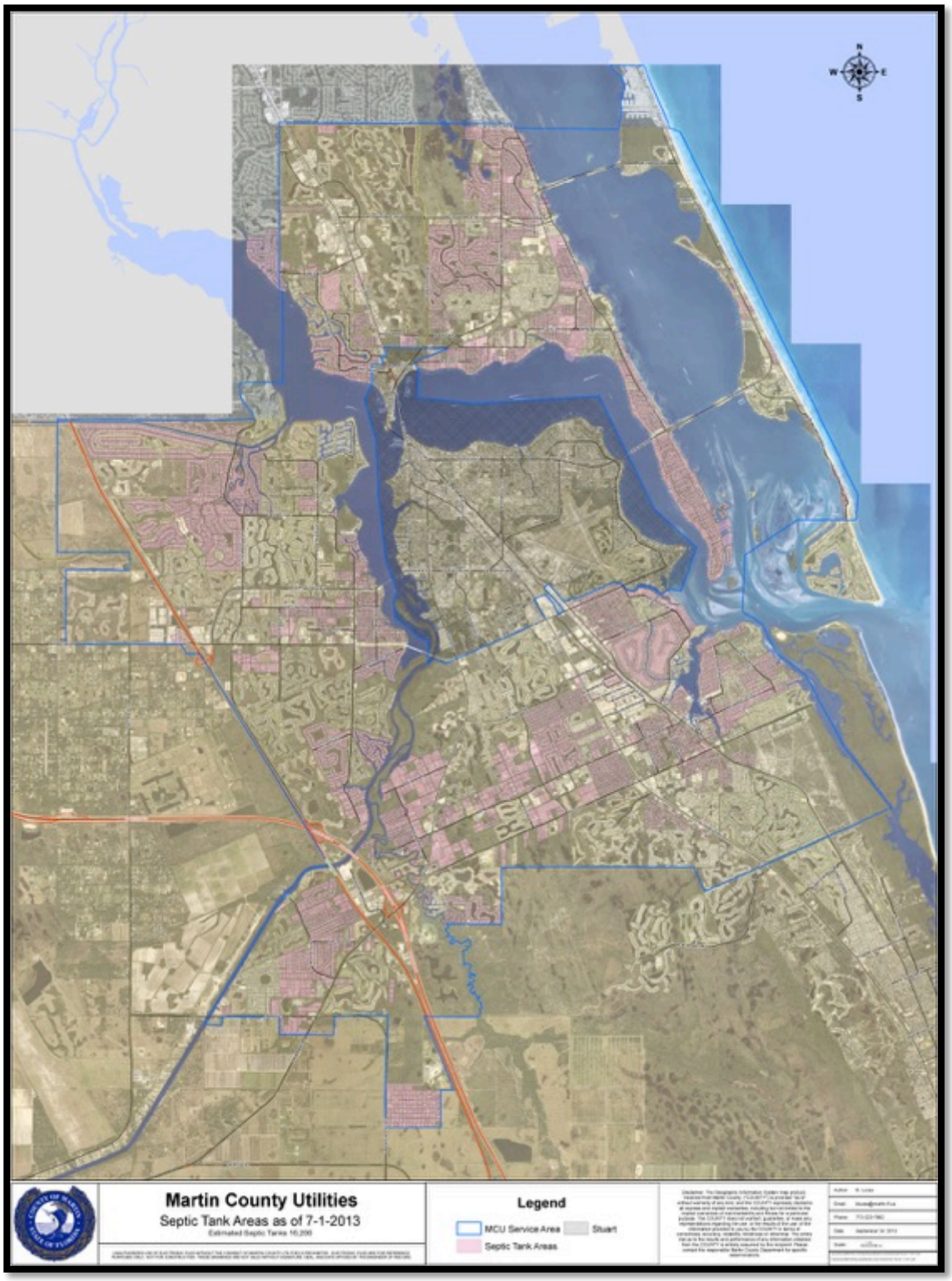

Fig. 18. Areas supporting an estimated 16,200 septic tanks within the Martin County Utilities Service Area as of July 1, 2013 are shown in pink. Map provided by Martin County.

5.0 Conclusions

1. *Enterococcus* counts measured by Martin County Department of Heath followed an upstream to downstream gradient in the SLE. The highest counts recorded just downstream of the Old Palm City sampling complex (Leighton Park), followed by Roosevelt Bridge, then Sandsprit Park (just downstream of the Golden Gates Estates Complex), and the Stuart Sandbar in St. Lucie Inlet (near the Crossroads site).

2. *Enterococcus* counts at sites just downstream of the Old Palm City and Golden Gates Estates sampling complexes were in the moderate to poor range (36 - >105 cfu/100mL) for human health 56% and 7% of the time, respectively, between January 1and September 30, 2015.

3. Dissolved nutrient concentrations in groundwater at the Old Palm City and Golden Gates Estates residential wells were significantly higher than those recorded at the reference wells during both the Dry and Wet seasons.

4. DIN in groundwater was comprised primarily of nitrate, an indicator of sewage pollution, at the residential sites and ammonium at the reference sites.

5. Groundwater P concentrations were high at the residential sites, driving the N:P ratios down to values known to support *Macrocystis aeroginosa* blooms.

6. Groundwater NH_4 and NO_3 were primarily associated with a wastewater source at both residential and reference sites with one exception. The NO_3 during the Wet season at the reference sites was primarily originating from either atmospheric or fertilizer N.

7. Sucralose concentrations were only detected in the residential wells at Old Palm City and Golden Gates Estates; none at the reference wells.

8. Surface water dissolved nutrient concentrations were significantly higher in the Wet season than they were in the Dry season, regardless of the analyte or location.

9. The highest regional mean nutrient concentrations in both the Wet and Dry seasons were primarily documented at one or both of the residential sampling networks (Old Palm City or Golden Gates Estates) followed by lower concentrations in the primary canals.

10. Surface water DIN was high in both residential sampling complexes, but especially in Golden Gates Estates, whereas SRP was highest in Old Palm City and the C-23 canal.

11. The presence of nutrient indicator species, such Clionid sponges, urchins, and the green alga *Codium intertextum*, on the reefs provides evidence of nutrient enrichment of the coastal area in Martin County.

12. Overall mean aqueous $\delta^{15}N$ - NO_3 values were >10x more enriched at residential sites than reference sites.

13. Sucralose was detected at all surface water sites in Old Palm City and Golden Gates Estates during the Dry and Wet seasons. The highest concentrations were documented in All American Ditch and the Golden Gates Estates retention pond. A dilution effect, where the concentrations decreased from upstream to downstream, was especially noticeable at Golden Gates Estates sampling complex.

14. Acetaminophen was not detected in the groundwater samples associated with the two homes in this study, but it was detected in low concentrations at five of the eight surface water sites; three in Old Palm City and two in Golden Gates Estates.

15. Overall, macroalgae collected at all sites except the southernmost St. Lucie Reef site (SLR-S), had enriched $\delta^{15}N$ values > +3 $^{o}/_{oo}$, indicating widespread uptake of wastewater N in the system that extends to the nearshore reefs adjacent to St. Lucie Inlet.

16. The overall mean $\delta^{15}N$ value for macroalgae collected in the SLE was +5.7 $^{o}/_{oo}$ and +3.5 $^{o}/_{oo}$ on the nearshore reefs. The value was more depleted during the Dry (+5.1 $^{o}/_{oo}$) than the Wet (+6.3 $^{o}/_{oo}$) season.

17. $\delta^{15}N$ values in macroalgae were consistently most enriched at residential sites, followed by the outflow of the primary canals, IRL, and nearshore reefs, respectively.

18. The C:N:P ratios in macroalgae indicate N-limitation in the SLE, P-limitation at the Crossroads (IRL), and N and P-limitation on the nearshore reefs.

19. $\delta^{15}N$ values for phytoplankton collected in the SLE were enriched (+5.2 $^{o}/_{oo}$). Project-wide, the degree of enrichment did not change between the Dry and Wet (+5.0 $^{o}/_{oo}$) seasons. On the nearshore reefs phytoplankton had an isotopic ratio of +4.8 $^{o}/_{oo}$.

20. C:N:P ratios in phytoplankton indicated consistent N-limitation throughout the study with the exception of the nearshore reefs in the Wet season where a switch to P-limitation was observed.

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APPENDIX A
REFERENCE WELL LOCATION MAPS

Jensen Beach Reference Monitor Well

Pendarvis Park Reference Monitor Well

APPENDIX B
Construction Photograph Log and Depth Table

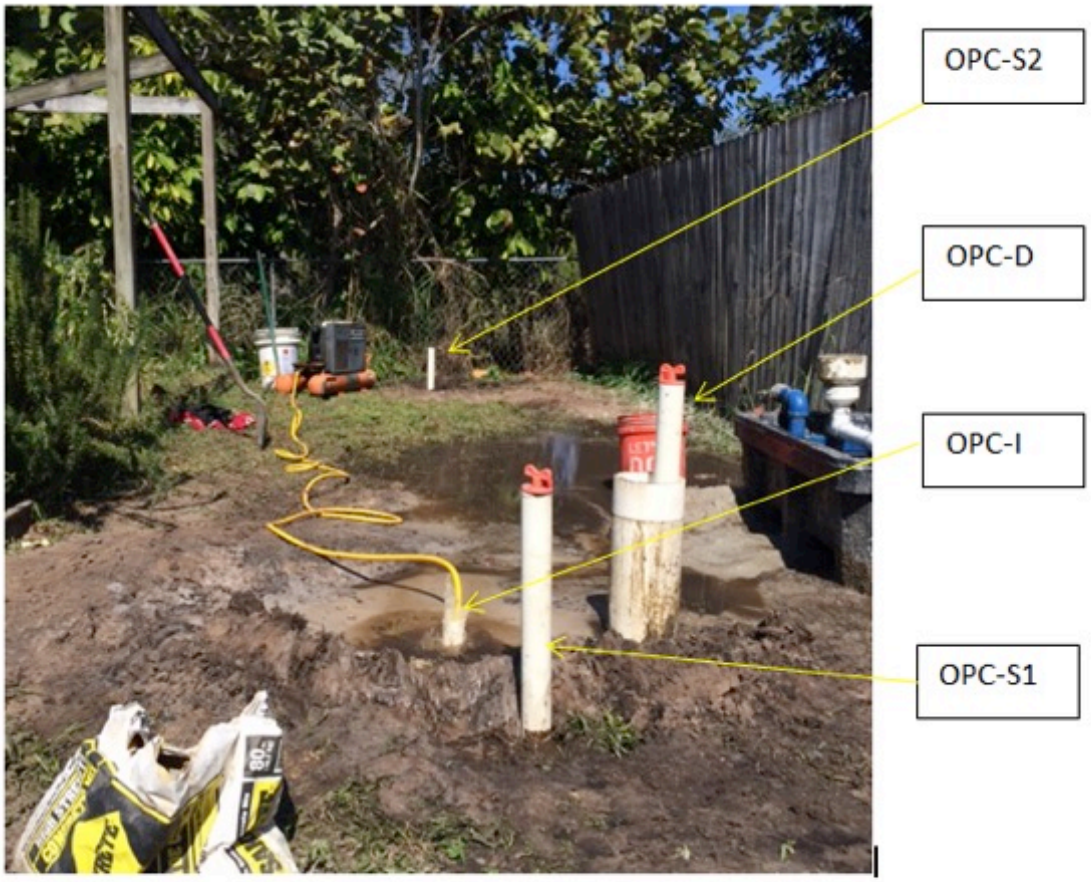
Martin County Septic Tank Study
Construction Photograph Log
February 2015

GG-S1 (L); GG-D (C); GG-I (R)

GGMW-S3

GG-S2 (Between well cluster and GG-S3 near water)

Pre-Final Surface Completion Photos
Golden Gate Monitor Wells
3352 Clayton Street



Pre-Final Surface Completion Photos
Old Palm City Monitor Wells
1135 All American Boulevard

Typical Monitor Well Surface Completions

Protective well riser with flush mount manhole and bolt down lid

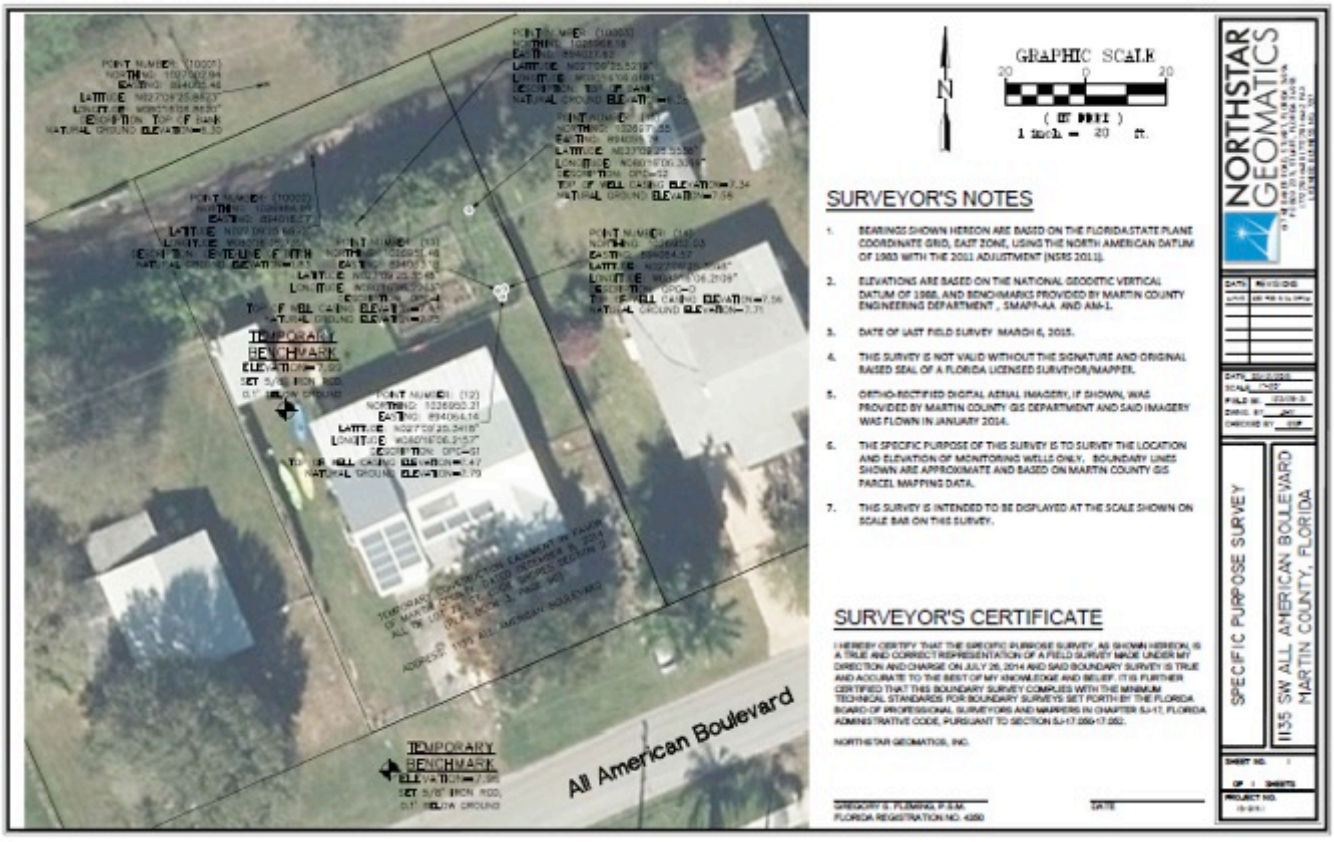
Top of monitor well survey (water level measuring point) is at top of PVC casing with well plug removed

Monitor well with removable plug

Groundwater Monitor Well Construction Summary Martin County Septic Tank Study				
Site Description	**Well ID**	**Total Borehole Depth (Feet Below Ground Level)**	**PVC Casing Diameter (Inches)**	**PVC Screen Length (Feet)**
Old Palm City	OPC-S1	12	2	2
	OPC-S2	12.2	2	2
	OPC-I	24.4	2	5
	OPC-D	57.5	2	10
Golden Gate	GG-S1	12	2	2
	GG-S2	12.3	2	2
	GG-S3	12	2	2
	GG-I	24	2	5
	GG-D	57	2	10
Pendarvis Park	PCP-C	29.5	2	5
Jensen Beach	W4B	49	2	5

APPENDIX C
Monitor Well Surveys

Old Palm City Site



Golden Gates Estates Site