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Research paper

Septic systems contribute to nutrient pollution and harmful algal blooms in the St. Lucie Estuary, Southeast Florida, USA

Brian E. Lapointe*, Laura W. Herren, Armelle L. Paule

Harbor Branch Oceanographic Institute at Florida Atlantic University, Marine Ecosystem Health Program, 5600 US 1 North, Fort Pierce, FL, 34946, USA

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ABSTRACT

Nutrient enrichment is a significant global-scale driver of change in coastal waters, contributing to an array of problems in coastal ecosystems. The St. Lucie Estuary (SLE) in southeast Florida has received national attention as a result of its poor water quality (elevated nutrient concentrations and fecal bacteria counts), recurring toxic *Microcystis aeruginosa* blooms, and its proximity to the northern boundary of tropical coral species in the United States. The SLE has an artificially large watershed comprised of a network of drainage canals, one of which (C-44) is used to lower the water level in Lake Okeechobee. Public attention has primarily been directed at nutrient inputs originating from the lake, but recent concern over the importance of local watershed impacts prompted a one-year watershed study designed to investigate the interactions between on-site sewage treatment and disposal systems (OSTDS or septic systems), groundwaters, and surface waters in the SLE and nearshore reefs. Results provided multiple lines of evidence of OSTDS contamination of the SLE and its watershed: 1) dissolved nutrients in groundwaters and surface waters were most concentrated adjacent to two older (pre-1978) residential communities and the primary canals, and 2) sucralose was present in groundwater at residential sites (up to 32.0 μg/L) and adjacent surface waters (up to 5.5 μg/L), and 3) $\delta^{15}N$ values in surface water (+7.5 ‰), macroalgae (+4.4 ‰) and phytoplankton (+5.0 ‰) were within the published range (>+3 ‰) for sewage N and similar to values in OSTDS-contaminated groundwaters. Measured $\delta^{15}N$ values in *M. aeruginosa* became increasingly enriched during transport from the C-44 canal (~5.8 ‰) into the mid-estuary (~8.0 ‰), indicating uptake and growth on sewage N sources within the urbanized estuary. Consequently, there is a need to reduce N and P loading, as well as fecal loading, from the SLE watershed via septic-to-sewer conversion projects and to minimize the frequency and intensity of the releases from Lake Okeechobee to the SLE via additional water storage north of the lake. These enhancements would improve water quality in both the SLE and Lake Okeechobee, reduce the occurrence of toxic harmful algal blooms in the linked systems, and improve overall ecosystem health in the SLE and downstream reefs.

1. Introduction

Despite their ability to provide invaluable ecological services to human populations, coastal and estuarine ecosystems are being degraded on a global scale. Humans have significantly 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, harmful algal blooms (HABs), and subsequent habitat loss (NRC, 2000; Glibert et al., 2005; Bricker et al., 2007; Heisler et al., 2008). The complexity of this problem was exemplified by Rothenberger et al. (2009) who showed that, while hog farming practices were an important contributor of eutrophic and unsafe conditions in the Neuse River (i.e. toxic *Pfiesteria* blooms (Burkholder and Glasgow, 2001)), wastewater treatment plants (WWTPs) and population growth were also significant nutrient contributors. Similarly, increasing nutrient inputs from urban, agricultural, and industrial sources have synergistically promoted blooms of the potentially toxic cyanobacterium *Microcystis aeruginosa* on a global scale (Paerl and Otten, 2013; Li et al., 2017; Liyanage et al., 2016; Preece et al., 2017). Some of the most chronic blooms have occurred in Lake Erie (Wynne and Stumpf, 2015), San Francisco Estuary (Lehman et al., 2015), Cape Fear River (Isaacs et al., 2014, Polera, 2016), Patos Lagoon Estuary, Brazil (Yunes et al., 1996), and Lake Taihu, China (Chen et al., 2003).

Findings from these and other impacted areas showed that both growth and toxicity of non-nitrogen fixing (Paerl et al., 2011)

* Corresponding author.
E-mail address: blapoin1@fau.edu (B.E. Lapointe).

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M. aeruginosa are directly linked to eutrophication and exemplify the need for simultaneous reduction of N and P inputs to freshwater and estuarine systems (Conley et al., 2009; Ma et al., 2014; Gobler et al., 2016). In estuarine waters with salinity >10 this species experiences a decrease in cellular growth and abundance and an increase in cell mortality ultimately prompting toxin release (Warhurst, 2014; Preece et al., 2017). Until recently, high P inputs alone associated with low N:P ratios (<44:1) have been thought to promote growth of *M. aeruginosa* blooms (Downing et al., 2005; Horst, 2014; Horst et al. 2014; Parrish, 2014). Recent work by Lehman et al. (2015) and a review by Gobler et al. (2016) also clearly demonstrated the importance of inorganic N, especially ammonium (NH_4^+), in the formation of *Microcystis* blooms. Contrary to previous consensus, Gobler et al. (2016) reports that *Microcystis* has multiple physiological adaptations that allow bloom formation in inorganic P – depleted waters. Like growth, production of the N-rich hepatotoxin microcystin is also driven by N:P ratios, but primarily as they relate to N assimilation (Downing et al., 2005; Gobler et al., 2016). Because microcystin is N-rich, toxic strains of *Microcystis* require more N than non-toxic strains (Davis et al., 2010). Downing et al. (2005) show that N:P ratios between 8 and 51 promoted the highest microcystin content. The direct relationship between microcystin levels and dissolved reactive N concentrations, both NH_4^+ (Donald et al., 2011) and nitrate (NO_3^-; Horst et al., 2014), provided evidence of wastewater (also referred to as sewage) inputs during these blooms. Like wastewater itself, elevated microcystin levels exacerbated by wastewater have the potential to impact both human and ecosystem health (Rastogi et al., 2014).

In southeast Florida, the St. Lucie Estuary (SLE) has received national attention and has been the subject of litigation for chronic human health impacts and severely degraded ecosystem health. The system is exceptional in its anthropogenic complexity, reoccurrences of economically and ecologically devastating *M. aeruginosa* blooms, frequent health advisories for high fecal bacteria counts, and proximity to the northern extent of tropical coral species along the east coast of the United States (Fig. 1A, B). Through a partnership between the U.S. Environmental Protection Agency and the Florida Department of Environmental Protection (FDEP), the SLE has been identified as an impaired waterbody and Total Maximum Daily Loads have been established for total nitrogen (TN), total phosphorus (TP), dissolved oxygen (DO), and fecal coliforms (Parmer et al., 2008; White and Turner, 2012). 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

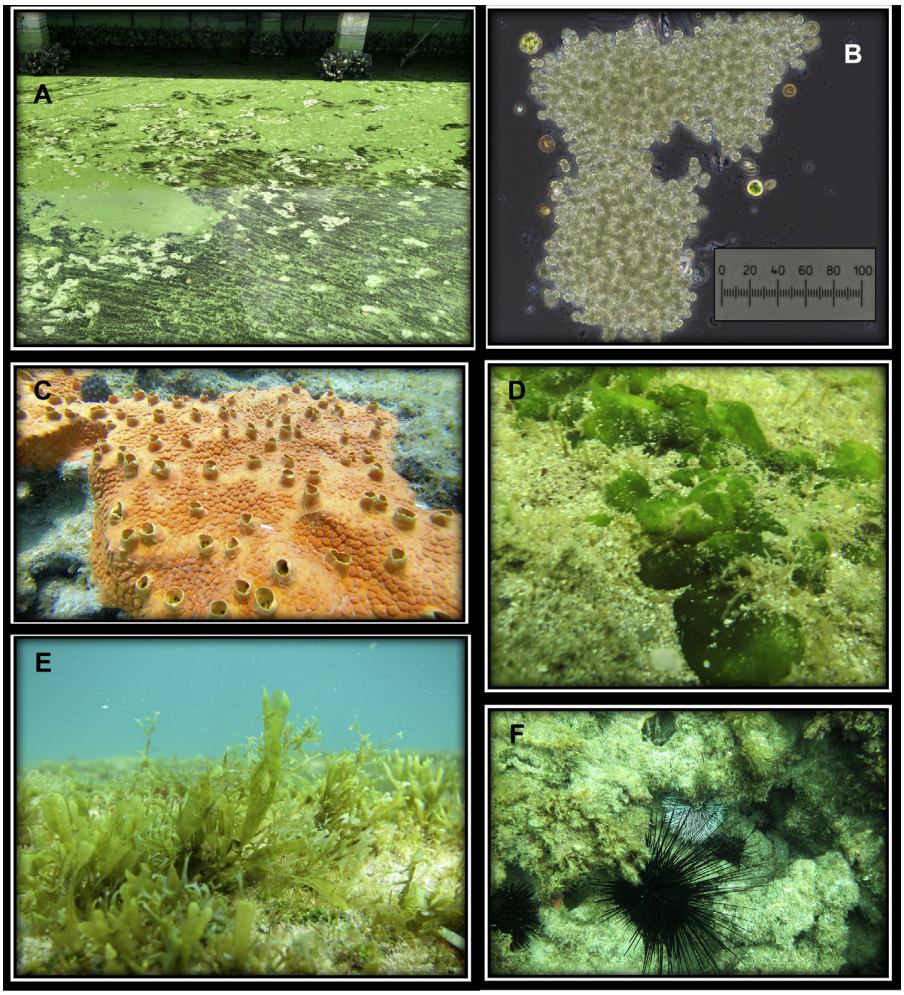

Fig. 1. Ecosystem responses to eutrophication in the St. Lucie Estuary (SLE) and nearshore reefs: (A, B) *Microcystis aeruginosa* in the SLE with 40x magnification scale bar in micrometers (μm) and (C) Clionid sponge, (D) *Codium intertextum*, (E) *Dictyota* spp., (F) four common species of sea urchins (*Diadema antillarum*, *Tripneustes ventricosus*, *Echinometra viridis*, *Eucidaris tribuloides*) along the nearshore reefs. Photo credits: (A, B) James Sullivan, (D) Brian Lapointe, (C, E, F) Laura Herren.

(FDEP, 2009; SFWMD, FDEP, FDACS, 2009). Hydrological alterations began in 1924 when the South Fork of the SLE was connected to Lake Okeechobee via the C-44 canal to reduce water levels in the lake (Fig. 2; Blake, 1980).

Massive fresh water releases from Lake Okeechobee and the C-44 watershed in 2005, 2013, and 2016 lowered the salinity in the estuary, seeded the system with *M. aeruginosa*, and ultimately resulted in the formation of three unprecedented *M. aeruginosa* blooms that extended from the estuary downstream to the nearshore reefs. Consistent with the literature, the TDN:TDP ratios during each of these blooms were <33 (Lapointe et al., 2012; Lapointe, *unpublished data*; FDEP, unpublished data). While emphasis has been placed on the nutrient inputs from Lake Okeechobee and the subsequent ecological impacts the additional load brings, Lapointe et al. (2012) suggested that there were sufficient local nutrient loads from the SLE watershed itself to support bloom development and toxicity. To this point, $\delta^{15}N$ values of *M. aeruginosa* samples collected in the mid-estuary during the 2013 and 2016 blooms were highly enriched (+8.6 $^{o}/_{oo}$ and +7.0 $^{o}/_{oo}$, respectively) compared to samples from the C-44 canal (< 6.0 $^{o}/_{oo}$), indicating wastewater N as a primary N source fueling the blooms in the SLE. Furthermore, the 2005, 2013, and 2016 blooms were confirmed to be comprised of toxic strains of *M. aeruginosa* (Ross et al., 2006; Phlips et al., 2012; Oehrle et al., 2017), ultimately suggesting that there were high enough concentrations of nitrate in the SLE to promote toxin production. During the 2016 bloom, Oehrle et al. (2017) documented that most (>85%) of the total microcystins were microcystin-LR, a form that was found at concentrations as high as 4500 μg/L. The World Health Organization drinking water and recreational water contact standards are set at 1 μg/L and 20 μg/L, respectively. Their research also showed that toxin concentrations increased from the C-44 (i.e. seed algae from Lake Okeechobee and the canal) to the Middle Estuary where high concentrations of nitrate from the South Fork and ammonium and phosphorus from the North Fork (Lapointe et al., 2012) converge to enrich the bloom. With such high nutrient availability, *Microcystis* has the potential to double its biomass in approximately two days (Nicklisch and Kohl, 1983; Li et al., 2014). This combination of a toxic gradient and nutrient availability provides additional evidence that significant bloom development has been occurring in the SLE itself rather than the upstream seed sources.

In addition to phytoplankton-based HABs, degradation of nearshore reefs by macroalgal HABs likewise result from inputs of land-based sources of N and P (Lapointe et al., 2005; Littler et al., 2006; Lapointe and Bedford, 2010; Lapointe et al., 2011). Along the east coast, the Florida Reef Tract extends from the Florida Keys north to St. Lucie Inlet in Martin County. While reefs supporting tropical coral species are exclusively found south of the inlet,

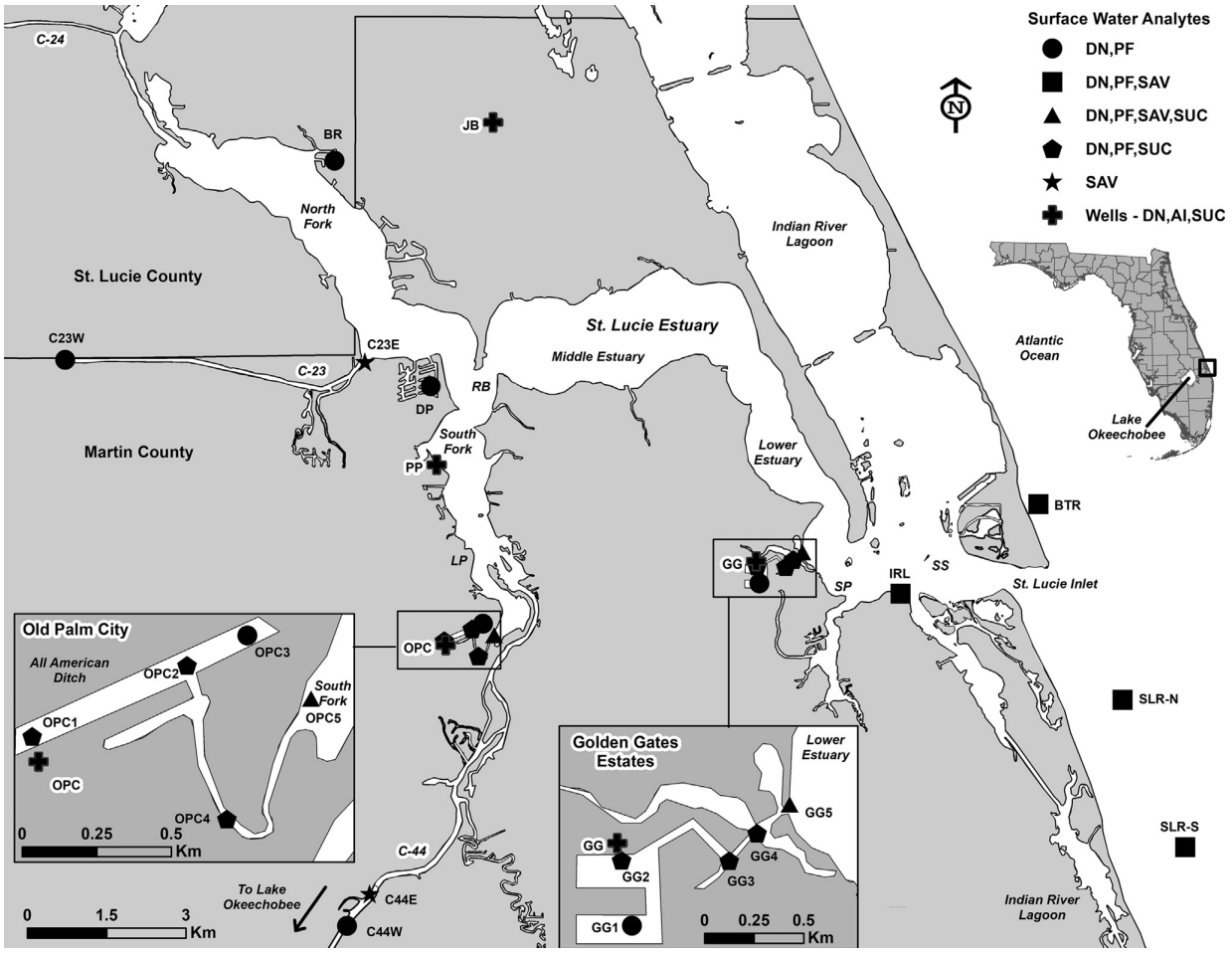

Fig. 2. Martin County watershed to reef project site map with subsets showing the Old Palm City (OPC) and Golden Gates Estates (GG) residential communities. Analytes measured at each site are represented by shapes as indicated by the key. DN = dissolved nutrients, AI = aqueous isotopes, PF = phytoplankton, SAV = submerged aquatic vegetation, SUC = sucralose. Martin County Department of Health *enterococcus* monitoring stations are italicized; LP = Leighton Park, RB = Roosevelt Bridge, SP = Sandsprit Park, and SS = Stuart Sandbar.

Sabellariid wormrock (*Phragmatopoma lapidosa*) reefs span both north and south. The nearshore reefs adjacent to St. Lucie Inlet are exposed to eutrophic water discharged from the Indian River Lagoon (IRL), SLE, and periodic freshwater releases from Lake Okeechobee located west of the SLE (Lapointe et al., 2012, 2015b). Smith (2016) concluded that during an ebbing tide most (~91%) of the water flowing out of the SLE continues east through the St. Lucie Inlet ultimately bathing nearshore reefs while the other ~9% flows north in the IRL towards Fort Pierce Inlet. Land-based discharges to these nearshore reefs likely contribute to biological indicators of stress, including unusually high abundances of species indicative of high nutrient environments such as clinoid (boring) sponges, macroalgal blooms (*Codium intertextum* and *Dictyota* spp.), and sea urchins (Herren and Monty, 2006; Fig. 1C–F). At these reef sites, corals also show negative physiological responses associated with changes in water chemistry and light associated with prolonged releases from Lake Okeechobee (Beal et al., 2012). Thus, to improve conditions along these biodiverse nearshore reefs, there is a pressing need to identify and subsequently manage upstream nutrient sources (Lapointe et al., 2012).

The health of the SLE and the underlying causes of its impairment, including reoccurrence of toxic HABs, high fecal bacteria, and degradation of downstream nearshore reefs, have been debated for decades. One emerging issue is the potential nutrient loading associated with the application of biosolids (domestic wastewater residuals; Tetra Tech, 2017). No Class AA biosolids are produced and no Class B biosolids are applied in Martin County, however, the practice does occur in other counties (but not significantly) along the IRL (FDEP, 2014). This has raised concerns as a potential nutrient source supporting HABs and introduction of pathogens and chemical contaminants into surface waters. While not without risk, when properly treated and applied this method of recycling waste material has been deemed safe for both humans and the environment (AMS, 2011). There has also been an increasing interest in Florida to understand the often overlooked role of on-site sewage disposal systems (OSTDS; septic systems and shallow injection wells) in enrichment and microbial contamination of shallow groundwaters and adjacent surface waters via submarine groundwater discharge (SGD; Lapointe et al., 1990, 2012; Lapointe and Krupa, 1995a,b; Paul et al., 1995a,b; Griffin et al., 1999; Tarnowski, 2014). In addition to SGD, SLE water quality is also affected by tidal creeks and primary canals (C-44, C-23, and C-24; Fig. 2) and, in turn, the sediments that can sequester incoming N and P from these sources (Howes et al., 2008; Havens et al., 2016). The C-23 and C-24 deliver water solely from the SLE watershed. Conversely, inputs from the C-44 originate from both the watershed (C-44 basin) and, periodically, Lake Okeechobee via freshwater releases up to 10,000 cfs (Doering, 1996; Lapointe et al., 2012). While Lapointe et al. (2012) previously documented the deleterious effects of prolonged, high-volume releases to the SLE, the 2005–2006 study simultaneously indicated local septic system contributions. Furthermore, tidal creeks were found to be a significant source of fecal coliform, total coliform, and enterococcus bacteria; both showing high to low count gradients from upstream (residential areas) to downstream (SLE). The human fecal source marker qPCR Bacteroidales HF183 was also documented at multiple sites throughout the SLE during a 2014 microbial source tracking study performed by FDEP (2015d).

The combination of sources interacts to exacerbate the chronically poor water quality conditions and susceptibility of the SLE and downstream nearshore reefs to HABs. The ability to distinguish between water quality impacts from the groundwater and stormwater runoff derived from the SLE watershed versus impacts directly related to periodic discharges from Lake Okeechobee is an important water management issue in this region. Recent water and nutrient budgets for the St. Lucie Estuary indicate that, between Water Year 1997 and 2015, about 30% of the N came from Lake Okeechobee, compared to 70% from the St. Lucie River watershed and tidal basin (Zheng et al., 2016). Although more attention has been given to the contribution of freshwater releases from Lake Okeechobee to this system, it is also important to understand the significance of nutrients (e.g., atmosphere, fertilizers, wastewater) from local watersheds to achieve nutrient mitigation for the SLE and the downstream ecosystems (Badruzzaman et al., 2012).

When combined, multiple analytical approaches can provide corroborative evidence of eutrophication and nutrient sources. While dissolved nutrient concentration data may suggest biological thresholds for nutrient pollution, artificial substances consumed by humans and evident in waste streams and receiving waters, and stable isotope analyses of water and algal tissue are reliable protocols for tracking nutrient sources. For example, analysis of water samples for the artificial sweetener sucralose provides an indicator of human wastewater. Sucralose is not broken down by any treatment process (including the body) and is transported conservatively through WWTPs and OSTDS (Oppenheimer et al., 2011). Meanwhile, aqueous stable isotopes of N, both $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}NO_3^-$, can be utilized to discriminate different sources of dissolved inorganic N. Previously, Lapointe et al. (2015a) documented stormwater runoff from urban and agricultural land uses within the SLE watershed with depleted mean $\delta^{15}N$ values within the accepted range for inorganic fertilizers (−2 to +2 ‰).

Similarly, macroalgae and phytoplankton are commonly used indicator organisms for assessment of the relative importance of N sources and algal tissue nutrient contents may indicate the 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 (wastewater, fertilizer) nutrient sources (Risk et al., 2009). The published ranges are generally as follows: natural N-fixation (0 ‰; Heaton, 1986; France et al., 1998), offshore upwelled nitrate (~+2.0 ‰; Knapp et al., 2008), atmospheric N (−3 ‰ to +1 ‰; Paerl and Fogel, 1994), and synthetic fertilizer N (−2 ‰ to +2 ‰; Bateman and Kelly, 2007). All of these N sources are depleted relative to enriched values of +3 ‰ to +19 ‰ for human wastewater (Heaton, 1986; Costanzo et al., 2001) and +10 ‰ to +20 ‰ for livestock waste (Kreitler, 1975, 1979; Heaton, 1986). These livestock waste values depend on if the effluent is nitrified, or not, as values can be much lower. Nitrogen in OSTDS effluent is primarily in the form of ammonium (Bicki et al., 1984; Lapointe et al., 1990; Valiela et al., 1997) with $\delta^{15}N$ values of +4 − 5 ‰ (Lapointe and Krupa, 1995a,b; Hinkle et al., 2008; Katz et al., 2010), but through ammonia volatilization and microbial processing values can become more enriched (i.e. treated wastewater). Because it is difficult to discern overlapping signatures (i.e. ≥+10 ‰) one must consider land use in the adjacent watershed. Accordingly, enriched macroalgae $\delta^{15}N$ values >+3 ‰ have been reported in a wide variety of sewage-polluted coastal waters, including Florida's densely-developed IRL (Lapointe et al., 2015b), nearshore reefs off urban areas of east-central Florida (Barile, 2004), and coastal urban areas of southeast (Lapointe et al., 2005) and southwest (Lapointe and Bedford, 2007) Florida.

In addition to stable isotope analyses, measurement of C:N:P content in macroalgae and phytoplankton 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 adsorb P (Bicki et al., 1984). When this occurs, groundwater and, ultimately, surface water P concentrations become higher, thereby lowering the N:P ratio and increasing *M. aeruginosa* bloom potential (Horst, 2014; Horst et al., 2014; Parrish, 2014).

A one-year watershed to reef study was designed to document sources of nutrients causing eutrophication in the SLE, the periodic *M. aeruginosa* blooms seeded by Lake Okeechobee discharges, and the subsequent downstream decline of the nearshore Sabellariid wormrock and coral reefs. The study was a comprehensive analysis of the interactions of septic systems, groundwaters, and surface waters that included: 1) groundwater sampling in two residential areas identified as high priority septic to sewer conversion sites and undeveloped reference sites for dissolved nutrients, aqueous N isotopes, and sucralose; 2) surface water sampling in the C-44 and C-23 canals, throughout the SLE, and nearshore reefs for dissolved nutrients and sucralose; and 3) collection and analysis of macrophytes and phytoplankton for stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analysis and algal tissue elemental composition (C:N:P).

2. Materials and methods

Located on Florida's east coast, the SLE is the largest tributary to the 251 km long IRL. The upper SLE includes the lower-salinity North and South forks which converge to form the higher-salinity Middle Estuary. The Middle Estuary flows east through the Lower Estuary to the IRL and out to tide through St. Lucie Inlet (Fig. 2). There are three primary canals (C-44, C-23, and C-24) that drain directly into the SLE that have ultimately increased the natural extent of the SLE watershed.

Four SLE watershed to reef sampling events were performed during ebbing tides in 2015; April 7–10, 17, 20 (Dry 1), May 11–14 (Dry 2), August 5–7 (Wet 1), September 21, 23, 24 (Wet 2). During each event, 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. 2). Upstream to downstream sampling networks were created within and adjacent to two older (pre-1978) residential communities previously identified as high-priority septic to sewer conversion sites (Keene, 2015; Lapointe et al., 2016). In Old Palm City (OPC), 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 (GG), 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. 2). 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 analyses were conducted on samples collected during each of the four sampling events (Table 1). The University of Georgia's Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL) in Athens, Georgia conducted all analyses except sucralose. Concentrations of this artificial sweetener were analyzed by Florida Department of Environmental Protection's Central Laboratory in Tallahassee, Florida.

2.1. Freshwater inputs and enterococcus bacteria counts

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 National Oceanic and Atmospheric Administration 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. To ensure a complete dataset, missing data were obtained from U.S. Climate Data's station Stuart 1 s (http://www.usclimatedata.com/climate/stuart/florida/united-states/usfl0468) located 1 km west of the above station (27.1897, −80.2397). Daily total precipitation (mm) was plotted to indicate seasonal rainwater inputs relative to sampling events.

2.1.2. Canal discharges

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 (SFWMD) online database DBHYDRO. Flow data for the S-80 structure (Key: DJ238) at the confluence of the C-44 canal and the South Fork were monitored by the U.S. Army Corps of Engineers (USACE), 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 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 separated into Dry (January 1 to May 31, 2015) versus Wet (June 1 to September 30, 2015) seasons. The USACE and SFWMD were informed of the study and requests were made by Martin County to stop releases during the sampling events.

2.1.3. Fecal bacteria distribution and abundance

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]/100 mL river water) were reported for four monitoring stations: 1) Roosevelt Bridge, 2) Sandsprit Park, 3) Leighton Park, and 4) the Stuart sandbar (near Sailfish Point and St. Lucie Inlet).

Table 1
Analytes measured during the 2015 Martin County watershed to reef study.

General Analysis	Media Collected (# Stations)	Analytes
dissolved nutrients	ground (11), surface (18) water	ammonium, nitrate, phosphate, total nitrogen, total phosphorus
stable aqueous isotopes	groundwater (11)	$\delta^{15}N$-ammonium, $\delta^{15}N$-nitrate
dissolved artificial substances	ground (11), surface (8) water	sucralose
stable tissue isotope	macroalgae (8), phytoplankton (18)	$\delta^{13}C$, $\delta^{15}N$
elemental tissue composition	macroalgae (8), phytoplankton (18)	C:N, C:P, N:P

The water quality scale based on count data set by Florida DOH includes the following categories, good (0 to 35 cfu/100 mL), moderate (36 to 70 cfu/100 mL), and poor (≥71 cfu/100 mL).

2.2. Groundwater

To clarify the potential effects of OSTDS on surface waters in the SLE and downstream nearshore reefs, nine wells were installed at two residential sites; OPC and GG as described by Lapointe and Herren (2016); Fig. 2). 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 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. 2). Two existing reference or control wells, one managed by SFWMD (PCP-C) and the other by the Martin County Utilities Department (W4B), were also incorporated into the study to investigate anthropogenic effects of residential septic systems on water quality. PCP-C (9.0 m) was located at Pendarvis Park along the South Fork and W4 B (14.9 m) was installed along Jensen Beach Boulevard adjacent to the Savannas Preserve State Park in Jensen Beach (Fig. 2). Because of the high degree of mixing between the surface, intermediate, and deep wells (especially the two former), results were pooled and presented by community (OPC, GG) and reference (Pendarvis Park, Jensen Beach Boulevard). Protocols for well purging and stabilization prior to sample collection were outlined in Lapointe and Herren (2016). After stabilization criteria were met, groundwater samples were collected using the following procedures.

2.2.1. Groundwater dissolved nutrients and aqueous N isotopes

Dissolved nutrient and aqueous N isotopes samples were collected from the same pump after a 0.45 μm high capacity cartridge filter was fitted to the discharge tube of the pump. A new cartridge filter was used for each well. From each well, three 1 L replicates for both forms of N aqueous isotopes ($\delta\ ^{15}N\text{-}NH_4^+$ and $\delta\ ^{15}N\text{-}NO_3^-$) were collected in a total of six acid washed high-density polyethylene (HDPE) bottles. Samples 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 to the UGA-SIEL for analysis. Once received, the samples were immediately frozen. Prior to analysis, the samples were thawed, homogenized, and ~100 mL of sample was removed from three of the six 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 soluble reactive phosphorus [SRP or PO_4^{3-}]). 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 per Koroleff (1983) methods as modified by Qualls (1989) (UGA-SIEL, 2015a). Once digested, all nutrient forms (NH_4^+, NO_x, 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 NH_4^+, then proceeding with the standard diffusion and ammonia capture on an acidified filter. The filter was then analyzed as a typical solid sample on a Carlo Erba Isotope Ratio Mass Spectrometer (IRMS) for $\delta^{15}N\text{-}NH_4^+$ and $\delta^{15}N\text{-}NO_3^-$.

2.2.2. Groundwater sucralose

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 °C through shipping. The coolers, with accompanying chain of custody forms, were returned overnight to the FDEP Central Laboratory the same day that the samples were collected. At the laboratory, water samples were filtered through a 0.75 μm glass fiber (GF/F) filter. A 250 mL aliquot of filtered sample was then passed through a graphitized carbon-based, solid-phase extraction (SPE) column. After extraction, the absorbed analytes were eluted from the SPE column with a mixture of 80% methylene chloride: 20% methanol. The extract was reduced to near dryness and brought to a final volume of 1 mL with 10% acetonitrile in deionized water. Analytical standards were prepared in the same final solvent mixture. Each extract was analyzed by high performance liquid chromatography/tandem mass spectrometry in the negative ion mode for the determination of sucralose (method detection limits of 0.01 μg/L). This analytical procedure is based on U.S. Geological Survey (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 their website (http://www.dep.state.fl.us/labs/library/lab_sops.htm).

2.3. Surface water and algal tissue

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

2.3.1. Surface water dissolved nutrients

Surface water samples were collected in triplicate just below the surface into acid-washed 250 mL HDPE bottles and covered with ice in a dark cooler until return to the laboratory for processing. The samples were filtered (0.7 μm GF/F filters) and frozen until analysis. At UGA-SIEL, samples were thawed, homogenized, and subsampled for either persulfate digestion (TN/TP) or direct analyses (NO_x, NH_4^+, and PO_4^{3-}) as mentioned for groundwater in 2.2.1. The resulting data were used to characterize ambient dissolved inorganic N (DIN), TN and TP concentrations, DIN:SRP ratios, and total dissolved N (TDN):total dissolved P (TDP) ratios at the 18 surface water collection sites. Calibrated YSI Model 1030 and ProODO hand-held meters were used to document salinity, temperature, DO, and pH at the time each water sample was collected.

2.3.2. Surface water sucralose

Unique surface water samples were collected for sucralose at four sites in both OPC and GG. Each of the samples were collected in 1 L amber bottles and kept on ice throughout overnight shipment to the FDEP Central Laboratory. Processing of the surface water samples was identical to the groundwater samples described above in Section 2.2.2.

2.3.3. Macroalgal tissue $\delta^{13}C$, $\delta^{15}N$ and C:N:P

Triplicate samples of macroalgae were collected at eight submerged aquatic vegetation sampling stations. Because of the inconsistent presence of macroalgae in some sections of the SLE, especially in the upper reaches, cages were deployed for 4 weeks to hold replicates of the red alga *Gracilaria tikvahiae* provided by the Marine Botany Laboratory at HBOI-FAU. Once the algae were 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 were ground to a fine powder using a Thompson Scientific Wiley Mini-Mill® and stored in plastic screw- top vials. Sub-samples were shipped to UGA-SIEL for stable C and N isotope analysis and tissue C, N, and P content. At UGA-SIEL, samples were split into two. One half was analyzed for stable C and N isotopes and total C and N content on a Thermo Delta V IRMS coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo Conflo III Interface (see http://sisbl.uga.edu/ratio.html#top; Thermo Scientific, 2007). National Institute of Standards and Technology reference materials 8549, 8558, 8568, and 8569 (NIST, 2008) were used to routinely calibrate or check working standards prepared in the laboratory. The working laboratory standards with a range of N isotope-ratios were produced by the methods described by Böhlke et al. (1993) and Böhlke et al. (2003). QA/QC results were incorporated into the raw data reports received by UGA-SIEL. The other half of each sample was analyzed for total P content, 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 (Allen et al., 1974; Jones et al., 1990; UGA-SIEL, 2015b). The acid extracts were then diluted 41:1 with pure water for TP (as PO_4-P) analysis on an Alpkem 300 series analyser. The resulting stable C and N isotope and C:N:P data of the macroalgae were used to make inferences regarding nutrient availability in relation to various natural and anthropogenic N sources (Atkinson and Smith, 1983; Lapointe, 1997; Costanzo et al., 2001; Lapointe et al., 2015b). C:N:P data were compared to a modified Redfield ratio of 360:30:1 to characterize temporal and spatial variation in tissue nutrient status, 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 (Atkinson and Smith, 1983).

2.3.4. Phytoplankton tissue $\delta^{13}C$, $\delta^{15}N$ and C:N:P

Three 1 L bottles of surface water were collected at the 18 fixed sites. Each sample was pre-filtered through 200 μm nylon netting in the field to remove macrodetritus and macrozooplankton (Savoye et al., 2003). At HBOI-FAU, the pre-filtered samples were passed through 47 mm GF/F filters to capture the phytoplankton and the filters were sent to UGA-SIEL for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes and C:N:P analysis (note: the water was used 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 content using the same equipment mentioned above for macroalgal tissue. The other half was ashed for Aqua Regia extraction and diluted for TP analysis according to the same process outlined above for macroalgae TP. The resulting stable isotope and C:N:P data were 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 increasing P-limitation, and N:P ratios >16 indicate increasing P-limitation.

2.4. Statistical analysis

Because of the non-normal distribution of data collected during this study, non-parametric tests were used to determine significance of spatial and temporal differences in measured variables. In IBM Statistical Package for the Social Sciences (SPSS) v23, the *t*-test was used to identify significant differences (p 0.05) in mean enterococcus counts between the Dry and Wet seasons and groundwater dissolved nutrient concentrations in residential areas versus reference sites. The Kruskall Wallis test was used to identify overarching significant ($p < 0.05$) spatial and temporal differences in measured variables. The Mann-Whitney *U* test was used to identify where the significant differences in spatial and temporal data occurred.

3. 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 little rainfall fell 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. 3). The cumulative rainfall five days prior to the Dry 1, Dry 2, Wet 1, and Wet 2 sampling events were 0 mm, 178 mm, 488 mm, and 306 mm, respectively. The average (±S.E.) daily rainfall between January 1 and September 30,

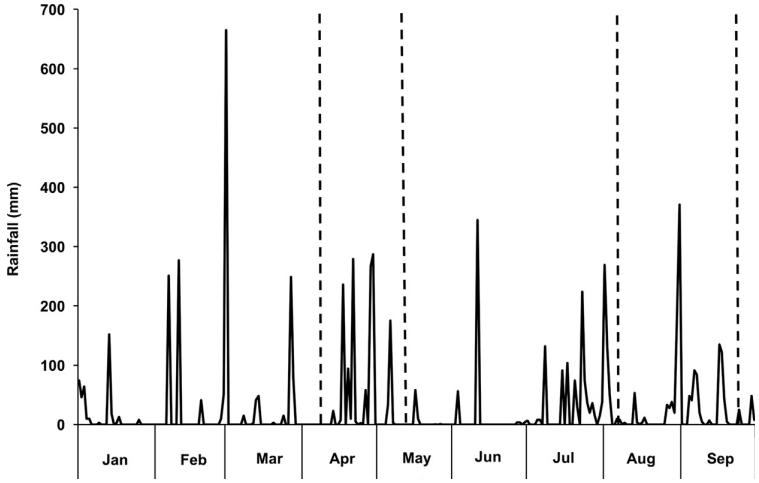

Fig. 3. Daily precipitation (mm) at centrally located weather stations. Dashed bars represent sampling events.

2015 was 25.4 ± 4.4 mm, with a range between 0 and 665 mm. During the Dry season (January 1 to May 31), rainfall ranged between 0 and 665 mm and averaged 24.6 ± 6.5 mm. The Wet season (June 1 to October 30) had an average rainfall of 26.4 ± 5.6 mm and daily precipitation ranged from 0 to 371 mm.

3.1.2. Canal discharges

Discharges from the S-80 structure on the C-44, S-48 structure on the C-23, and the S-49 on the C-24 occurred 50.6%, 57.9%, and 48.4% of the time, respectively during the study. Mean (±S.E.) flow rates at these three structures were 312.5 ± 27.4 cfs, 472.7 ± 14.3 cfs, and 155.2 ± 18.2 cfs, respectively. The C-44 was unique in that more water was released during the Dry (65.6% of the time; mean flow rate 394.0 ± 33.2 cfs) than the Wet (31.9% of the time; mean flow rate 209.7 ± 44.0) season. Conversely, most water was released from the C-23 and C-24 canals during the Wet season when water was discharged 63.9% and 67.2% of the time, respectively, with mean flow rates of 136.4 ± 30.2 cfs and 396.5 ± 35.9, respectively. During the Dry season water was released from the C-23 and C-24 canals 53.0% and 33.1% of the time, respectively, with mean flow rates of 27.5 ± 5.9 cfs and 43.0 ± 7.5 cfs, respectively.

The amount of water released from each of the three canals varied during the four collection periods (Fig. 4A–C). No water was released during the Dry 1 from any of the canals. While little water was released from the C-44 for one month prior to the Dry 2 sampling event, water managers released at a rate of 650 to 1250 cfs during the Dry 2 collection period (Fig. 4A). Water was also discharged in low volumes from the C-23 (<130 cfs) and C-24 (<215 cfs) during the Dry 2 collection. No water was released from the C-44 and C-23 during the Wet 1 collection, however, there were low-level releases (<215 cfs) from the C-23 just prior to the sampling event (Fig. 4B). The C-24 releases during the Wet 1 collection were <300 cfs. The Wet 2 samples were collected during C-44 releases ranging from 450 to 850 cfs, with higher-level releases ranging from 1500 to 3500 cfs the week prior to sample collection (Fig. 4A). Water was also released during the Wet 2 collection from the C-23 (<900 cfs), with heavier releases (between 1000 and 2700 cfs) for eight consecutive days prior to Wet 2 sampling. The C-24 flow rates were >2,440 cfs just two days prior to the Wet 2 sample collection, but ranged from 560 to 1040 cfs during the Wet 2 collection period (Fig. 4C).

3.1.3. Fecal bacteria distribution and abundance

Throughout the study, mean enterococcus counts (cfu/100 mL ± SE) followed an upstream to downstream gradient with highest values 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; Fig. 2). Although there was no significant difference in mean enterococcus counts (cfu/100 mL) between the Dry and Wet seasons ($t = 0.31$, $p = 0.76$), the highest enterococcus counts at the Leighton Park (390 cfu/100 mL), Roosevelt Bridge (281 cfu/100 mL), and the Stuart Sandbar (33 cfu/100 mL) occurred during the Dry season. Conversely, the highest counts at Sandsprit Park (80 cfu/100 mL) were collected on September 21, 2015, overlapping with the last sampling event. The concentrations at the Leighton Park, Roosevelt Bridge, Sandsprit Park, and Stuart Sandbar sites were in the moderate to poor range (≥36 cfu/100 mL) 56.1%, 19.5%, 7.3%, and 0% of the time, respectively between January 1 and September 30, 2015 (Fig. 5A–D).

3.2. Groundwater

3.2.1. Groundwater environmental parameters

Environmental measurements for pH, temperature, conductivity, DO, and turbidity varied by location and sampling event

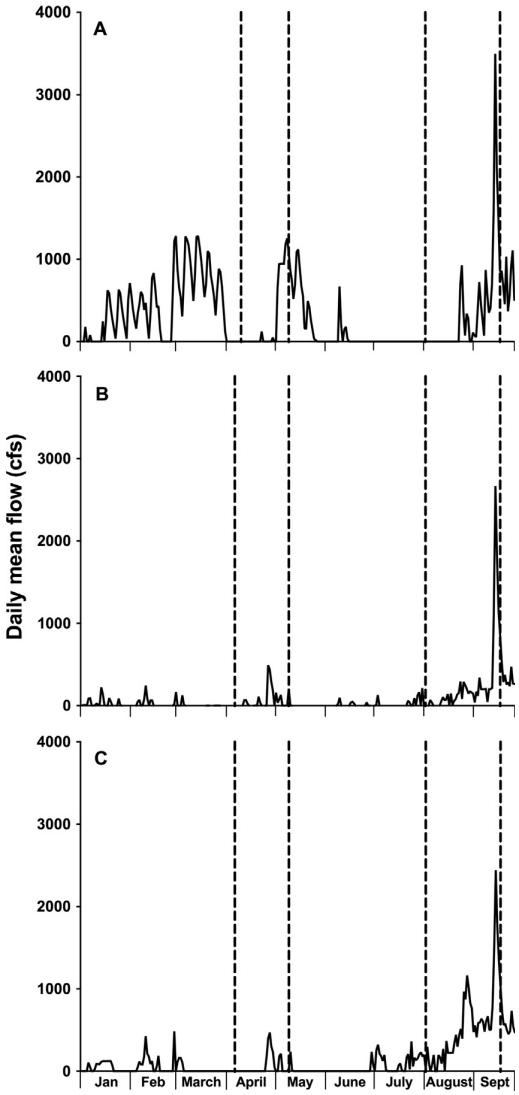

Fig. 4. Daily mean flows (cfs) from the a) S-80 water control structure along the C-44 canal, b) S-48 structure along the C-23 canal, and c) S-49 structure along the C-24 canal between January 1 and September 30, 2015. Black dashed bars represent sampling events.

(Table 2). The mean (±S.E.) pH in all groundwater wells was 6.9 ± 0.1. These overall means were lowest in the reference wells (6.5 ± 0.3), slightly higher in OPC (6.9 ± 0.2), and highest in GG (7.2 ± 0.1). The pH was consistently lower during the Wet (6.6 ± 0.1) than the Dry (7.3 ± 0.1) season throughout the study, especially at the two residential sites (Table 2). pH was generally lowest in the shallow wells (Table 2). The mean (±S.E.) water temperature in all wells was 26.2 ± 0.2 °C with consistently lower temperatures in the two residential well clusters (~25.8 °C) than the reference (27.7 ± 0.5 °C) wells (Table 2). Water temperature was lower during the Dry (25.3 ± 0.3 °C) than the Wet (27.0 ± 0.2 °C) season. The mean (±S.E.) conductivity in all groundwater wells was 404.2 ± 28.8 μmhos/cm where both OPC (417.3 ± 47.6 μmhos/cm) and GG (412.6 ± 48.3 μmhos/cm) well clusters had higher conductivity than the reference (357.3 ± 45.0 μmhos/cm) wells. The conductivity was consistently lowest during the Dry season (Table 2). DO concentration remained relatively consistent with an overall mean (±S.E.) and seasonal

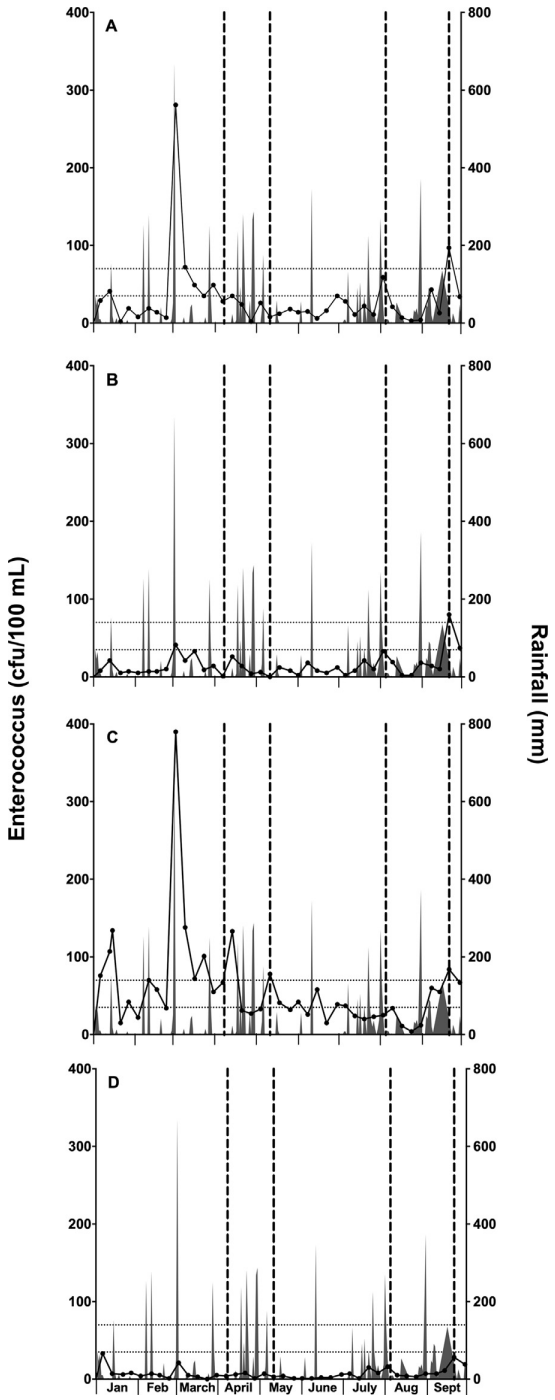

Fig. 5. Enterococcus counts (cfu/100 mL; black line with data points) versus daily rainfall (mm; filled gray line) between January 1 and September 30, 2015 at a) Leighton Park, b) Roosevelt Bridge, c) Sandsprit Park, and d) the Stuart Sandbar. Data were supplied by Martin County Department of Health. 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 70 cfu/100 mL; light gray), and Poor (≥71 cfu/100 mL; white). The vertical dashed lines represent the four project sampling events.

means all of ~0.7 mg/L (Table 2). Two exceptions were observed during the Dry 2 and Wet 1 sampling events the concentrations at OPC and Pendarvis Park (>1 mg/L) wells adjacent to the South Fork were higher than those concentrations documented in GG (<0.5 mg/L). The mean (±S.E.) turbidity level for all wells was 4.4 ± 1.5 NTU. The OPC wells had the highest turbidity (9.2 ± 4.0 NTU) followed by those in GG (2.1 ± 0.6 NTU) and at the reference (1.3 ± 0.4 NTU) sites. Overall, turbidity was higher during the Dry (6.5 ± 3.0 NTU) than the Wet (2.4 ± 0.7 NTU) season. Overarching event means were highest during the Dry 1 sampling event because of especially turbid conditions (61.9 NTU) in the OPC deep well at that time (Table 2).

3.2.2. Groundwater dissolved nutrients

Overall, dissolved nutrients documented at residential groundwater monitoring sites located at OPC and GG were significantly higher than those recorded at the non-residential reference sites ($t = 5.617$, $p < 0.001$). This remained true during both the Dry ($t = 3.018$, $p = 0.003$) and Wet ($t = 5.799$, $p < 0.001$) seasons (Fig. 6A–F). Overall mean DIN and TDN concentrations (±S.E.) in the residential neighborhoods were 258.9 ± 64.3 μM and 303.4 ± 65.7 μM, respectively, versus 21.1 ± 1.4 μM and 49.1 ± 4.9 μM at the reference sites. With the exception of OPC during the Wet season, the DIN at the OPC and GG well sites were 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 OPC 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 GG 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 P (both SRP and TDP) concentrations were high in the residential areas (22.1 ± 3.2 μM and 24.7 ± 3.4 μM, respectively) compared to the reference sites (0.6 ± 0.1 μM and 1.9 ± 0.3 μM, respectively). Both SRP and TDP concentrations were especially elevated in OPC during the Wet season (Fig. 6B,E). The majority of the P documented in the groundwater at OPC and GG was in the reactive (PO_4^{3-}) 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 OPC well sites ranged from 0.4 ± 0.1 μM during the Dry season to 106.9 ± 7.9 μM during the Wet season. At GG, values ranged from 0.6 ± 0.0 μM during the Dry season to 99.4 ± 2.4 μM during the Wet season. The highest mean SRP concentration (±S.E.) measured at the reference sites was 1.3 ± 0.0 μM (along Jensen Beach Boulevard).

The overall mean groundwater DIN:SRP and TDN:TDP ratios in the residential neighborhoods (14.7 ± 1.6 and 20.1 ± 2.6, respectively) were significantly lower than the reference sites (76.6 ± 14.9 and 51.5 ± 9.2, respectively; $t = -3.958$; $p = 0.001$ and $t = -2.305$; $p = 0.024$, respectively). While the overall mean (± S.E.) groundwater DIN:SRP ratios were lower at OPC (11.9 ± 1.9) than GG (17.0 ± 2.5), the TDN:TDP ratios at OPC (28.4 ± 5.4) were higher than GG (13.5 ± 1.6; Fig. 6C,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 OPC and GG 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 GG.

3.2.3. Groundwater nitrogen sources

The mean values (±S.E.) for groundwater aqueous stable isotope values of ammonium (NH_4^+; +7.8 ± 0.4 ‰) and nitrate (NO_3^-; +8.8 ± 1.5 ‰) were within the ranges of wastewater N

Table 2
Mean (±S.E.) field measurements for the Old Palm City (OPC), Golden Gates Estates (GG), and reference (REF) groundwater wells 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)			
	pH	Temp. (° C)	Conductivity (μmhos/cm)	DO (mg/L)	Turbidity (NTU)	pH	Temp. (°C)	Conductivity (μmhos/cm)	DO (mg/L)	Turbidity (NTU)	pH	Temp. (°C)	Conductivity (μmhos/cm)	DO (mg/L)	Turbidity (NTU)	pH	Temp. (°C)	Conductivity (μmhos/cm)	DO (mg/L)
OPC-S1	6.90	23.6	603	0.62	9.1	7.42	25.1	525	1.49	2.0	6.26	27.7	712	1.46	1.7	6.40	27.4	789	0.58
OPC-S2	6.73	24.1	237	0.68	*	7.52	24.8	362	1.19	20.4	6.40	27.8	460	1.17	3.9	6.48	26.7	259	0.76
OPC-I	6.63	24.2	164	0.58	7.6	7.62	26	158	1.14	2.1	5.98	27.4	288	1.28	5.6	5.36	26.1	205	0.47
OPC-D	7.53	24.2	470	0.57	61.9	7.40	25.6	461	1.20	0.5	8.30	26.4	509	1.35	4.2	7.24	25.5	474	0.30
OPC Event Mean	**7.0 ± 0.2**	**24.0 ± 0.1**	**368.5 ± 101.8**	**0.6 ± 0.0**	**26.2 ± 17.9**	**7.5 ± 0.1**	**25.4 ± 0.3**	**376.5 ± 80.2**	**1.3 ± 0.1**	**6.2 ± 4.7**	**6.7 ± 0.5**	**27.3 ± 0.3**	**492.3 ± 87.2**	**1.3 ± 0.1**	**3.9 ± 0.8**	**6.4 ± 0.4**	**26.4 ± 0.4**	**431.8 ± 132.5**	**0.5 ± 0.1**
OPC Seasonal Mean	**7.2 ± 0.1**	**24.7 ± 0.3**	**372.5 ± 60.0**	**0.9 ± 0.1**	**14.8 ± 8.3**						**6.6 ± 0.3**	**26.9 ± 0.3**	**462.0 ± 74.3**	**0.9 ± 0.2**	**4.3 ± 1.5**				
OPC Project Mean	**6.9 ± 0.2**	**25.8 ± 0.3**	**417.3 ± 47.6**	**0.9 ± 0.1**	**9.2 ± 4.0**														
GG-S1	7.35	24.3	885	0.65	0.5	7.54	28	256	0.33	1.1	6.58	27.7	568	0.43	1.3	6.49	27.3	599	0.51
GG-S2	7.05	23.6	225	0.60	0.6	7.42	25.3	171	0.52	0.5	6.45	27	339	0.39	0.7	6.37	26.7	321	0.70
GG-S3	6.94	23.6	131	0.52	2.0	7.78	25.1	149	0.48	1.3	6.45	26.8	447	0.31	0.7	6.31	26.3	235	0.60
GG-I	8.02	24.7	428	0.51	8.8	7.82	25.8	289	0.22	3.7	7.84	26.6	342	0.35	0.4	7.11	26.1	300	0.55
GG-D	7.93	24.8	694	0.50	7.5	7.49	26	474	0.34	1.0	7.25	26.5	628	0.40	1.4	7.09	26.1	770	0.42
GG Event Mean	**7.15 ± 0.2**	**24.2 ± 0.3**	**472.6 ± 141.3**	**0.6 ± 0.0**	**3.9 ± 1.8**	**7.6 ± 0.1**	**26.0 ± 0.5**	**267.8 ± 57.7**	**0.4 ± 0.1**	**1.5 ± 0.6**	**6.9 ± 0.3**	**26.9 ± 0.2**	**464.8 ± 58.5**	**0.4 ± 0.0**	**0.9 ± 0.2**	**6.7 ± 0.2**	**26.5 ± 0.2**	**445.0 ± 102.4**	**0.6 ± 0.1**
GG Seasonal Mean	**7.5 ± 0.1**	**25.1 ± 0.4**	**370.2 ± 79.6**	**0.5 ± 0.0**	**2.7 ± 1.0**						**6.8 ± 0.2**	**26.7 ± 0.2**	**454.9 ± 55.7**	**0.5 ± 0.0**	**1.5 ± 0.6**				
GG Project Mean	**7.2 ± 0.1**	**25.9 ± 0.3**	**412.6 ± 48.3**	**0.5 ± 0.0**	**2.1 ± 0.6**														
JBB	5.27	26	190	0.71	3.1	6.65	28.8	177	0.50	2.2	5.61	27.5	259	0.45	2.1	6.41	30.2	401	0.86
PP	7.54	26.1	438	0.74	1.0	7.19	27.8	447	1.18	0.5	6.39	27.7	469	1.22	1.0	7.04	27.4	477	0.38
REF Event Mean	**6.4 ± 1.1**	**25.6 ± 0.4**	**314.0 ± 124.0**	**0.7 ± 0.0**	**2.1 ± 1.1**	**6.9 ± 0.3**	**28.3 ± 0.5**	**312.0 ± 135.0**	**0.8 ± 0.4**	**1.3 ± 0.9**	**6.0 ± 0.4**	**27.6 ± 0.1**	**364.0 ± 105.0**	**0.8 ± 0.4**	**1.6 ± 0.5**	**6.7 ± 0.3**	**28.8 ± 1.4**	**439.0 ± 38.0**	**0.6 ± 0.2**
REF Seasonal Mean	**6.7 ± 0.2**	**27.2 ± 0.7**	**313.0 ± 74.8**	**0.8 ± 0.1**	**1.7 ± 0.6**						**6.4 ± 0.3**	**28.2 ± 0.7**	**401.5 ± 50.5**	**0.7 ± 0.2**	**1.0 ± 0.4**				
REF Project Mean	**6.5 ± 0.3**	**27.7 ± 0.5**	**357.3 ± 45.0**	**0.8 ± 0.1**	**1.3 ± 0.4**														
Overall Event Mean	**7.1 ± 0.2**	**24.5 ± 0.3**	**405.9 ± 73.8**	**0.6 ± 0.0**	**10.2 ± 5.9**	**7.4 ± 0.1**	**26.2 ± 0.4**	**315.4 ± 43.3**	**0.8 ± 0.1**	**3.2 ± 1.8**	**6.7 ± 0.2**	**27.2 ± 0.2**	**456.5 ± 43.1**	**0.8 ± 0.1**	**2.1 ± 0.5**	**6.6 ± 0.2**	**26.9 ± 0.4**	**439.1 ± 62.1**	**0.6 ± 0.1**
Overall Seasonal Mean	**7.3 ± 0.1**	**25.3 ± 0.3**	**360.6 ± 42.9**	**0.7 ± 0.1**	**6.5 ± 3.0**						**6.6 ± 0.1**	**27.0 ± 0.2**	**447.8 ± 36.9**	**0.7 ± 0.1**	**2.4 ± 0.7**				
Overall Project Mean	**6.9 ± 0.1**	**26.2 ± 0.2**	**404.2 ± 28.8**	**0.7 ± 0.1**	**4.4 ± 1.5**														

Embolden values represent Event, Seasonal, or Project means.

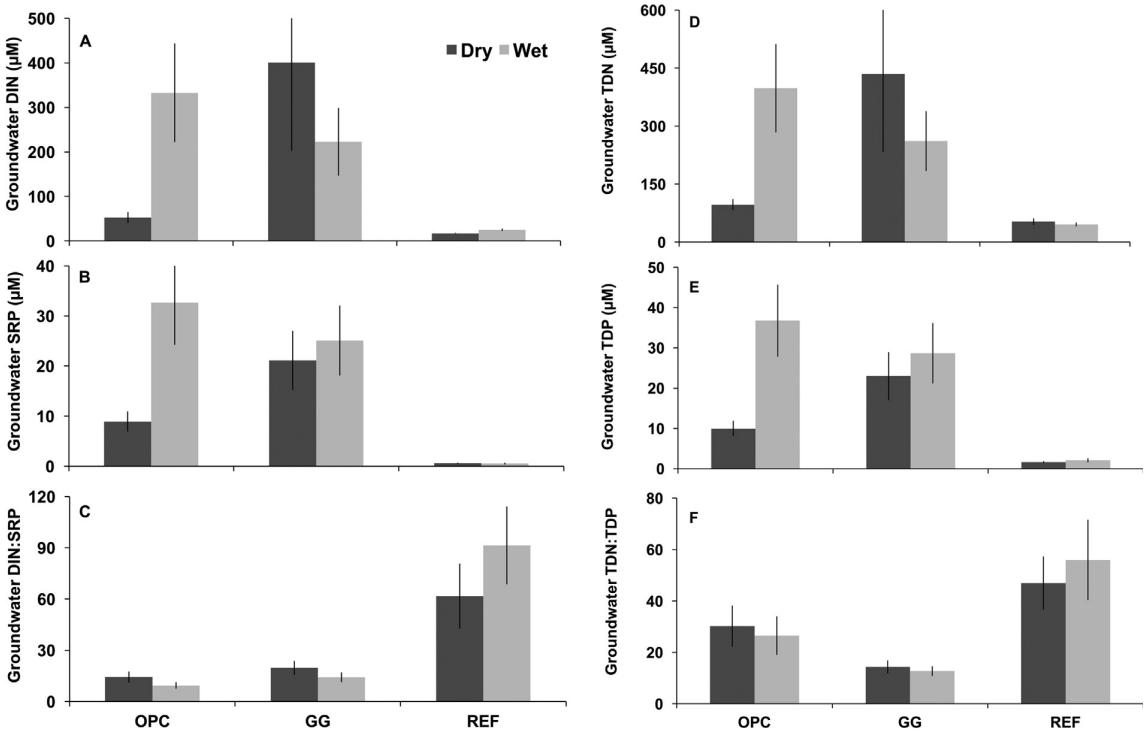

Fig. 6. Dissolved nutrient concentrations and ratios of inorganic N and P (A-C) and total N and P (D-F) recorded in groundwater at the Old Palm City and Golden Gates Estates well clusters and the reference sites.

(>+3 $^{o}/_{oo}$). Overall, the isotopic signatures for both forms of N varied spatially ($X^2 = 17.642$; $p < 0.001$ and $X^2 = 5.766$; $p < 0.056$, respectively), but not temporally ($X^2 = 2.581$; $p = 0.108$ and $X^2 = 1.556$; $p = 0.212$, respectively). For both forms, the residential sites had significantly higher signatures than the reference sites ($p = 0.001$ and $p = 0.058$, respectively). The seasonal mean $\delta^{15}N\text{-}NH_4^+$ isotopic signature was also within the wastewater N range during the Dry and Wet seasons at both the residential and reference sites (Fig. 7). For $\delta^{15}N\text{-}NO_3^-$, 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. 7).

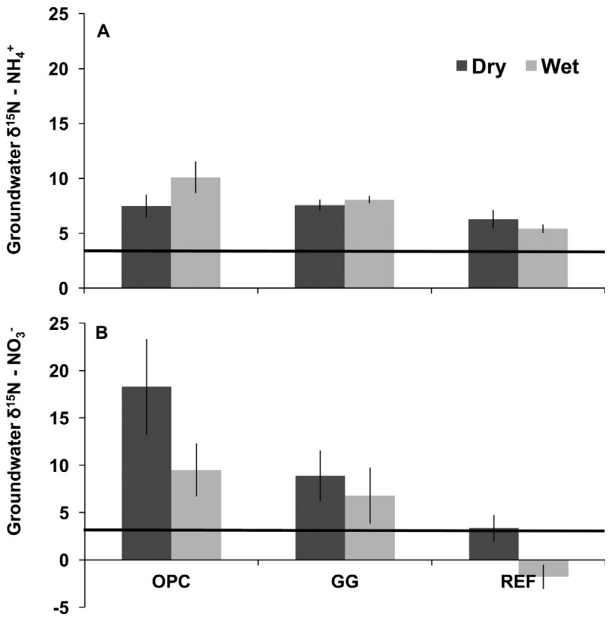

Fig. 7. Stable isotopic ratios of A) $\delta^{15}N\text{-}NH_4^+$ and B) $\delta^{15}N\text{-}NO_3^-$ in groundwater at the Old Palm City and Golden Gates Estates residential well clusters and the reference wells during the 2015 Dry and Wet seasons.

3.2.4. Groundwater sucralose

Sucralose was only detected in the shallow groundwater wells within OPC and GG, not in the deeper residential wells or at the reference sites. In OPC, the mean (±S.E.) concentration was 5.9 ± 3.7 µg/L and ranged from 1.8 to 30.0 µg/L in the Dry season. The mean Wet season concentration was 2.3 ± 1.2 µg/L and ranged from 2.7 to 8.8 µg/L. Similar concentrations were seen in GG where the mean concentration was 3.9 ± 3.2 µg/L in the Dry season with a range of 0.11 to 32.0 µg/L and an overall Wet season mean of 5.3 ± 2.3 µg/L with values ranging from undetectable to 18 µg/L.

3.3. Surface water and algal tissue

3.3.1. Surface water environmental parameters

Environmental measurements for salinity, temperature, DO, and pH varied by location and sampling event (Table 3). The mean (±S.E.) salinity showed an upstream to downstream gradient with the lowest values in the primary canals (0.3 ± 0.1) followed by the OPC complex (1.3 ± 0.6), GG complex (8.7 ± 2.6), the IRL (22.8 ± 5.4), and the nearshore reefs (32.2 ± 0.4). Overall, the salinity was higher during the Dry (10.8 ± 2.1) than the Wet (8.3 ± 1.9) season. Salinity was also consistently higher (~11) during both the Wet 1 and Dry 1 collections, the two events not associated with freshwater releases (Table 3). The project-wide

Table 3
Mean (±S.E.) 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)							Wet 1 (August 2015)				Wet 2 (September 2015)			
	Salinity	Temp. (°C)	DO (mg/L)	pH	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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**	**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**											

Embolden values represent Event, Seasonal, or Project means.

mean (±S.E.) water temperature for the primary canals (29.0 ± 0.7 °C) were higher than the two residential complexes, the IRL, and the nearshore reefs (all ~27 °C). Overall, the temperature was lower during the Dry (26.6 ± 0.3 °C) than the Wet (29.4 ± 0.2 °C) season and gradually increased from the Dry 1 to the Wet 2 collections (Table 3). The lowest mean (±S.E.) DO concentrations were at the OPC (3.6 ± 0.5 mg/L) and GG (4.2 ± 0.5 mg/L) sampling complexes (Table 3). These were followed by slightly higher concentrations in the primary canals (5.3 ± 0.8 mg/L), the IRL (6.1 ± 0.3 mg/L), and the nearshore reefs (7.6 ± 0.1 mg/L). Overall, the DO concentrations generally remained around 5.5 mg/L throughout the study, except when the DO concentration decreased to 1.2 mg/L in the C-23 canal during the Wet 2 sampling resulting in a fish kill estimated to affect 2000 individuals (Table 3; Florida Fish and Wildlife Conservation Commission Fish Kill Database Directory: https://publictemp.myfwc.com/FWRI/FishKillReport/ReportWorkSheet.aspx). The mean (±S.E.) pH followed an upstream to downstream to upstream gradient between the SLE and nearshore reefs. pH was lowest in the OPC (7.0 ± 0.1) and GG (7.7 ± 0.1) complexes followed by the IRL (8.0 ± 0.1) and the nearshore reefs (8.1 ± 0.0). The overall pH in the primary canals (7.6 ± 0.1 each) was most similar to values documented in GG. Overall, the pH did not vary between the Dry season (7.6 ± 0.1) and the Wet (7.5 ± 0.1) seasons (Table 3).

3.3.2. Surface water dissolved nutrients

Regardless of the analyte or location, mean surface water dissolved nutrient concentrations were higher (some greater than three-fold) in the Wet season than the Dry season (Figs. 8 AB, 9 AB). With few exceptions, the highest regional mean nutrient concentrations in the Dry and Wet seasons were primarily documented at one or both of the primary canals (C44W, C23W) or residential sampling networks (OPC, GG) followed by lower concentrations in the IRL and nearshore reefs (Figs. 8 and 9).

Overall mean (±S.E.) concentrations of reactive DIN (11.5 ± 0.9 μM) and TDN (64.4 ± 2.2 μM) were high and varied by segment ($X^2 = 39.676$; $p < 0.001$ and $X^2 = 79.572$; $p < 0.001$, respectively; Figs. 8 A, 9 A). The concentrations of both analytes were significantly higher in the primary canals and residential neighborhoods than along the nearshore reefs ($p < 0.001$; Figs. 8 A,

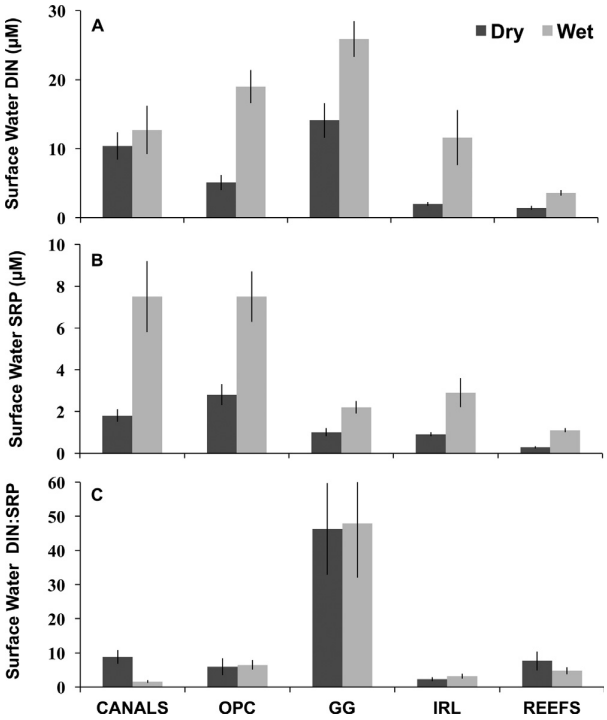

Fig. 8. Dissolved inorganic nutrient concentrations and ratios of A) dissolved inorganic N, B) soluble reactive P, and C) DIN:SRP ratios recorded at the 18 surface water sites located in the primary canals, St. Lucie Estuary, Indian River Lagoon, and nearshore reefs.

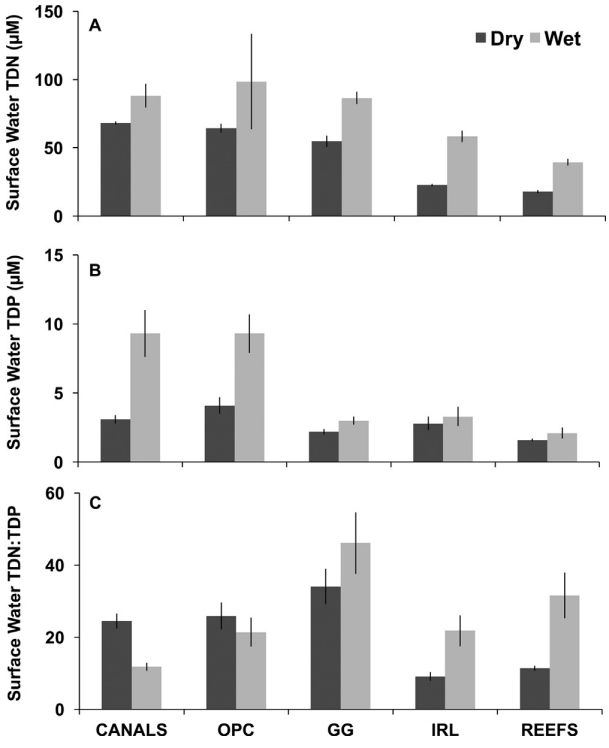

Fig. 9. Dissolved nutrient concentrations and ratios of A) total N, B) total P, and C) TDN:TDP ratios recorded at the 18 surface water sites located in the primary canals, St. Lucie Estuary, Indian River Lagoon, and nearshore reefs.

9 A). This was especially true for GG, which had significantly higher DIN concentrations than any other segment ($p \leq 0.016$). Both overall DIN and TDN concentrations were lower in the Dry (6.9 ± 0.9 μM and 49.7 ± 2.2 μM, respectively) than the Wet (16.0 ± 1.4 μM and 79.0 ± 3.2 μM, respectively) seasons ($t = -5.271$; $p < 0.001$ and $t = -6.830$; $p < 0.001$, respectively). The highest mean (±S.E.) DIN and TDN concentrations documented in the primary canals were 31.2 ± 0.2 μM and 124.1 ± 0.4 μM during the Wet season. The surface water DIN in OPC was primarily comprised of NO_3^- ($67.1 \pm 13.1\%$) during the Dry season and NH_4^+ ($56.3 \pm 10.9\%$) during the Wet season. Most of the surface water DIN at GG was NH_4^+ during both the Dry ($65.8 \pm 7.0\%$) and Wet ($57.9 \pm 7.8\%$) seasons. Like OPC, the DIN in the two primary canals was comprised primarily of NO_3^- ($87.1 \pm 9.3\%$) during the Dry and NH_4^+ ($69.5 \pm 14.3\%$) during the Wet seasons. Nearshore reef mean (±S.E.) concentrations of DIN and TDN were 1.4 ± 0.3 μM and 17.8 ± 1.1 μM, respectively, during the Dry and 3.6 ± 0.4 μM and 39.4 ± 2.5 μM, respectively, during the Wet seasons.

Similar to N, mean (±S.E.) SRP and TDP concentrations were high (3.0 ± 0.3 μM and 4.2 ± 0.3 μM, respectively) and varied among segments ($X^2 = 51.730$; $p < 0.001$ and $X^2 = 46.844$; $p < 0.001$). Both SRP and TDP concentrations were highest in the primary canals and OPC ($p \leq 0.05$ and $p \leq 0.05$, respectively) and lowest along the nearshore reefs ($p \leq 0.004$ and $p \leq 0.003$, respectively). There was no significant difference between GG and the IRL for either SRP ($p = 0.319$) or TDP ($p = 0.257$). In the Wet season both SRP (4.3 ± 0.5 μM) and TDP (5.5 ± 0.5 μM) were significantly higher (twice as high) than Dry season SRP (1.6 ± 0.2 μM) and TDP (2.9 ± 0.2 μM) concentrations ($t = -5.151$; $p < 0.001$ and $t = -4.408$; $p < 0.001$). Data for both analytes show a general dilution effect, especially during the Wet season, where higher concentrations for each were documented furthest upstream in the primary canals and along the South Fork (OPC) and the lowest furthest downstream along the nearshore reefs (Figs. 8 B, 9 B). The mean SRP and TDP concentrations were especially high in Old Palm City's All American Ditch (OPC3; 13.3 ± 1.8 μM and 16.6 ± 1.9 μM, respectively) and the C-23 primary canal (C23W; 6.2 ± 1.7 μM and 7.2 ± 1.9 μM, respectively). During the Wet season, the mean SRP and TDP concentrations reached 19.3 ± 0.4 μM and 23.4 ± 0.2 μM, respectively in All American Ditch (OPC3) and 15.9 ± 0.5 μM and 17.8 ± 0.3 μM, respectively, in the C-23 canal (C23W). Despite the Wet season spike at OPC3, the seasonal SRP mean (±S.E.) concentrations were the same (7.5 μM) at both OPC and the primary canals during the Wet season, but the primary canals had lower (1.8 μM) concentrations than those documented in OPC (2.8 μM) during the Dry season (Fig. 8B). The SRP and TDP concentrations were significantly lower in the GG network than OPC ($p < 0.001$) and the primary canals ($p \leq 0.001$). The highest mean (±S.E.) concentrations in GG were 3.3 ± 0.1 μM and 3.8 ± 0.1 μM, respectively during the Dry season and 5.7 ± 0.2 μM and 6.3 ± 0.3 μM, respectively during the Wet season. Along the nearshore reefs, mean (±S.E.) SRP and TDP concentrations were 0.3 ± 0.1 μM and 1.6 ± 0.1 μM, respectively during the Dry season and 1.1 ± 0.1 μM and 2.1 ± 0.4 μM, respectively during the Wet season.

The overall mean ratios (±S.E.) for inorganic DIN:SRP and TDN:TDP were 17.5 ± 3.3 and 25.7 ± 1.8 μM, respectively and showed slight spatial variation ($X^2 = 32.687$; $p < 0.001$ and $X^2 = 23.735$; $p < 0.001$, respectively). GG had significantly higher DIN:SRP and TDN:TDP ratios ($p \leq 0.002$ and $p \leq 0.005$, respectively) than all other statistically similar ($p \geq 0.100$ and $p \geq 0.212$, respectively) segments (Figs. 8 C, 9 C). Seasonal comparisons show that while overall mean DIN:SRP ratios (±S.E.) were slightly higher in the Dry (18.6 ± 4.5) than during the Wet (16.5 ± 4.8) season, the difference was not significant ($t = 0.346$; $p = 0.730$). Conversely, TDN:TDP ratios were slightly lower during the Dry season (23.2 ± 1.9) than during the Wet season (28.2 ± 3.0) but, again, it was not significant ($t = -1.333$; $p = 0.185$). The DIN:SRP ratios were low in the primary canals where the collective mean ratio was 8.8 ± 2.0 in the Dry season and 1.6 ± 0.4 in the Wet season (Fig. 8C). With the exception of the Wet 1 sampling event, the DIN:SRP ratios were consistently lower in the C-23 canal than in the C-44 canal. Similarly, with the exception of the Dry 1 sampling, the TDN:TDP were also consistently lower in the C-23 canal when compared to the C-44 canal. DIN:SRP remained consistently low at OPC and high at GG between the Dry (~6 and ~46, respectively) and Wet (~6 and ~48, respectively) seasons (Fig. 8C). Although not as consistent as DIN:SRP, TDN:TDP ratios were also lower at OPC than GG ($p = 0.001$; Fig. 9C). While the nearshore reefs had slightly higher inorganic DIN:SRP ratios during the Dry (7.7 ± 2.8) than the Wet (4.8 ± 1.0) seasons, the differences were not significant ($t = 0.992$; $p = 0.335$). Conversely, the TDN:TDP ratios were significantly lower during the Dry (11.4 ± 0.7) than the Wet (31.6 ± 6.4) season ($t = -3.136$; $p = 0.006$).

3.3.3. Surface water sucralose

Sucralose was detected at each of the eight surface water sites tested in OPC and GG (4 stations in each neighborhood) during both the Dry and Wet seasons. During each season and in both neighborhoods, a dilution effect occurred where the highest mean (±S.E.) concentrations were documented upstream and the lowest at the downstream-most site. In Old Palm City's All American Ditch (OPC2) the concentrations were 1.0 ± 0.4 μg/L and 2.8 ± 2.1 μg/L during the Dry and Wet seasons, respectively, and fell to 0.1 ± 0.1 μg/L during both seasons at OPC5 in the South Fork. At the GG retention pond (GG2) concentrations were 5.2 ± 0.4 μg/L and 5.3 ± 0.1 μg/L during the Dry and Wet seasons, respectively, and declined to 0.5 ± 0.4 μg/L and 0.1 ± 0.0 μg/L during the Dry and Wet seasons, respectively, at GG5 in the Lower Estuary. During the

Dry season individual sucralose concentrations ranged from 0.1 to 1.3 μg/L at OPC and from 0.1 to 5.5 μg/L at GG. In the Wet season, individual concentrations ranged from 0.1 to 4.9 μg/L at OPC and from just above detection (0.03 μg/L) to 5.4 μg/L at GG.

3.3.4. Surface water carbon and nitrogen sources

Overall, stable carbon isotope ($\delta^{13}C$) enrichment varied spatially in both macroalgae ($X^2 = 101.899$; $p < 0.001$) and phytoplankton ($X^2 = 83.307$; $p < 0.001$). The spatial variation in macroalgae $\delta^{13}C$ suggests an upstream to downstream gradient with the lightest values near the primary canals (-24.8 ± 0.6 °/₀₀; $p \leq 0.019$) and a gradually heavier signature at the residential sites (-22.7 ± 0.5 °/₀₀; $p < 0.001$), IRL (-19.6 ± 0.3 °/₀₀; $p < 0.001$), and the along the nearshore reefs (-15.3 ± 0.4 °/₀₀; $p < 0.001$; Fig. 10A). This carbon isotope gradient reflects the lighter, more depleted terrestrial dissolved inorganic carbon (DIC) source coming from the freshwater primary canals to the more enriched marine DIC sources in the coastal waters. For phytoplankton, the lightest values were recorded in the statistically similar primary canals (-27.2 ± 0.4 °/₀₀) and residential communities (OPC -28.2 ± 0.3 °/₀₀ and GG -27.6 ± 0.5 °/₀₀; $p \geq 0.085$). The most enriched values were recorded in the IRL (-21.9 ± 0.3 °/₀₀) and along the statistically similar nearshore reefs (-22.8 ± 0.5 °/₀₀; $p < 0.001$). Within the GG community, there was a consistent upstream to downstream gradient of lighter values in the community retention pond (overall mean -31.8 ± 0.6 °/₀₀) to heavier values in the Lower Estuary (overall mean -22.5 ± 0.6 °/₀₀). 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. 12A). The overall project mean (±S.E.) for carbon stable isotopes ($\delta^{13}C$) in macroalgae (-19.0 ± 0.4 °/₀₀) were depleted compared to phytoplankton (26.9 ± 0.3 °/₀₀). This is indicative of more terrestrial DIC assimilated by macroalgae versus a predominantly marine DIC source fueling phytoplankton. With the canals, IRL, and nearshore reefs removed, the overall SLE mean (±S.E.) was depleted in both macroalgae (-23.5 ± 0.4 °/₀₀) and phytoplankton (-28.2 ± 0.3 °/₀₀) suggesting primarily terrestrial sources of DIC in the SLE proper. No significant temporal variation was documented in macroalgae or phytoplankton $\delta^{13}C$ values over the duration of the study ($p \geq 0.482$). While not significant, the seasonal SLE mean in macroalgae was slightly lighter during the Dry season (-24.3 ± 0.6 °/₀₀) than during the Wet season (-22.7 ± 0.5 °/₀₀), possibly the result of freshwater releases from the C-44 during the Dry season. Conversely, the phytoplankton mean was slightly heavier during the Dry (-27.6 ± 0.4 °/₀₀) than during the Wet (-28.8 ± 0.4 °/₀₀) season.

The overall means (±S.E.) for nitrogen stable isotopes ($\delta^{15}N$) in both macroalgae (+4.4 ± 0.1 °/₀₀) and phytoplankton (+5.0 ± 0.3 °/₀₀) were within the accepted range of wastewater N. While overall means in macroalgal tissue showed spatial ($X^2 = 91.558$; $p < 0.001$) variation in $\delta^{15}N$ enrichment, those for phytoplankton did not ($p = 0.374$). The most enriched macroalgae were collected adjacent to the residential neighborhoods (+5.9 ± 0.2 °/₀₀; $p \leq 0.05$), followed by those near the primary canals (+5.4 ± 0.3 °/₀₀; $p \leq 0.05$), the IRL (+4.3 ± 0.2 °/₀₀; $p \leq 0.005$), and most depleted were collected on the nearshore reefs (+3.5 ± 0.1 °/₀₀; $p < 0.001$). Regardless of their relatively depleted condition, the signatures on the nearshore reefs closest to St. Lucie Inlet (BTR, SLR-N) were still consistently > +3 °/₀₀, the lower threshold for wastewater N (Fig. 10B). When comparing individual sites within these groupings, the macroalgae collected at OPC5 (+6.5 ± 0.2 °/₀₀) were significantly more enriched than those collected at GG5 (+5.3 ± 0.3 °/₀₀; $p = 0.006$), macroalgae collected adjacent to the C-44 canal (+5.6 ± 0.4 °/₀₀) were more enriched than those collected adjacent to the C-23 canal (+5.0 ± 0.3 °/₀₀; $p = 0.003$), and individuals collected at Bathtub Reef (+3.9 ± 0.1 °/₀₀) were significantly more enriched than those collected at SLR-N (+3.4 ± 0.2 °/₀₀) and SLR-S (+3.1 ± 0.2 °/₀₀; $p = 0.005$; Fig. 10B).

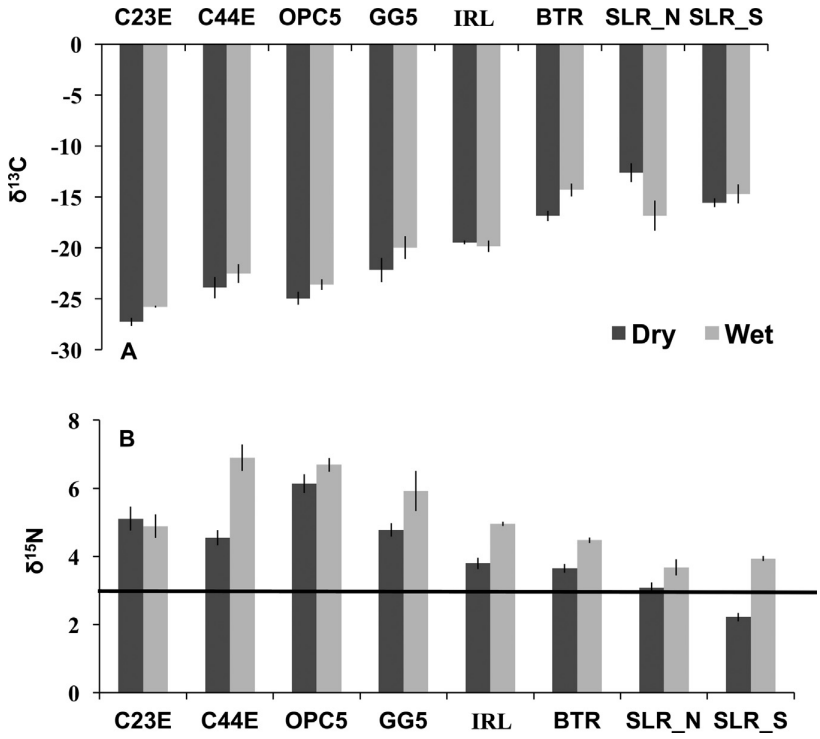

Fig. 10. A comparison of mean stable isotopic ratios (°/₀₀ ± S.E.) in macroalgae collected throughout the St. Lucie Estuary and nearshore reefs broken down by sampling season. The black line represents the lower $\delta^{15}N$ threshold (+3 °/₀₀) for wastewater N.

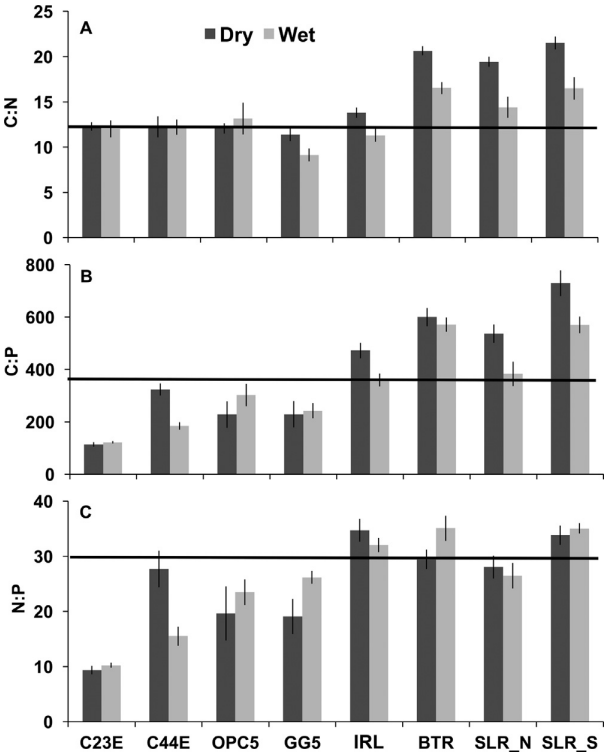

Fig. 11. A comparison of mean (±S.E.) C:N:P ratios in macroalgae collected throughout the St. Lucie Estuary and nearshore reefs broken down by sampling season. The black lines represent difference between N and P limitation where C:N >12 represents N-limitation, C:P >360 represents P-limitation, N:P >30 represents P-limitation, and N:P <30 represents N-limitation.

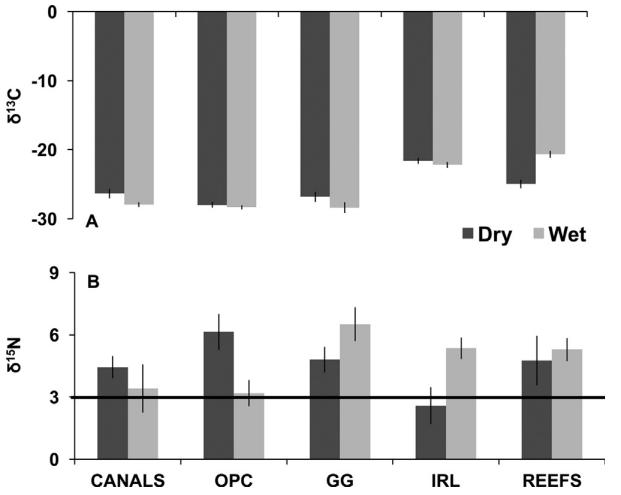

Fig. 12. A comparison of mean stable isotopic ratios (‰ ±S.E.) in phytoplankton collected in the primary canals, the St. Lucie Estuary, Indian River Lagoon, and nearshore reefs broken down by sampling season. The black line represents the lower $\delta^{15}N$ threshold (+3 ‰) for wastewater N.

While not significant, phytoplankton $\delta^{15}N$ values were most enriched near OPC (+4.7 ± 0.6 ‰) and GG (+5.6 ± 0.5 ‰) and along the nearshore reefs (+5.0 ± 0.6 ‰) than in the primary canals and IRL (both +4.0 ‰). Like macroalgae, with the exception of SLR-S, the phytoplankton means were > +3 ‰ at each site throughout the study. The most enriched signatures (+7 to +11 ‰) were consistently at GG2 in the Golden Gates community retention pond during both the Dry and Wet seasons (Fig. 12B). When looking at temporal variation, the overall seasonal mean for macroalgae was depleted during the Dry (+3.9 ± 0.1 ‰) compared to the Wet (+5.1 ± 0.2 ‰) season (X^2 = 25.375; $p < 0.001$), but there was no seasonal change in phytoplankton enrichment (+5.0 ‰; X^2 = 0.189; p = 0.664). With the sites near the primary canals removed, both mean seasonal macroalgae $\delta^{15}N$ values went from an enriched upstream signal in the South Fork at OPC to a more depleted downstream signal at SLR-S (Fig. 10B). Unlike macroalgae $\delta^{15}N$ signatures, no upstream to downstream gradient was seen in phytoplankton during either season.

3.3.5. Nutrient limitation

Overall, C:N ratios in macroalgae showed both spatial (X^2 = 74.535; $p < 0.001$) and temporal (X^2 = 14.310; $p < 0.001$) variation, whereas phytoplankton only varied by season (X^2 = 102.927; $p < 0.001$). The mean (±S.E.) C:N ratio in both macroalgae (15.2 ± 0.4) and phytoplankton (8.7 ± 0.3) indicated that the system was N-limited (ratios >12 and >6.6, respectively). The C:N ratios of macroalgae collected on the nearshore reefs were significantly more N-limited (18.4 ± 0.4; $p < 0.001$) than those collected in the SLE and IRL, which were statistically similar and consistently on the cusp of N-limitation (~12; $p > 0.05$; Fig. 11A). The phytoplankton ratios were similar in the primary canals (8.1 ± 0.5), SLE proper (8.9 ± 0.3), and IRL (9.2 ± 0.9). Although there was a slight decrease in ratio along the nearshore reefs (7.8 ± 0.7), the decrease was not statistically significant. Mean (±S.E.) seasonal C:N ratios were significantly lower in both macroalgae and phytoplankton during the Dry (16.5 ± 0.5 and 10.5 ± 0.4, respectively) season, indicating more N-limitation, than in the Wet (13.5 ± 0.5 and 6.8 ± 0.1, respectively) season when more dissolved N was available. Lower macroalgae C:N ratios (higher N inputs) in the Wet season were documented only in the lower system (GG, IRL, and the nearshore reefs; Fig. 11A). Conversely, phytoplankton C:N ratios were lower during the Wet season in all segments of the system (Fig. 13A). While the lowest phytoplankton C:N ratios (highest N concentrations) were in the lower reaches of the study area (GG, IRL, and nearshore reefs) during the Wet season, the lowest Dry season C:N ratios were documented in the upper reaches (primary canals and OPC; Fig. 13A).

Like C:N ratios, C:P ratios in macroalgae showed both spatial (X^2 = 91.148; $p < 0.001$) and temporal (X^2 = 4.957; $p < 0.026$) variation, but phytoplankton only varied by location (X^2 = 54.361; $p < 0.001$). The macroalgae collected near the C-23 canal had the lowest overall C:P ratio (highest P concentrations; 117 ± 6; $p \leq 0.011$) followed by macroalgae collected near the C-44 canal (260 ± 26), OPC (276 ± 33), and GG (235± 31; $p \leq 0.001$), then the IRL (426 ± 23) and SLR-N (463 ± 32; $p \leq 0.011$). The highest macroalgae C:P ratios (lowest P concentrations) were documented at Bathtub Reef (591 ± 24) and SLR-S (650 ± 34; $p \leq 0.001$). Likewise, the highest phytoplankton C:P ratios were also documented on the nearshore reefs (81 ± 5; $p \leq 0.001$) and lowest were in the primary canals (41 ± 3), OPC (47 ± 3), and GG (50 ± 4; $p \leq 0.013$). The mean (±S.E.) project-wide C:P ratio for macroalgae (420 ± 17) suggests P-limiting conditions (ratio > 360). When the IRL and nearshore reefs were removed, however, the SLE proper (233 ± 17) was not P-limited. Neither the overall project-wide phytoplankton C:P ratio (55 ± 2) nor the project-wide SLE proper C:P ratio (50 ± 2) suggested P-limitation (ratios < 106; Fig. 13B). The overall seasonal mean for macroalgae C:P was significantly higher during the Dry (457 ± 25) than the Wet (376 ± 22) season, but was constant in phytoplankton (54 ± 3 and 55 ± 2, respectively). The individual sites that showed lower macroalgae C:P ratios (higher P concentrations) during the Wet season were near the C-44 canal, in the IRL, and along the nearshore reefs south of the inlet

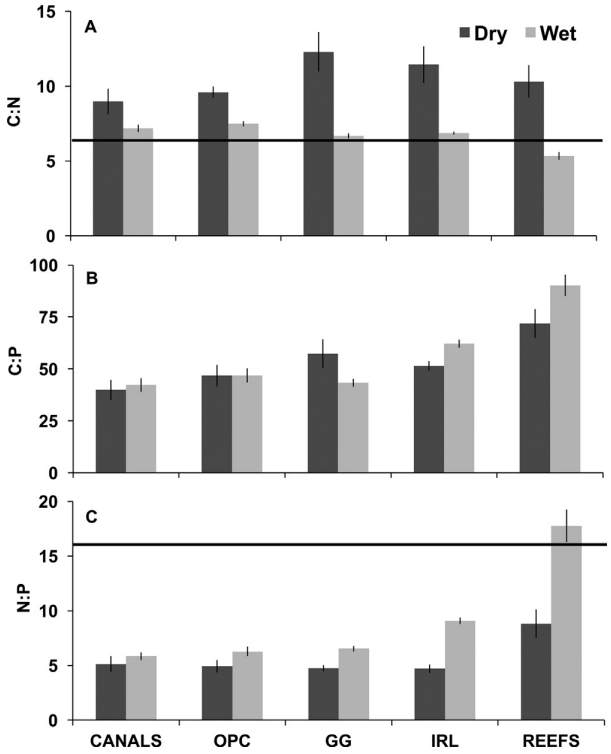

Fig. 13. A comparison of mean (±S.E.) C:N:P molar ratios in phytoplankton collected in the primary canals, the St. Lucie Estuary, Indian River Lagoon, and nearshore reefs broken down by sampling season. The black lines represent difference between N and P limitation where C:N >6.6 represents N-limitation, C:P >106 represents P-limitation, N:P >16 represents P-limitation, and N:P <16 represents N-limitation.

(SLR-N and SLR-S; Fig. 11B). Conversely, phytoplankton C:P ratios were only lower during the Wet season in GG. Both the mean macroalgae and phytoplankton C:P ratios gradually increased in an upstream (within or immediately adjacent to the primary canals) to downstream (nearshore reefs) gradient, again suggesting exposure to higher P concentrations near the canals and in the SLE than on the reefs (Figs. 11B, 13B).

Overall N:P ratios in macroalgae varied only by location ($X^2 = 59.452$; $p < 0.001$), whereas phytoplankton N:P ratios showed both spatial ($X^2 = 45.723$; $p < 0.001$) and temporal ($X^2 = 40.488$; $p < 0.001$) variation. The macroalgae collected at the confluence of the C-23 canal and the North Fork had the lowest mean N:P ratios (9.7 ± 0.5; $p < 0.001$), suggesting long-term N-limiting conditions or higher P inputs from this canal. This was followed by macroalgae collected at the confluence of the C-44 canal and the South Fork (22.2 ± 2.7), OPC (22.1 ± 2.3), GG (22.2 ± 2.0), and SLR-N (27.3 ± 1.5; $p \leq 0.004$). The least relative N-limitation in macroalgae was documented in the IRL (33.6 ± 1.3), Bathtub Reef (31.3 ± 1.5) and SLR-S (34.5 ± 1.0; $p \leq 0.009$). Phytoplankton were also relatively least N-limited on the nearshore reefs (13.4 ± 1.2; $p \leq 0.006$) with no significant difference documented between the other four regions (Fig. 13C). The mean project-wide macroalgae (27.2 ± 0.8) and phytoplankton (7.1 ± 0.3) N:P ratios both suggest that the system is generally N-limited (ratios <30 and 16, respectively). Macroalgae N:P ratios remained ~27 throughout the study whereas phytoplankton had significantly lower N:P ratios in the Dry (5.5 ± 0.3) than in the Wet (8.6 ± 0.5) season (Fig. 13C). Unlike the macroalgae, which had N:P ratios of ~10 in both the Dry and Wet seasons, near the confluence of the C-23 canal and the North Fork, phytoplankton N:P ratios were typically lower within the C-44 canal (1.4 to 5.5 in the Dry and 4.4 to 6.8 in the Wet seasons) than within the C-23 canal (6.1 to 7.6 in the Dry and 5.5 to 6.6 in the Wet seasons).

4. Discussion

The origin of excessive nutrient loads and increased contamination of fecal bacteria in the SLE is an important management question. While FDEP has identified the SLE as impaired for TN, TP, DO, and fecal coliforms (Parmer et al., 2008; White and Turner, 2012), Lake Okeechobee has only been deemed impaired for TP (FDEP, 2001). Because of the artificial connection to Lake Okeechobee, the SLE watershed also technically incorporates the lake watershed, including the lands extending north through central Florida to Orlando. While there is a widespread misconception that Lake Okeechobee *Microcystis* blooms ultimately responsible for seeding the SLE result from sugar farm back pumping along the southern edge of the lake (https://weather.com/news/news/florida-toxic-lake-okeechobee), Barnett (Barnett, *personal communication*; raw data available at https://www.sfwmd.gov/science-data/dbhydro) estimates that >90% of water and nutrient loads entering into the lake originates from the 12,950 km^2 basin to the north. Current storage capacity in this basin is only 0.18 km^3 (~7–10 cm in the lake). Deep well injection and aquifer storage and recovery has been recommended as a way to significantly increase storage north of the lake and subsequently reduce discharges by as much as 90% (SFWMD, FDEP, FDACS, 2008; Graham et al., 2015). In addition, the ongoing Kissimmee River restoration project (expected to be completed in 2020) will provide additional dynamic storage and filtration through floodplain restoration including nearly 8100 ha of wetlands and 71 km of historic river channel (USACE, 2017). Increased storage, especially during climatic events, such as hurricanes and strong El Niño Southern Oscillation would reduce water input and nutrient pulses to the lake. Havens et al. (2016) explains how shallow low-lying lakes, such as Lake Okeechobee, are particularly susceptible to oscillations in rainfall and runoff that may directly impact the intensity of cyanobacteria-dominated HABs. For example, the record El Niño-associated rainfall event during the 2016 Dry season (January–April) in South Florida likely supplied the pulse of nutrients that fueled the subsequent, nationally-recognized *M. aeruginosa* bloom in Lake Okeechobee and waters discharged to the SLE in May and June 2016 (Fig. 3). Therefore, facilitating nutrient removal through increased storage (both dynamic and static) and filtration in the northern basin would not only reduce harmful discharges but it would also help moderate the potentially toxic *M. aeruginosa* blooms in both the lake and downstream estuaries (SLE and Caloosahatchee River). Previous observations of higher nutrient concentrations in the SLE compared to the Caloosahatchee Estuary could help explain the greater bloom development and toxicity of *M. aeruginosa* in the SLE (Lapointe et al., 2012).

Although releases from Lake Okeechobee are known to exacerbate poor water quality conditions in the SLE, the data show that the majority of the nutrient loading originates in the adjacent St. Lucie River and tidal basin watersheds (Graham et al., 2015; Zheng et al., 2016). Zheng et al. (2016) identified 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 local basin watersheds. Thiel et al. (2016) report that the two Martin County WWTPs within the local watershed remove an average of 92% of the N and 66% of the P in wastewater effluent. This is higher than the N (10–30%) and P (25–50%) removed by properly working septic systems (Bicki et al., 1984; Toor et al., 2011). Because reactive forms of N (DIN) and P (SRP) are principally responsible for fueling eutrophication and community shifts, including HABs that impair waterways, it is

important to approximate contributions of these readily assimilated forms. Florida DOH reports 17,687 known OSTDS in the Martin County portion of the watershed. Based on this number, Lapointe and Herren (2016) conservatively estimated that reactive forms of N (~190,927 kg) and P (25,159 kg) derived from the watershed surpass the amounts of reactive N (93,744 kg) and reactive P (18,620 kg) loading from Lake Okeechobee releases. If the total estimated number of OSTDS (31,634) in Martin County were used, then the N and P loading estimates increased by a factor of 79%. If the total estimated OSTDS in adjacent St. Lucie County were also included, then the total loading would be another two-fold higher. These estimates indicate why OSTDS are now considered the second largest N source to Florida's surface waters (Badruzzaman et al., 2012). These impacts also include the degradation of Florida's oligotrophic springs where the FDEP has recently recognized that a significant portion of the N loading responsible for supporting freshwater algal blooms is derived from OSTDS in the spring's recharge basins (Kuphal, 2005; Harrington et al., 2010; MACTEC, 2010; FDEP, 2015a,b,c). It is clear from recent research in the springs, the IRL (Lapointe et al., 2015a), and the SLE (Lapointe et al., 2012; this study) that the role of OSTDS in nutrient loading to ground and surface waters has been underestimated.

Results from this watershed to reef study also clearly indicate that wastewater contamination is a significant driver of water quality decline and ecological dysfunction in the SLE and downstream nearshore reefs. Furthermore, this study provides three separate lines of evidence that *M. aeruginosa* blooms are primarily driven by wastewater within the urbanized SLE: 1) low N:P ratios as a result of simultaneous input of both N and P, 2) the cyanobacterium's greater affinity for NH_4^+ over NO_3^-, and 3) stable N isotopes ($\delta^{15}N$) signatures in water and algal tissue. While previously believed that *M. aeruginosa* bloom potential is solely correlated with high P inputs resulting in low N:P ratios, it is now recognized that its productivity is optimized by simultaneous contributions of both N (Conley et al., 2009; Ma et al., 2014; Gobler et al., 2016) and P (Horst, 2014; Horst et al., 2014). During this study, reactive forms of both dissolved N and P concentrations increased during the Wet season when *M. aeruginosa* blooms have been most prevalent in this system; reactive N was predominantly derived from the residential sites and reactive P from the primary canals and septic systems in OPC (Figs. 6 and 8). While discussions regarding nutrient impacts from septic systems primarily address N contamination, this study clearly shows that P is also discharged to the adjacent ground and surface waters. Interestingly, P was banned from residential fertilizers via the Urban Turf Fertilizer Rule effective December 31, 2007 (5E-1.003 F.A.C., revised January 8, 2015). The discontinued use of P-based fertilizers in residential communities eight years prior to this study suggests a higher contribution of P to surface waters from septic systems in the study area than previously thought. This notion is further supported by N isotope data showing strong wastewater influence throughout the SLE, but especially just downstream of residential communities relying on septic systems such as those along the C-44 (C44E), OPC, and GG (Figs. 10 and 12). The low N:P ratios downstream of septic systems that result from inputs of both N and P create an ideal environment for *Microcystis*. Dissolved nutrients in both the surface water and algal (i.e. macroalgae plus phytoplankton) C:N:P ratios independently suggest relatively high levels of P and subsequent N-limitation throughout the SLE compared to the nearshore reefs (Figs. 8, 11, 13). This is likely the result of low elevation and high water tables along the OSTDS-rich SLE watershed that, over time, have inhibited the ability of P to bind to soil. 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.

In addition to OPC, Lapointe et al. (2012) documented high P concentrations in the North Fork during the prolonged releases following hurricanes Charley, Frances, and Jeanne in 2004 and Dennis, Katrina, Rita, and Wilma in 2005. It was suspected that high amounts of P were coming into the system through the C-23 and C-24 canals and the Ten Mile Creek headwaters from both agricultural (non-tidal upstream areas) and urban (tidally influenced areas along the North Fork) sources (Graves et al., 2004; He et al., 2006; Yang et al., 2008). Historically, stormwater runoff from citrus farms was a significant source of P to the North Fork; however, the combination of a marked decrease in citrus farming (~72% reduction IRL-wide since 2000) along with best management practices (Boman et al., 2000) have reduced P runoff to the SLE from citrus in recent years. While samples were not collected in the C-24 canal or Ten Mile Creek, this 2015 study confirmed that elevated SRP concentrations still exist in the C-23 canal. Additionally, in 2016, Land/Ocean Biogeochemical Observatories (LOBOs) were deployed in both the North and South Forks as a reliable way to automate real-time water quality and HAB monitoring (Zamankhan et al., 2017). These publically accessible data (http://fau.loboviz.com) showed that reactive P concentrations were up to six-fold greater in the North Fork than the South Fork during the June-July 2016 *M. aeruginosa* blooms. These continuous data corroborate the earlier *in situ*-based findings by Lapointe et al. (2012) and this 2015 study. The LOBO data again showed that reactive P concentrations were highest in the North Fork during outgoing tides, but highest in the South Fork during high tide as a result of ebbing water from the North Fork being advected into the South Fork via the flooding tide. Further analysis of the LOBO data indicates that the high freshwater inputs from the primary canals in late spring and summer 2016 formed a fresh water bubble (salinities <10) in the upper and middle estuary, which increased residence time of the nutrient-rich freshwater "bioreactor" for a six-week period (late May through mid-July 2017) facilitating bloom development. Considering the high availability of P in the North Fork and the increased residence time of freshwater in the upper estuary, it is likely that the *Microcystis* populations increased their biomass exponentially within the SLE itself. In addition to reactive P, Lapointe et al. (2012) also documented exceptionally high NH_4^+ concentrations in the North Fork during heavy releases from the primary canals in 2004–2005 following multiple hurricanes. It is again suspected that once transported into the SLE with the freshwater discharges, blooms of *M. aeruginosa* are likely fueled by nutrient inputs (especially PO_4^{3-} and NH_4^+) from the surrounding watershed and perpetuated by longer residence times of the nutrient-rich freshwater.

Like most phytoplankton, *M. aeruginosa* has a much higher affinity for NH_4^+ than NO_3^- (Parrish, 2014; Glibert et al., 2016), which suggests that NH_4^+ is likely the primary form of N supporting these blooms. Horst et al. (2014b) shows that while the other major form of inorganic N, NO_3^-, 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 NH_4^+ and NO_3^- (up to 30.3 ± 3.3 μM and 15.1 ± 3.5 μM, respectively) entering into the system. This was especially true during the Wet season for the entire system and throughout the year (both Wet and Dry seasons) for the residential neighborhoods. The proportions of these two N species in surface waters varied temporally during the study. The surface water DIN during the Dry season in the primary canals, OPC, and GG were predominantly comprised of NO_3^- (87%, 67%, and 66%, respectively). Conversely, the surface waters in these same segments had more NH_4^+ (69%, 56%, and 58%, respectively) during the Wet season when *M. aeruginosa* blooms are known to occur. The lower water table during the Dry season likely provided additional time for NH_4^+ in septic systems to be aerobically 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). A review by Glibert et al. (2016) demonstrates the importance of both NH_4^+ and NO_3^- use on growth and productivity of phytoplankton and the potential suppression of productivity in estuaries by elevated NH_4^+ loading. Because the Wet season NH_4^+ percentages were moderate and the Dry season was dominated by NO_3^-, the NH_4^+ loading and the percentages of NO_3^- in these three segments (31%, 44%, and 42%, respectively) were still potentially high enough to influence both productivity and toxicity had a bloom formed during the 2015 study. These results suggest wastewater contamination and further evidence of the need for advanced wastewater treatment (N-removal; e.g., ANNAMOX®) within the watershed.

In addition to DIN concentrations, stable isotopes of $\delta^{15}N$ in surface water, macroalgae, and phytoplankton, and the abundance of the artificial sweetener sucralose all independently suggest wastewater contamination in the SLE that extends out to the nearshore reefs adjacent to St. Lucie Inlet. Groundwater aqueous stable isotopes of $\delta^{15}N$-NH_4^+ and $\delta^{15}N$-NO_3^- were enriched in both residential areas compared to the reference sites and were predominantly from a wastewater source ($\delta^{15}N$ values $>+3$ ‰). Groundwater $\delta^{15}N$-NO_3^- were consistently less enriched during the Wet season (Fig. 7). This is likely the result of a higher water table in the Wet season reducing the amount of nitrification (conversion of NH_4^+ to NO_3^-), which would ultimately reduce the amount of NO_3^- from septic systems in the groundwater. The water tables at OPC and GG well clusters were as much as 0.27 m (1.31 m to 1.04 m) and 0.34 m (1.31 m to 0.97 m) higher during the Wet season, respectively. Florida standards require >0.61 m of dry zone between the Wet season water table and the bottom of the drain field (64E-6.006 F.A.C.) and >1.22 m overall between Wet season water table and ground level (64E-6.009(3)(f) F.A.C.). During the September 2016 sampling event the mean distance ($\pm$S.E.) was <1.22 m between ground level and water table in both OPC (0.97 ± 0.03 m) and GG (1.03 ± 0.03 m), and therefore in violation of these standards. Another potential consequence of the higher Wet season water tables is increased leaching of fecal bacteria from OSTDS (FDEP, 2015d). One could also expect higher concentrations of atmospheric (-3 to +1 ‰) and inorganic fertilizer (-2 to +2 ‰) N from rainfall and stormwater runoff (both of which can also have high NO_3^- concentrations), respectively, during the Wet season that would deplete the relatively enriched wastewater N signal ($>+3$ ‰). Although not the case in this study, findings of elevated $\delta^{15}N$-NO_3^- Dry season groundwater values (Fig. 7) also typically extends to system biological response variables, such as the $\delta^{15}N$ signature in macroalgae and phytoplankton (Figs. 10 B, 12 B). For example, depleted Wet season $\delta^{15}N$ values were documented during similar Florida studies in Lee (Lapointe and Bedford, 2007) and Indian River (Tarnowski, 2014) counties and the Florida Keys (Lapointe et al., 2004). This is logical as the NO_3^- pulses primarily occur during the Wet season while wastewater N from septic systems is a year-round factor.

As indicated by the enriched $\delta^{15}N$ values in most phytoplankton and benthic macroalgae, the assimilated N also was also derived from a wastewater source. The mean SLE $\delta^{15}N$ values for phytoplankton (+5.2 ‰) and macroalgae (+5.7 ‰) were slightly lower than the overall mean $\delta^{15}N$ value (+6.3 ‰) for macroalgae collected throughout the IRL in 2011–2012 (Lapointe et al., 2015b). This likely reflects 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 immediately downstream of the S-80 structure (C44E; +5.6 ‰) and in OPC (OPC5; +6.5 ‰). Both sites are near communities relying on septic systems and strongly suggest wastewater contamination, however, the signature was most enriched in the vicinity of OPC. While not as pronounced, aqueous isotopes of $\delta^{15}N$-NH_4^+ in surface waters simultaneously showed evidence of wastewater contamination and enrichment between the C44W (6.9 ± 0.9 ‰) and OPC5 (7.3 ± 1.2 ‰) sites (Lapointe and Herren, 2016). Overall, these surface water aqueous $\delta^{15}N$-NH_4^+ signatures matched the macroalgae and phytoplankton $\delta^{15}N$ signature more closely than the aqueous $\delta^{15}N$-NO_3^- signatures (Lapointe and Herren, 2016). This provides further evidence that ammonium, rather than nitrate, is driving the HABs (both *Microcystis* and macroalgal blooms) in this system and along the nearshore reefs. Interestingly, the highest enterococcus bacteria counts documented in the SLE during the study by Martin County DOH were also adjacent to OPC where the counts ranged from 4 to 390 cfu/100 mL and concentrations were in the moderate to poor range ($\geq$36 cfu/100 mL) 56.1% of the time (Lapointe and Herren, 2016). Regardless of season, all mean $\delta^{15}N$ values in macroalgae were enriched ($>+3$ ‰) with the exception of SLR-S in the Dry season (Fig. 10). This enrichment was evidenced by the significant increase in $\delta^{15}N$ values of the transplanted *Gracilaria* from cultures at HBOI, which increased by >2 ‰ in the Dry season and >3 ‰ in the Wet season. The enrichment is the result of human waste, not livestock (i.e. cattle). This is evidenced by the sucralose and $\delta^{15}N$ dilution gradients and the significant $\delta^{15}N$ enrichment from the upstream primary canals to the west and the downstream human-dominated study sites to the east. Similarly, *Microcystis aeruginosa* also became enriched (>2 ‰) and more toxic (Oehrle et al., 2017) as it was transported from the C-44 canal into the SLE indicating uptake of sewage N sources within the urbanized estuary.

In addition to enriched algal nitrogen stable isotope values in the range suggestive of wastewater contamination, sucralose was detected in the surface wells in both the OPC and GG well clusters during the Dry and Wet seasons. To put these findings into perspective, Oppenheimer et al. (2011) reported sucralose concentrations in the following sources: 1) WWTP effluent from multiple states including Florida (mean of 27 μg/L with 30% relative standard deviation), 2) grab samples from active septic systems in Florida (12–69 μg/L), and 3) waterbodies impacted by upstream wastewater discharge (0.12–10 μg/L). Sucralose was undetectable in waters not impacted by upstream municipal wastewater discharges. In this study, the highest mean sucralose concentration (~6 μg/L) was documented in the groundwater at OPC during the Dry season. Although lower than the 24 μg/L documented at an Indian River County, Florida WWTP (Tarnowski, 2014) and the 67 μg/L at a Charlotte County, Florida lift station (Lapointe et al., 2016), the sucralose was entering into the surface waters in both of these communities. In OPC, the average sucralose concentration in the groundwater (~8 μg/L) was 8 x higher than those in the surface waters of All American Ditch (~1 μg/L) adjacent and just downstream of the well cluster. In GG, the mean concentration in the groundwater (~7 μg/L) was similar to that documented in the surface water in the community retention pond adjacent to the well cluster (GG2; ~5 μg/L), suggesting high levels of sewage contamination. The surface water concentrations documented in the GG retention pond (up to 6 μg/L) and Old Palm City's All American Ditch (up to 5 μg/L) in were higher than those documented by Tarnowski (2014) in the nearby Indian River County canals and St. Sebastian River (generally <1 μg/L). This may be because the Indian River County canals and St. Sebastian River were larger and likely more diluted than the small drainage systems in OPC and GG. Like the dissolved nutrients results, there was evidence of an upstream to downstream gradient in sucralose concentrations between GG2 and GG5, which suggests a dilution factor and may explain the differences in the two studies. The surface water sucralose data from this study corroborate those found during a 2014 microbial source tracking study by FDEP (2015d), which also documented the detection of human fecal

source marker qPCR Bacteroidales HF183 at both the OPC and GG sites.

Like sucralose, fecal bacterial contamination of both ground and surface waters have been associated with high densities of OSTDS (Yates, 1985; Lipp et al., 2001a,b; Meeroff et al., 2008; Mallin, 2013; Verhougstraete et al., 2015) – more so than treated WWTP effluent which has relatively few fecal bacteria (Hamaidi et al., 2014). Along coastal North Carolina, Duda and Cromartie (1982) found that watershed septic system densities greater than 0.62 OSTDS/ha (0.25 OSTDS/acre) were associated with high fecal bacterial counts and subsequent shellfish bed closures. Along the west coast of Florida in Charlotte Harbor, Lipp et al. (2001b) found that a watershed septic system density of 0.94 OSTDS/ha (0.38 OSTDS/acre) resulted in microbial pollution. The specific communities of interest in this study, OPC (1078 parcels) and GG (775 parcels), had septic system densities of 4.19 and 4.45 OSTDS/ha (1.7 and 1.8 OSTDS/acre), respectively (Keene, 2015). Furthermore, several of the parcels in the GG community had septic systems that serviced duplexes rather than a single family home. This was especially true adjacent to the GG community retention pond that drains to the Lower Estuary. Unsuitable soils provide another explanation. Lapointe et al. (2012) suggested that the high bacterial transport to the SLE in 2005 were likely facilitated by moderate to excessively drained soils associated with Paola and St. Lucie sands. A study by the U.S. Department of Agriculture (2017) confirmed that these soil types had high Ksat rates and drained up to 1 m of water per hour. The county health departments are responsible for posting warnings based on bacterial counts. Throughout this study, mean enterococcus counts (cfu/100 mL ± SE) followed and upstream to downstream gradient in the SLE where they were highest near Leighton Park by OPC (59.1 ± 9.8) and lowest at the Stuart Sandbar (7.2 ± 1.2; Lapointe and Herren, 2016). Because of the generally poor conditions in this system several warnings are issued each year.

Downstream of the SLE, multiple lines of evidence support nutrient enrichment of the Martin County nearshore reefs. The biological composition of these reefs corroborates the environmental chemistry presented above. The reefs support multiple nutrient indicator species including at least four species of clionid sponges (Herren and Monty, 2006; Fig. 1C). These indicators of wastewater N (Ward-Paige et al., 2005) are commonly observed boring into the brain coral *Diploria clivosa* on the Martin County reefs. These reefs also support diverse and abundant populations of sea urchins (Herren and Monty, 2006; Fig. 1F) and the green alga *Codium intertextum* (Fig. 1D). *Codium* 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. The macroalgae $\delta^{15}N$ values were consistently enriched on these nearshore reefs in both 2006–2007 (~+4 ‰; Lapointe, 2007) and the current study (>+3 ‰). Aqueous $\delta^{15}N$ isotopes were also enriched during the 2015 Wet season at St. Lucie Inlet ($\delta^{15}N\text{-}NO_3^-$ >+14 ‰) and along the nearshore reefs ($\delta^{15}N\text{-}NH_4^+$ >+6 ‰; Lapointe and Herren, 2016), respectively. These enriched values support the presence of the aforementioned biological indicators of wastewater pollution. The study by Lapointe (2007) also consistently documented higher macroalgal abundance (~58% in Spring 2007) and DIN concentrations (~3.5 μM during Winter 2007) and SRP (~0.7 μM in Spring 2007) on the reefs adjacent to the St. Lucie Inlet than along the southern Florida Reef Tract. During this 2015 study, concentrations of both DIN and SRP along the nearshore reefs were higher during the Wet season and were associated with lower Wet season N:P ratios (Fig. 8). The mean DIN and SRP (±SE) reached 5.5 ± 0.5 μM and 2.0 ± 0.1 μM, respectively at SLR-N during the Wet 2 sampling event. While these concentrations were more dilute than seen in the SLE, Lapointe (1997) showed that Caribbean and Southeast Florida reefs with >1 μM DIN and >0.1 μM SRP were more prone to becoming and remaining ecologically compromised macroalgal-dominated systems. These data support the hypothesis that elevated nutrients, which favor competitive growth of macroalgae over reef corals, might set the upper latitudinal limits to coral reef growth (Johannes et al., 1983).

In conclusion, these results provide evidence of wastewater originating from OSTDS contamination derived from the SLE watershed that is ultimately impacting not only the ecological integrity of the SLE, but also the nearshore reefs located downstream of this system. Stormwater-driven contributions of both N and P appear to fuel periodic, but intense *M. aeruginosa* blooms originating from Lake Okeechobee. Sinha et al. (2017) predict that climate-change induced increases in precipitation alone will increase N loading from world rivers by ~20%. They estimate that offsetting this increase will require a 33% reduction in N inputs, representing a major challenge to planners and resource managers. In the SLE study area, septic-to-sewer programs, which include nutrient (N and P) removal from advanced wastewater treatment, could help meet this challenge and mitigate future HAB events. These improvements within the local watershed combined with increased storage and nutrient management north of Lake Okeechobee would collectively improve the conditions in both the SLE, adjacent portions of the IRL and the nearshore reefs downstream of the St. Lucie Inlet while better protecting human health.

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References

AMS (American Society for Microbiology), 2011. Land Application of Organic Residuals: Public Health Threat or Environmental Benefit? 28 pp.
Allen, S.E., Grimshaw, H.M., Parkinson, J.A., Quarmby, C., 1974. Chemical Analysis of Ecological Materials. Blackwell Scientific Publications, Oxford.
Atkinson, M.J., Smith, S.V., 1983. C:N: P ratios of benthic marine plants. Limnol. Oceanogr. 28, 568–574.
Badruzzaman, M., Pinzon, J., Oppenheimer, J., Jacangelo, J.G., 2012. Sources of nutrients impacting surface waters in Florida: a review. J. Environ. Manag. 109, 80–92.
Barile, P.J., 2004. Evidence of anthropogenic nitrogen enrichment of the littoral waters of east central Florida. J. Coast. Res. 20, 1237–1245.
Bateman, A.S., Kelly, S.D., 2007. Fertilizer nitrogen isotope signatures. Isotopes Environ. Health Stud. 43, 237–247.
Beal, J., Voss, J., Edge, S., Cohen, L., 2012. Assessment of Coral Stressors on St. Lucie Reef: Florida's Northermost Coral Reef. Final Report to the US Fish and Wildlife Service for State Wildlife Grant USFWS T-19-1.
Bicki, T.J., Brown, R.B., 1990. On-site sewage disposal: the importance of the wet season water table. J. Environ. Health 52, 277–279.
Bicki, T.J., Brown, R.B., Collins, M.E., Mansell, R.S., Rothwell, D.F., 1984. Impact of On-site Sewage Disposal Systems on Surface and Ground Water Quality. Report to Florida Department of Health and Rehabilitative Services under contract number LC170, Florida.
Blake, N.M., 1980. Land into Water –water into Land: A History of Water Management in Florida. University Press of Florida, Tallahassee.

Böhlke, J.K., Gwinn, C.J., Coplen, T.B., 1993. New reference materials for nitrogen-isotope-ratio measurements. Geostand. Newslett. 17, 159–164.
Böhlke, J.K., Mroczkowski, S.J., Coplen, T.B., 2003. Oxygen isotopes in nitrate: new reference materials for 18O:17O:16O measurements and observations on nitrate-water equilibration. Rapid Comm. Mass Spectrom. 17, 1835–1846.
Boman, B., Wilson, C., Hebb, J. (Eds.), 2000. Water Quality/Quantity BMPs for Indian River Area Citrus Groves. University of Florida, Institute of Food and Agriculture, Indian River Research and Education Center, Fort Pierce, Florida.
Bricker, S., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C., Woerner, J., 2007. Effects of Nutrient Enrichment in the Nation's Estuaries: A Decade of Change. National Centers for Coastal Ocean Science, Silver Spring, Maryland.
Burkholder, J.M., Glasgow, H.B., 2001. History of toxic *Pfiesteria* in North Carolina estuaries from 1991 to the present. Bioscience 10, 827–841.
Chen, Y., Qin, B., Teubner, K., Dokulil, M.T., 2003. Long-term dynamics of phytoplankton assemblages: *Microcystis*-domination in Lake Taihu, a large shallow lake in China. J. Plankton Res. 25 (4), 445–453.
Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Karl, E., Lancelot, C., Gene, E., 2009. Controlling eutrophication: nitrogen and phosphorus. Science 123, 1014–1015.
Costanzo, S.D., O'Donohue, M.J., Dennison, W.C., Loneragan, N.R., Thomas, M., 2001. A new approach for detecting and mapping sewage impacts. Mar. Pollut. Bull. 42, 149–156.
Davis, T.W., Harke, M.J., Marcoval, M.A., Goleski, J., Orano-Dawson, C., Berry, D.L., Gobler, C.J., 2010. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. Aquat. Microb. Ecol. 61, 149–162.
Doering, P.H., 1996. Temporal variability of water quality in the St. Lucie Estuary, south florida. J. Am. Water Resour. Assoc. 32, 1293–1306.
Donald, D.B., Bogard, M.J., Finlay, K., Leavitt, P.R., 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. 56 (6), 2161–2175.
Downing, T.G., Sember, C.S., Gehringer, M.M., Leukes, W., 2005. Medium N:P ratios and specific growth rate comodulate microcystin and protein content in Microcystis aeruginosa PCC7806 and M. aeruginosa UV027. Microb. Ecol. 49, 468–473.
Duda, A.M., Cromartie, K.D., 1982. Coastal pollution from septic tank drainfields. J. Environ. Eng. 108 (6), 1265–1279.
FDEP (Florida Department of Environmental Protection), 2001. Total Maximum Daily Load for Total Phosphorus Lake Okeechobee, Florida. Tallahassee, Florida. Submitted to the U.S. Environmental Protection Agency Region IV, Atlanta, Georgia. 53p.
FDEP (Florida Department of Environmental Protection), 2009. North Fork St. Lucie River Aquatic Preserve Management Plan: 2009–2019. Office of Coastal and Aquatic Managed Areas, Tallahassee, Florida.
FDEP (Florida Department of Environmental Protection), 2014. Biosolids in Florida: Summary. Domestic Wastewater Program, Tallahassee, Florida.
FDEP (Florida Department of Environmental Protection), 2015a. Final Basin Management Action Plan for the Implementation of the Total Maximum Daily Load for Nutrients (Biology) by the Florida Department of Environmental Protection in the Upper Wakulla River and Wakulla Springs Basin. Prepared by the Division of Environmental Assessment and Restoration, Tallahassee, Florida.
FDEP (Florida Department of Environmental Protection), 2015b. Final Basin Management Action Plan for the Implementation of the Total Maximum Daily Load Adopted by the Florida Department of Environmental Protection in the Silver Springs Basin Management Area for Silver Springs, Silver Springs Group, and Upper Silver River. Prepared by the Division of Environmental Assessment and Restoration, Tallahassee, Florida.
FDEP (Florida Department of Environmental Protection), 2015c. Final Basin Management Action Plan for the Implementation of the Total Maximum Daily Load for Nutrients by the Florida Department of Environmental Protection in the Middle St. Johns River Basin for Wekiva River, Rock Springs Run, and Little Wekiva Canal. Prepared by the Division of Environmental Assessment and Restoration, Tallahassee, Florida.
FDEP (Florida Department of Environmental Protection), South Fork St. Lucie Estuary and River Microbial Source Tracking Study, 2015d, Prepared by the Division of Environmental Assessment and Restoration. Tallahassee, Florida.
France, R., Holmquist, J., Chandler, M., Cattaneo, A., 1998. $\delta^{15}N$ evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove-coral reef system. Mar. Ecol. Prog. Ser. 167, 297–299.
Furlong, E.T., Anderson, B.D., Werner, S.L., Soliven, P.P., Coffey, L.J., Burkhardt, M.R., 2001. Methods of Analysis by the USGS National Water Quality Laboratory – Determination of Pesticides in Water by Graphitized Carbon-based Solid-phase Extraction and High-performance Liquid Chromatography/mass Spectrometry. US Geological Survey Water-Resources Investigations Report 01-4134.
Glibert, P.M., Seitzinger, S., Heil, C.A., Burkholder, J.M., Parrow, M.W., Codispoti, L.A., Kelly, V., 2005. The role of eutrophication in the global proliferation of harmful algal blooms: new perspectives and new approaches. Oceanography 18 (2), 198–209.
Glibert, P.M., Wilkerson, F.P., Dugdale, R.C., Raven, J.A., Dupont, C.L., Leavitt, P.R., Parker, A.E., Burkholder, J.M., Kana, T.M., 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen-enriched conditions. Limnol. Oceanogr. 61 (1), 165–197.
Gobler, C.J., Burkholder, J.M., Davis, T.W., Harke, M.J., Johengen, T., Stow, C.A., Van de Waal, D.B., 2016. The dual role of nitrogen supply in controlling the growth and toxicity of cyanobacterial blooms. Harmful Algae 54, 87–97.
Graham, W.D., Angelo, M.J., Frazer, T.K., Frederick, P.C., Havens, K.E., Reddy, K.R., 2015. Options to Reduce High Volume Freshwater Flows to the St. Lucie and Caloosahatchee Estuaries and Move Water from Lake Okeechobee to the Southern Everglades: An Independent Technical Review by the University of Florida Water Institute. Prepared for the Florida Senate, Tallahassee, Florida.
Graves, G.A., Wan, Y., Fike, D.L., 2004. Water quality characteristics of storm water from major land uses in South Florida. J. Am. Water Resour. Assoc. 40 (6), 1405–1419.
Griffin, D.W., Gibson III, C.J., Lipp, E.K., Riley, K., Paul III, J.H., Rose, J.B., 1999. Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in canals of the Florida Keys. Appl. Environ. Microbiol. 65, 4118–4125.
Hamaidi, M.S., Hamaidi-Chergui, F., Errahmani, M.B., Lamroussi, A., Benmira, H., 2014. Efficiency of indicator bacteria removal in a wastewater treatment plant (Algiers, Algeria). Proceedings of the 2nd International Conference ?Water Resources and Wetlands, Tulcea, Romania, pp. 503–509.
Harrington, D., Maddox, G., Hicks, R., Florida Springs Initiative Monitoring Network Report and Recognized Sources of Nitrate, 2010, Prepared by Florida Department of Environmental Protection Division of Environmental Assessment and Restoration Bureau of Watershed Restoration Ground Water Protection Section. Tallahassee, FL.
Havens, K., Paerl, H., Phlips, E., Zhu, M., Beaver, J., Srifa, A., 2016. Extreme weather events and climate variability provide a lens to how shallow lakes may respond to climate change. Water 8 (229) 18 pp.
He, Z.L., Zhang, M., Stofella, P.J., Yang, X.E., 2006. Vertical distribution and water solubility of phosphorus and heavy metals in sediments of the St. Lucie Estuary, South Florida, USA. Environ. Geol. doi:http://dx.doi.org/10.1007/s00254-006-0205-5.
Heaton, T.H.E., 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. Chem. Geol. 5, 87–102.
Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W. C., Dortch, Q., Gobler, C.J., Heil, C.A., Humphries, E., Lewitus, A., 2008. Eutrophication and harmful algal blooms: a scientific consensus. Harmful Algae 8 (1), 3–13.
Herren, L., Monty, J., 2006. St. Lucie Inlet Preserve State Park Benthics. St. Lucie Inlet Preserve State Park Symposium, October 18, 2006, Stuart, Florida.
Hinkle, S.R., Böhlke, J.K., Fisher, L.H., 2008. Mass balance and isotope effects during nitrogen transport through septic tank systems with packed-bed (sand) filters. Sci. Total Environ. 407, 324–332.
Horst, G.P., Sarnelle, O., White, J.D., Hamilton, S.K., Kaul, R.B., Bressie, J.D., 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. Water Res. 54, 188–198.
Horst, G., 2014. The Role of Nitrogen and Phosphorus on the Growth and Toxicity of the Bloom- Forming Cyanobacterium *Mycrocystis Aeruginosa*. Inland Lakes Convention, May 2, [2014,] Michigan.
Howes, B.H., Schlezinger, D., Samimy, R., 2008. The Characterization and Quantification of Benthic Nutrient Fluxes in the St. Lucie River and Estuary. Final Report prepared for South Florida Water Management District, West Palm Beach, Florida.
Isaacs, J.D., Strangman, W.K., Barbera, A.E., Mallin, M.A., McIver, M.R., Wright, J.L., 2014. Microcystins and two new micropeptin cyanopeptides produced by unprecedented *Microcystis aeruginosa* blooms in North Carolina's Cape Fear River. Harmful Algae 31, 82–86.
Johannes, R.E., Wiebe, W.J., Crossland, C.J., Rimmer, D.W., Smith, S.V., 1983. Latitudinal limits of coral reef growth. Mar. Ecol. Prog. Ser. 11, 105–111.
Jones Jr., J.B., Wolf, B., Mills, H.A., 1990. Organic matter destruction procedures. Plant Analysis Handbook, Micro-Macro Publishing, Inc., Athens, pp. 195–196.
Katz, B.G., Griffin, D.W., McMahon, P.B., Harden, H.S., Wade, E., Hicks, R.W., Chanton, J.P., 2010. Fate of effluent-borne contaminants beneath septic tank drainfield overlying a karst aquifer. J. Environ. Qual. 39, 1181–1195.
Keene, R., 2015. Martin County Septic System Elimination Report. Prepared for Martin County Utilities Department by Captec Engineering, Inc. Stuart, Florida.
Knapp, A.N., DiFiore, P.J., Deutsch, C., Sigman, D.M., Lipschultz, F., 2008. Nitrate isotopic composition between Bermuda and Puerto Rico: implications for N2 fixation in the atlantic ocean. Glob. Biogeochem. Cycles 22, GB3014.
Koroleff, F., 1983. Simultaneous oxidation of nitrogen and phosphorus compounds by persulfate, In: Grasshoff, K., Eberhardt, M., Kremling, K. (Eds.), Methods of Seawater Analysis. 2nd ed. Verlag Chemie Weinheim, Federal Republic of Germany, pp. 168–169.
Kreitler, C.W., 1975. Determining the Sources of Nitrate in Groundwater by Nitrate Isotope Studies: Texas Bureau of Economic Geology Report of Investigation #83. Texas Bureau of Economic Geology, Austin, Texas.
Kreitler, C.W., 1979. Nitrogen-isotope ratio studies of soils and groundwater nitrate from alluvial fan aquifers in Texas. J. Hydrol. 42, 147–170.
Kuphal, T., Quanitification of Domestic Wastewater Discharge and Associated Nitrate Loading in Marion County, FL., 2005, Prepared by the Marion County Planning Department. Ocala, Florida.
Lapointe, B.E., Bedford, B.J., 2007. Drift rhodophyte blooms emerge in Lee County, Florida, USA: Evidence of escalating coastal eutrophication. Harmful Algae 6, 421–437.
Lapointe, B.E., Bedford, B.J., 2010. Ecology and nutrition of invasive *Caulerpa brachypus f. parvifolia* blooms on coral reefs off southeast Florida. Harmful Algae 9, 1–12.

Lapointe, B.E., Herren, L.W., 2016. 2015 Martin County Watershed to Reef Septic Study. Prepared for Martin County Utilities Department. Stuart, FL.
Lapointe, B.E., Krupa, S., 1995a. Jupiter Creek Septic Tank Water Quality Investigation. Prepared for Loxahatchee River Environmental Control District, Jupiter, Florida.
Lapointe, B.E., Krupa, S., 1995b. Tequesta Peninsula Septic Tank/water Quality Investigation. Prepared for Loxahatchee River Environmental Control District, Jupiter, Florida.
Lapointe, B.E., O'Connell, J.D., Garrett, G.S., 1990. Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. Biogeochemistry 10, 289–307.
Lapointe, B.E., Littler, M.M., Littler, D.S., 1992. Nutrient availability to marine macroalgae in siliciclastic *versus* carbonate-rich coastal waters. Estuaries 15, 75–82.
Lapointe, B.E., Matzie, W.R., Barile, P.J., 2004. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the lower Florida Keys: discrimination of local versus regional nitrogen sources. J. Exp. Mar. Biol. Ecol. 308 (1), 23–58.
Lapointe, B.E., Barile, P.J., Littler, M.M., Littler, D.S., 2005. Macroalgal blooms on southeast Florida coral reefs: II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen. Harmful Algae 4 1106–1102.
Lapointe, B.E., Thacker, K., Hanson, C., Getten, L., 2011. Sewage pollution in Negril, Jamaica: effects on nutrition and ecology of coral reef macroalgae. Chin. J. Oceanol. Limn. 29 (4), 775–789.
Lapointe, B.E., Herren, L.W., Bedford, B.J., 2012. Effects of hurricanes, land-use, and water management on nutrient and microbial pollution: St. Lucie Estuary, southeast Florida. J. Coast. Res. 28, 1345–1361.
Lapointe, B.E., Herren, L., Lynch, K., 2015a. St. Lucie River Stormwater Project. Prepared for Indian River Lagoon National Estuary Program. Palm Bay, Florida.
Lapointe, B.E., Herren, L.W., Debortoli, D.D., Vogel, M., 2015b. Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. Harmful Algae 43, 82–102.
Lapointe, B., Herren, L., Paule, A., Sleeman, A., Brewton, R., 2016. Charlotte County Water Quality Assessment Phase I: Data Analysis and Recommendations for Long-term Monitoring. Prepared for Charlotte County Board of County Commissioners.
Lapointe, B.E., 1985. Strategies for pulsed nutrient supply to *Gracilaria* cultures in the Florida Keys: interactions between concentration and frequency of nutrient pulses. J. Exp. Mar. Biol. Ecol. 93, 211–222.
Lapointe, B.E., 1997. Nutrient thresholds for bottom-up control of macroalgal blooms on coral reefs in Jamaica and southeast Florida. Limnol. Oceanogr. 42 (5), 1119–1131.
Lapointe, B.E., 2007. Comparative Ecology of Harmful Macroalgal Blooms in South Florida's Coastal Waters (CEHAB). Final report prepared for Florida Wildlife Research Institute. St. Petersburg, FL.
Lehman, P.W., Kendall, C., Guerin, M.A., Young, M.B., Silva, S.R., Boyer, G.L., Teh, S.J., 2015. Characterization of the *Microcystis* bloom and its nitrogen supply in San Francisco Estuary using stable isotopes. Estuar. Coasts 38, 165–178.
Li, M., Nkrumah, P.N., Xiao, M., 2014. Biochemical composition of *Microcystis aeruginosa* related to specific growth rate: insight into the effects of abiotic factors. Inland Waters 4 (4), 357–362.
Li, X., Zhang, X., Xie, W., Zhou, C., Li, Y., Zhang, X., 2017. Alterations in transcription and protein expressions of HCC-related genes in HepG2 cells caused by microcystin-LR. Toxicol. In Vitro 40, 115–123.
Lipp, E.K., Farrah, S.A., Rose, J.B., 2001a. Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. Mar. Pollut. Bull. 42 (4), 286–293.
Lipp, E.K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S.R., Rose, J.B., 2001b. The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. Estuaries 24 (2), 266–276.
Littler, M.M., Littler, D.S., Brooks, B.L., 2006. Harmful algae on tropical coral reefs: bottom-up eutrophication and top-down herbivory. Harmful Algae 5 (5), 565–585.
Liyanage, H.M., Arachchi, D.M., Abeysekara, T., Guneratne, L., 2016. Toxicology of freshwater cyanobacteria. J. Environ. Sci. Health Part C 34 (3), 137–168.
MACTEC, 2010. Wekiva River Basin Nitrate Sourcing Study. Final Report. MACTECProject No. 6063090160A. Prepared for the St. Johns River Water Management District and Florida Department of Environmental Protection.
MEA (Marine Ecosystem Assessment), 2005. Marine and Coastal Ecosystems and Human Well-being: Synthesis. Island Press, Washington DC Prepared by UNEP World Conservation Monitoring Centre, Cambridge, UK. 137 pp.
Ma, J., Brookes, J.D., Qin, B., Paerl, H.W., Gao, G., Wu, P., Zhang, W., Deng, J., Zhu, G., Zhang, Y., Xu, H., 2014. Environmental factors controlling colony formation in blooms of the cyanobacteria *Microcystis* spp. in Lake Taihu, China. Harmful Algae 31, 136–142.
Mallin, M.A., 2013. Septic systems in the coastal environment: multiple water quality problems in many areas. In: Ahuja, S. (Ed.), Monitoring Water Quality: Pollution Assessment, Analysis and Remediation. Elsevier Publishers B.V., Amsterdam, pp. 81–102.
Meeroff, D.E., Bloetscher, F., Bocca, T., Morin, F., 2008. Evaluation of water quality impacts of on-site treatment and disposal systems on urban coastal waters. Water Air Soil Pollut. 192 (1–4), 11–24.
NIST (National Institute of Standards and Technology), 2008. Report of Investigation for Reference Materials 8549, 8558, 8568, and 8569 Nitrogen and Oxygen Isotopes in Nitrate. 4 pp.
NRC (National Research Council), 2000. Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution. National Research Council, National Academy Press, Washington, DC.
Nicklisch, A., Kohl, J.G., 1983. Growth kinetics of *Microcystis aeruginosa* (Kütz) Kütz as a basis for modelling its population dynamics. Int. Rev. Gesamten Hydrobiol. Hydrogr. 68 (3), 317–326.
Nixon, S.W., 1995. Coastal marine eutrophication: a definition, social causes, and future consequences. Ophelia 41, 199–219.
Oehrle, S., Rodriguez-Matos, M., Cartamil, M., Zavala, C., Rein, K.S., 2017. Toxin composition of the *Microcystis aeruginosa* bloom in the St. Lucie Estuary, Florida. Toxicon 138, 169–172.
Oppenheimer, J., Eaton, A., Badruzzaman, M., Haghani, A., Jacangelo, J., 2011. Occurrence and suitability of suitability of sucralose as an indicator compound of wastewater loading of surface waters in urbanized regions. Water Res. 45, 4019–4027.
Paerl, H.W., Fogel, M.L., 1994. Isotopic characterization of atmospheric nitrogen inputs as sources of enhanced primary production in coastal Atlantic Ocean waters. Mar. Biol. 119 (4), 635–645.
Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65 (4), 995–1010.
Paerl, H.W., Xu, H., McCarthy, M.J., Zhu, G., Qin, B., Li, Y., Gardner, W.S., 2011. Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): the need for a dual nutrient (N & P) management strategy. Water Res. 45 (5), 1973–1983.
Parmer, K., Laskis, K., McTear, R., Peets, R., 2008. Nutrient and Dissolved Oxygen TMDL for the St. Lucie Basin. Florida Department of Environmental Protection, Division of Water Resource Management, Bureau of Watershed Management, Tallahassee, Florida 70p.
Parrish, J., 2014. The Role of Nitrogen and Phosphorus in the Growth, Toxicity, and Distribution of the Toxic Cyanobacteria, *Microcystis Aeruginosa*. University of San Francisco, California (Master Thesis).
Paul, J.H., Rose, J.B., Brown, J., Shinn, E.A., Miller, S., Farrah, S.R., 1995a. Viral tracer studies indicate contamination of marine waters by sewage disposal practices in Key Largo, Florida. Appl. Environ. Microbiol. 61, 2230–2234.
Paul, J.H., Rose, J.B., Jiang, S., Kellog, C., Shinn, E.A., 1995b. Occurrence of fecal indicator bacteria in surface waters and the subsurface aquifer in Key Largo, Florida. Appl. Environ. Microbiol. 61, 2235–2241.
Phlips, E.J., Badylak, S., Hart, J., Haunert, D., Lockwood, J., O'Donnell, K., Sun, D., Viveros, P., Yilmaz, M., 2012. Climatic influences on autochthonous and allochthonous phytoplankton blooms in a subtropical estuary, St. Lucie Estuary, Florida, USA. Estuar. Coasts 35, 335–352.
Polera, M., 2016. Microcystis in the Cape Fear River: Where, When, and Why? Master's Thesis 42 pp. Department of Biology and Marine Biology, University of North Carolina, Wilmington.
Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in Estuarine and Marine waters: environmental implications and human health risk. Harmful Algae 61, 31–45.
Qualls, R.G., 1989. Determination of Total Nitrogen and Phosphorus in Water Using Persulfate Oxidation: a Modification for Small Sample Volumes Using the Method of Koroleff (19830). (Appendix A pp. 131–138. In the biogeochemical properties of dissolved organic matter in a hardwood forest ecosystem: their influence on the retention of nitrogen, phosphorus, and carbon. Ph.D dissertation, University of Georgia Institute of Ecology, Athens, Georgia, USA. University Microfilms, Inc., no. DEX9003448.).
Rastogi, R.P., Sinha, R.P., Incharoensakdi, A., 2014. The cyanotoxin-microcystins: current overview. Rev. Environ. Sci. Biotechnol. 13, 215–249.
Redfield, A.C., 1958. The biological control of chemical factors in the environment. Am. Sci. 46, 205–222.
Risk, M.J., Lapointe, B.E., Sherwood, O.A., Bedford, B.J., 2009. The use of $\delta^{15}N$ in assessing sewage stress on coral reefs. Mar. Pollut. Bull. 58, 793–802.
Ross, C., Santiago-Vázquez, L., Paul, V., 2006. Toxin release in response to oxidative stress and programmed cell death in the cyanobacterium *Microcystis aeruginosa*. Aquat.Toxicol. 78 (1), 66–73.
Rothenberger, M.B., Burkholder, J.M., Brownie, C., 2009. Long-term effects of changing land use practices on surface water quality in a coastal river and lagoonal estuary. J. Environ. Manag. 44, 505–523.
SFWMD, FDEP, FDACS (South Florida Water Management District, Florida Department of Environmental Protection, Florida Department of Agriculture and Consumer Services), 2008. Lake Okeechobee Watershed Construction Project: Phase II Technical Plan. West Palm Beach, Florida: South Florida Water Management District: Florida Environmental Protection and Florida Department of Agriculture and Consumer Services, Tallahassee, Florida.
SFWMD, FDEP, and FDACS (South Florida Water Management District, Florida Department of Environmental Protection, Florida Department of Agriculture and Consumer Services), 2009. St. Lucie River Watershed Protection Plan. West Palm Beach, Florida: South Florida Water Management District: Florida Environmental Protection and Florida Department of Agriculture and Consumer Services, Tallahassee, Florida.
Savoye, N., Aminot, A., Treguer, P., Fontugne, M., Naulet, N., Kerouel, R., 2003. Dynamics of particulate organic matter $\delta^{15}N$ and $\delta^{13}C$ during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France). Mar. Ecol. Prog. Ser. 255, 27–41.
Sinha, E., Michalak, A.M., Balaji, V., 2017. Eutrophication will increase during the 21 st century as a result of precipitation changes. Science 357 (6349), 405–408.
Smith, N.P., 2016. Transport pathways through southern indian river lagoon. Fla. Sci. 79 (1), 39–50.

Tarnowski, M., 2014. The Effects of On-site Sewage Treatment and Disposal Systems on the Relief Canals of Indian River County, the St. Sebastian River, and the Central Indian River Lagoon. Florida Atlantic University, Boca Raton, Florida (Master's Thesis).
Tetra Tech, 2017. Water Quality Needs Assessment for Martin County, Florida. Prepared for Martin County Engineering Department/Ecosystem Restoration and Management Division. Stuart, Florida.
Thermo Scientific, 2007. Conflo IV operating manual. Revision A-122 4730.
Thiel, W., Bassett, B., Sarno, R., 2016. Martin County Utilities North and Tropical Farms Wastewater Treatment Facilities: An Evaluation of the Removal Efficiencies and Achieved Loading Reduction of Major Nutrients. Prepared December 2016 for Martin County Utilities Department. 11 pp.
Toor, G.S., Lusk, M., Obreza, T., 2011. Onsite Sewage Treatment and Disposal Systems: Nitrogen. Document SL348 prepared by the Department of Soil and Water Sciences at the University of Florida Institute of Food and Agricultural Sciences Extension. 6 pp.
U.S Department of Agriculture Natural Resources Conservation Service, 2017. Custom Soil Resource Report for Martin County, Florida Town of Sewall's Point. .
UGA-SIEL (University of Georgia Stable Isotope Environmental Laboratory), 2015a. Persulfate Digest for Water Total N and Total P Standard Operating Procedure. September 2015.
UGA-SIEL, 2015b. Plant Dry Ash-acid Extraction for Total P and Metals. Standard Operating Procedure. September 2015.
USACE (U.S. Army Corps of Engineers), 2017. Kissimmee River Restoration Project Facts and Information Sheet. Jacksonville, Florida. March 2017.
Valiela, I., McClelland, J., Hauxwell, J., Behr, P.J., Hersh, D., Foreman, K., 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnol. Oceanogr. 42 (Part 2 (5)), 1105–1118.
Verhougstraete, M.P., Martin, S.L., Kendall, A.D., Hyndman, D.W., Rose, J.B., 2015. Linking fecal bacteria in rivers to landscape, geochemical, and hydrologic factors and sources at the basin scale. Proc. Natl. Acad. Sci. U. S. A. 12 (33), 10419–10424.
Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Technical Report: human alteration of the global nitrogen cycle: sources and consequences. Ecol. Appl. 7, 737–750.
Ward-Paige, C.A., Risk, M.J., Sherwood, O.A., Jaap, W.C., 2005. Clionid sponge surveys on the Florida Reef Tract suggest land-based nutrient inputs. Mar. Pollut. Bull. 51, 570–579.
Warhurst, B.C., 2014. Effects of Elevated Salinity and Oxidative Stress on the Physiology of the Toxigenic Cyanobacterium Microcystis Aeruginosa. University of North, Florida, Jacksonville, Florida (Master's Thesis).
Weiskel, P.K., Howes, B.L., 1992. Differential transport of sewage-driven nitrogen and phosphorus through a coastal watershed. Environ. Sci. Technol. 26, 352–360.
White, G., Turner, J., 2012. Fecal Coliform TMDL for North Fork St. Lucie River WBID 3194. Florida Department of Environmental Protection, Division of Water Resource Management, Bureau of Watershed Management, Tallahassee, Florida 48 p.
Wynne, T.T., Stumpf, R.P., 2015. Spatial and temporal patterns in the seasonal distribution of toxic cyanobacteria in western Lake Erie from 2002-2014. Toxins 7 (5), 1649–1663.
Yang, Y., He, Z., Lin, Y., Phlips, E.J., Yang, J., Chen, G., Stoffella, P.J., Powell, C.A., 2008. Temporal and spatial variations of nutrients in the Ten Mile Creek of South Florida, USA and effects on phytoplankton biomass. J. Environ. Monit. 10 (4), 508–516.
Yates, M.V., 1985. Septic tank density and ground-water contamination. Groundwater 23 (5), 586–591.
Yunes, J.S., Salomon, P.S., Matthiensen, A., Beattie, K.A., Raggett, S.L., Codd, G.A., 1996. Toxic blooms of cyanobacteria in the Patos Lagoon Estuary, southern Brazil. J. Aquat. Ecosyst. Health 5 (4), 223–229.
Zamankhan, H., Westrick, J., Anscombe, F.R., Stumpf, R., Wynne, T.T., Sullivan, J., Twardowski, M., Moore, T., Choi, H., 2017. Sustainable monitoring of algal blooms. In: Chen, D.H. (Ed.), Sustainable Water Management and Technologies Sustainable Water Management, Vol. 1. CRC Press, Taylor and Francis Group, Boca Raton, pp. 65–90.
Zheng, F., Bertolotti, L., Doering, P., 2016. South Florida Environmental Report, Chapter 10: St. Lucie and Caloosahatchee River Watershed Protection Plan Annual Updates. South Florida Water Management District (SFWMD), West Palm Beach, Florida.