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Article *in* Harmful Algae · March 2015

DOI: 10.1016/j.hal.2015.01.004

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Harmful Algae 43 (2015) 82–102

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal

Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon

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ARTICLE INFO

Article history:
Received 17 October 2014
Received in revised form 27 January 2015
Accepted 28 January 2015

Keywords:
HAB
Nitrogen
Phosphorus
Indian River Lagoon
Eutrophication
Stable isotopes

ABSTRACT

Nutrient pollution is a primary driver of eutrophication and harmful algal blooms (HABs) in estuaries and coastal waters worldwide. In 2011–2012, 20 sites evenly distributed throughout the 251-km long Indian River Lagoon (IRL) were assessed during three sampling events for dissolved nutrients (DIN, SRP, TDN, TDP) and chlorophyll *a*. Benthic macroalgae were also analyzed for $\delta^{13}C$, $\delta^{15}N$, and C:N:P contents to identify potential nutrient sources and gauge the type and degree of N and P limitation. The mean DIN and SRP concentrations throughout the IRL were high, averaging 4.24 ± 0.45 and 0.68 ± 0.06 µM, respectively, explaining the widespread occurrence of HABs during the study. High TDN concentrations (up to 152 µM) and TDN:TDP ratios (>100:1) in the poorly flushed northern IRL, Mosquito Lagoon and Banana River segments reflected the accumulation and cycling of N-rich groundwater inputs that produce P-limitation. These enriched nutrient conditions were associated with unprecedented chlorophyll *a* concentrations (>100 µg/L), dominated by *Resultor* sp. Ø. Moestrup in the Banana River in 2011 and *Aureoumbra lagunensis* D.A. Stockwell, DeYoe, Hargraves and P.W. Johnson in the Mosquito Lagoon and northern IRL in 2012. C:N, C:P, and N:P ratios in macroalgae averaged 15.9, 698.9, and 40.6, throughout the IRL, respectively; significantly higher C:P and N:P ratios in the northern IRL segments suggested strong P-limitation in these N-enriched waters. Macroalgae $\delta^{15}N$ values were enriched throughout the IRL (+6.3‰) and similar to values reported for macroalgae from other sewage-polluted coastal waters. Because point-source sewage inputs to the IRL were largely eliminated through the IRL Act of 1990, these results suggest that non-point source N enrichment from septic tanks (~300,000) represents a significant and largely ignored N-source to the IRL. The high degree of sewage N contamination of the IRL, combined with recent HABs, including toxic ecotypes of the red macroalga *Gracilaria tikvahiae* McLachlan, seagrass loss, and wildlife mortality, indicates a critical need for improved sewage collection and treatment, including nutrient removal.

1. Introduction

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

Located along Florida's east-central coast, the Indian River Lagoon (IRL) is a shallow (mean depth ~0.8 m) and narrow (~3 km wide) bar-built estuary extending 251 km between Jupiter and Ponce inlets (Steward and VanArman, 1987; Fig. 1). Because the IRL comprises a transition zone between temperate and subtropical biomes, the IRL is considered a regional-scale ecotone and one of the most species-diverse estuaries in North America (Swain et al., 1995). The basin includes the Mosquito Lagoon (ML) and Banana River (BR), which are located in the northern regions of the IRL. The climate of the IRL basin is humid subtropical with distinct dry and wet seasons. Rainfall within the basin averages 140–150 cm/yr,

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http://dx.doi.org/10.1016/j.hal.2015.01.004
1568-9883/

with 62% falling from June through October (IRLNEP, 1996). Freshwater enters the IRL basin from rainfall, surface water runoff, submarine groundwater discharge (SGD), and inflows from tributaries and canals.

The IRL watersheds have experienced dramatic changes in land-use over the past century. Historically, drainage of the IRL basin occurred through slow, meandering streams, creeks, rivers, and wetlands. Since the Drainage Acts of Florida (1916) that permitted the creation of canals to drain uplands for agriculture, reduce flooding, and control mosquitos, the IRL watershed has nearly tripled its size from 231,480 hectares to more than 566,560 hectares (SJRWMD, 2007). These changes greatly altered the hydrology and increased stormwater discharges to the IRL. This is especially evident in the St. Lucie Estuary (SLE; Fig. 1), which now receives periodic excessive freshwater discharges from Lake Okeechobee, especially following hurricanes and tropical storms (Lapointe et al., 2012). Since the drainage of the IRL watersheds, the increased land area and accompanying socio-economic opportunities directly contributed to rapid urbanization and population increases. The population in the IRL region has increased from ~250,000 in 1960 to ~1.7 million today (US Census Bureau, 2014). As a consequence, land use changes in the IRL watershed have been dramatic. In 1920, only 4% of the land was classified as low-density residential, while 95% of land was forest/grass/pasture and 1% agriculture (Kim et al., 2002). The IRL watershed is now dominated by urban land uses (39%), followed by agriculture (24%), range (20.8%), wetland (12.1%), and natural forest (4.5%; Bricker et al., 2007).

Eutrophication of the IRL resulting from widespread urbanization and population growth has long been a concern of scientists and resource managers. The number of tidal inlets that allow flushing with Atlantic coastal waters is limited and the residence time of water in the northern IRL can be high (>1 yr; Smith, 1993). High residence times combined with rapid population growth on its watersheds, has made the northern IRL especially susceptible to nutrient enrichment, eutrophication, and seagrass die-off (Briel

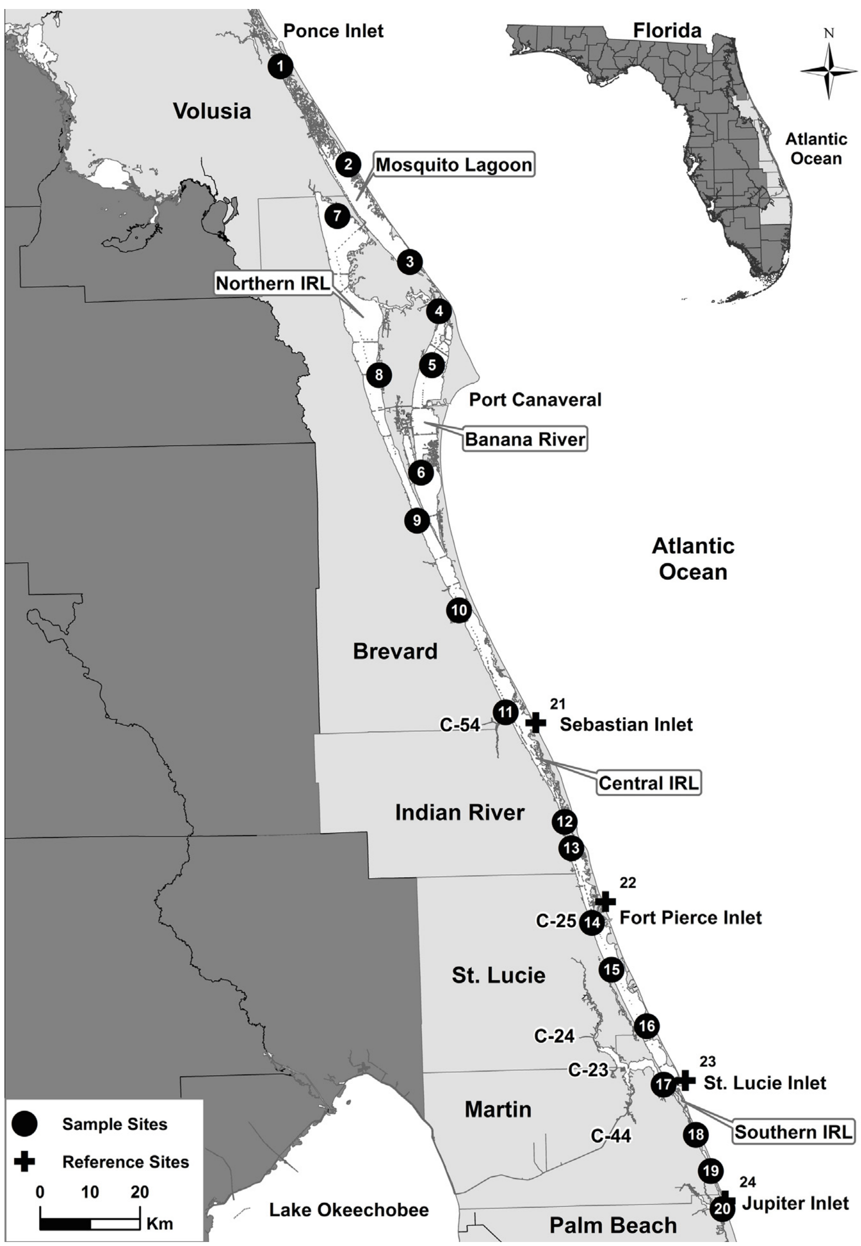

Fig. 1. Map of the Indian River Lagoon showing the five segments, 20 sampling sites, and four reference sites.

et al., 1973; Bricker et al., 2007; FDEP, 2008). Increasing land-based sources of nutrients in surface waters of the IRL primarily originate from stormwater and groundwater within agricultural and urban areas on the watershed. In recent decades, urbanization of the watershed, especially in the northern IRL segments, has increased rapidly with parallel decreases in agriculture (Duncan et al., 2004). In 1990, concerns of increased sewage-driven eutrophication led to the Indian River Lagoon Act of 1990 (IRL Act; Chapter 90-262, Laws of Florida) that required sewage treatment plants to cease discharging into surface waters of the IRL by July 1, 1995. The IRL Act also required municipalities to identify areas where package sewage treatment plants and septic tanks posed threats to the IRL and implement plans to provide centralized sewage treatment to these areas by July 1, 1994. By 1996, point-source sewage discharges (outfalls) into surface waters of the IRL were largely eliminated (IRLNEP, 2008). The Watershed Restoration Act of 1999 (403.067 F.S.) mandated the Florida Department of Environmental Protection (FDEP) to scientifically evaluate the quality of Florida's surface waters and promote the mechanisms necessary to reduce nutrient pollution through the implementation of the federal Total Maximum Daily Load (TMDL) program. The effort of the implementation was mainly geared toward the reduction of nutrient inputs from wastewater treatment plants (WWTPs) and stormwater runoff. With the exception of periodic wet weather discharges, all WWTPs in the IRL region are presently in compliance with current water quality protection codes, rules, and statues (IRLNEP, 2008).

Despite the elimination of point-source sewage inputs to the IRL through the IRL Act, non-point source sewage pollution from septic tanks (on-site sewage treatment and disposal systems, OSTDS) has continued to expand and remains a serious environmental and human health concern (Bicki et al., 1984; Lapointe and Krupa, 1995a,b; Belanger et al., 2007; Lapointe et al., 2012). Federal and state agencies have long recognized the harmful impacts of OSTDS to groundwaters and surface waters in Florida, including the IRL (Bicki et al., 1984; Woodward-Clyde, 1994; IRLNEP, 1996; Kroening, 2007; IRLNEP, 2008). Currently, there are an estimated 300,000 OSTDS installed in the counties adjacent to the IRL, with the majority of these located in Brevard and Volusia counties, which border the poorly-flushed ML, BR, and northern IRL (Fig. 1). Contamination of groundwaters with nutrients derived from non-sewered human wastewater has long been known to contribute to eutrophication of lakes, estuaries, and coastal waters (Brezonik, 1972; Bicki et al., 1984; Lapointe et al., 1990; Valiela et al., 1992; Weiskel and Howes, 1992; NRC, 1993; US EPA, 2002) and could be an important pathway for nutrient pollution of the IRL (Martin et al., 2007). Such groundwater-borne nutrient inputs would negatively impact the seagrass communities and biodiversity of the IRL by fueling harmful algal blooms (HABs), including macroalgae and/or epiphytes (Lapointe et al., 1994; Burkholder et al., 2007) and phytoplankton (Phlips et al., 2011; Lapointe et al., 2012; Gobler et al., 2013). Groundwaters contaminated by OSTDS have impacted a wide variety of other urbanized estuaries and coastal waters, including Bermuda (Lapointe and O'Connell, 1989; McGlathery, 1995), the Florida Keys (Lapointe et al., 1990; Tomasko and Lapointe, 1991; Lapointe et al., 1994), Cape Cod, MA (Valiela et al., 1992; Weiskel and Howes, 1992; Valiela et al., 1997), Outer Banks, North Carolina (Mallin, 2013), Chesapeake Bay (Reay, 2004), and Puerto Rico (Olsen et al., 2010). In addition, bacterial contamination of groundwaters and surface waters has been associated with unsuitable soil characteristics and high densities of OSTDS on the IRL watersheds (Lapointe and Krupa, 1995a,b; Belanger et al., 2007; Lapointe et al., 2012) and is considered a primary source of bacterial contamination associated with immunologic perturbations in populations of bottlenose dolphins, *Tursiops truncatus* Montagu, in the IRL (Schaefer et al., 2009; Bossart et al., 2014). Schaefer et al. (2011) found a positive correlation between the number of IRL bottlenose dolphins colonized by *Escherichia coli* and the number of septic tanks in the area they lived; with the highest number of both being in the northern segments of the IRL. Similarly, the health of juvenile green sea turtles *Chelonia mydas* Linnaeus as measured through blood parameters and the severity and prevalence of fibropapillomatosis (caused by an alphaherpesvirus) was significantly poorer in the IRL population than those captured on adjacent nearshore wormrock reefs (Hirama et al., 2014).

Blooms of benthic macroalgae have been symptomatic of N-driven eutrophication in the shallow waters of the IRL for decades (Lapointe and Ryther, 1979; Benz et al., 1979; Virnstein and Carbonara, 1985; Bricker et al., 2007) and provide effective indicator organisms to assess the relative importance of N sources and gauge the type and degree of N vs. P limitation. Macroalgae are 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). Measurement of stable carbon and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$) in macroalgal tissue have been widely used to discriminate among natural (upwelling, N-fixation) and anthropogenic (sewage, fertilizers) nutrient sources (Risk et al., 2008). Because natural N-fixation source values are close to 0‰ (Heaton, 1986; France et al., 1998), atmospheric N typically ranges from −3‰ to +1‰ (Paerl and Fogel, 1994) and synthetic fertilizer N ranges from −2‰ to +2‰ (Bateman and Kelly, 2007); these N sources are all depleted relative to enriched values of +3‰ to +19‰ for human sewage (Heaton, 1986; Costanzo et al., 2001; Table 1). Accordingly, enriched $\delta^{15}N$ values > +3‰ have been reported for macroalgae in a wide variety of sewage-polluted coastal waters, including Boston Harbor, MA (France et al., 1998), Childs River, Cape Cod, MA (McClelland and Valiela, 1998), Narrangansett Bay, RI (Thornber et al., 2008), Sarasota Bay, FL (Lapointe, 2013), Moreton Bay, Australia (Costanzo et al., 2001), and nearshore reefs off urban areas of east-central Florida (Barile, 2004), southeast Florida (Lapointe et al., 2005a), southwest Florida (Lapointe and Bedford, 2007), Jamaica (Lapointe et al., 2011), and Tobago (Lapointe et al., 2010; Table 1). In addition, measurement of C:N:P contents of macroalgae provides a measure of nutrient quantity and stoichiometry that is useful in assessing the relative importance of N vs. 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).

To assess spatial and temporal variability in N source(s) and overall patterns of eutrophication and nutrient limitation of HABs in the IRL, we performed a comprehensive two-year study throughout the IRL. Previous water quality studies of the IRL have largely focused on the region north of the Ft. Pierce Inlet (Sigua et al., 2000; Steward et al., 2005); in comparison, we chose to perform an IRL-wide study using 20 fixed stations evenly spaced among the five IRL segments (Mosquito Lagoon [ML], Banana River [BR], Northern IRL [NIRL], Central IRL [CIRL], and Southern IRL [SIRL]) extending from Ponce Inlet in Volusia County to Jupiter Inlet in northern Palm Beach County; we also included four reference stations located just outside the IRL on the nearshore sabellarid wormrock reefs (Fig. 1). The study involved sampling the water column for various forms of dissolved nutrients (N and P) and chlorophyll *a*, macroalgae for $\delta^{13}C$, $\delta^{15}N$, and C:N:P ratios, and seagrass communities for species composition and percent cover to assess nutrient stress in this key management endpoint.

Table 1
$\delta^{15}N$ levels reported for macroalgae in both sewage-impacted and relatively unpolluted reference waters worldwide.

Location	Species	Mean $\delta^{15}N$‰	Reference
Sewage impacted			
Boston Harbor, MA, USA	*Ceramium* sp.	$X = 6.5 \pm 0.7$ (SD)	France et al. (1998)
	Chondrus crispus		
	Desmarestia sp.		
	Ectocarpus sp.		
Narragansett Bay, RI, USA	*Ulva* spp.	$X = 7.5 - 15$	Thornber et al. (2008)
Childs River, Cape Cod, MA, USA	*Cladophora vagabunda*	5.4 ± 0.1 (SE)	McClelland and Valiela (1998)
	Enteromorpha sp.	8.4 ± 0.2 (SE)	
	Gracilaria tikvahiae	7.6 ± 0.4 (SE)	
Sage Lot Pond, Cape Cod, MA, USA	*Cladophora vagabunda*	3.4 ± 0.1 (SE)	McClelland and Valiela (1998)
	Enteromorpha sp.	4.9 ± 0.1 (SE)	
	Gracilaria tikvahiae	5.1 ± 0.6 (SE)	
Nearshore reefs, east-central FL, USA	*Botryocladia spinulifera*	$X = 8.7 - 9.9$	Barile (2004)
	Bryothamnion triquetrum		
	Caulerpa spp.		
	Chaetomorhpa linum		
	Codium isthmocladum		
	Colpomenia sinuosa		
	Enteromorhpa intestinalis		
	Gracilaria tikvahiae		
	Laurencia poiteaui		
	Ulva lactuca		
Coastal reefs, Southeast FL, USA	*Caulerpa* spp.	$X = 5.52 \pm 0.88$ (SE)	Lapointe et al. (2005)
	Codium isthmocladum	$X = 6.95 \pm 0.97$ (SE)	
Coastal Reefs, Southwest FL, USA	*Agardhiella subulata*	$X = 3.89 \pm 0.96 - 5.84 \pm 1.37$ (SD)	Lapointe and Bedford (2007)
	Botryocladia occidentalis		
	Cladophora sp.		
	Eucheuma isiforme var. denudatum		
	Gracilaria spp.		
	Hypnea musciformis		
	Rhodymenia divaricata		
Sarasota Bay, FL, USA	*Acanthophora spicifera*	$X = 3.76 \pm 0.13$	Lapointe (2013)
	Botryocladia occidentalis		
	Caulerpa spp.		
	Gracilaria spp.		
	Hypnea spp.		
Urbanized canals, Florida Keys, USA	*Chaetomorpha* spp.	$X = 4.31 \pm 0.35$ (SE)	Lapointe, unpublished data
	Caulerpa spp.		
	Halimeda opuntia		
Shallow reefs, South Negril, Jamaica	*Acanthophora spicifera*	$X = 4.79 \pm 0.53$ (SE)	Lapointe et al. (2011)
	Bryothamnion triquetrum		
	Chaetomorpha gracilis		
	Chaetomorpha linum		
	Cladophora fuliginosa		
	Codium isthmocladum		
	Sargassum spp.		
	Spyridia hypnoides		
Shallow reefs, Buccoo Reef Complex, Tobago	*Bryopsis* spp.	$X = 6.2 \pm 1.1$ (SD)	Lapointe et al. (2010)
	Caulerpa spp.		
	Dictyota spp.		
	Halimeda opuntia		
	Laurencia poiteaui		
Moreton Bay, Australia	*Catenella nipae*	2.5–11.3	Costanzo et al. (2001)
	Gracilaria edulis		
Reference sites			
Coral Reefs, Green Turtle Cay, Abacos, Bahamas	*Caulerpa verticillata*	$X = 1.75 \pm 0.06$ (SD)	Lapointe et al. (2005b)
	Caulerpa racemosa	$X = 0.78 \pm 0.37$ (SD)	
Coral Reefs, Puerto Rico	*Acanthophora spicifera*	$X = 0.3 \pm 1.0$ (SD)	France et al. (1998)
	Avrainvillea longicaulis		
	Halimeda incrassata		
	Penicillus capitatus		

2. Methods

Spatial and temporal variability in water quality throughout the IRL were documented by collection of seawater and macroalgal tissue from the two most abundant species at 20 fixed IRL sampling sites plus four fixed reference sites (REF) on nearshore sabellarid wormrock reefs (Fig. 1) during three sampling events in 2011 and 2012. The 20 IRL sites were grouped by waterbody segment, which included ML ($n = 3$), BR ($n = 3$), NIRL ($n = 4$), CIRL ($n = 5$), and SIRL ($n = 5$). Because the IRL has been increasingly impacted by human activities over the past century, rainfall now plays a significant role in stormwater-driven nutrient pollution and the overall health of the system. Rainfall patterns prior to and during the study showed the transition from the end of a prolonged multi-year drought followed by more typical "wet vs. dry" rainfall seasonality during the two-year study (SJRWMD et al., 2012; Fig. 2A). Accordingly, the

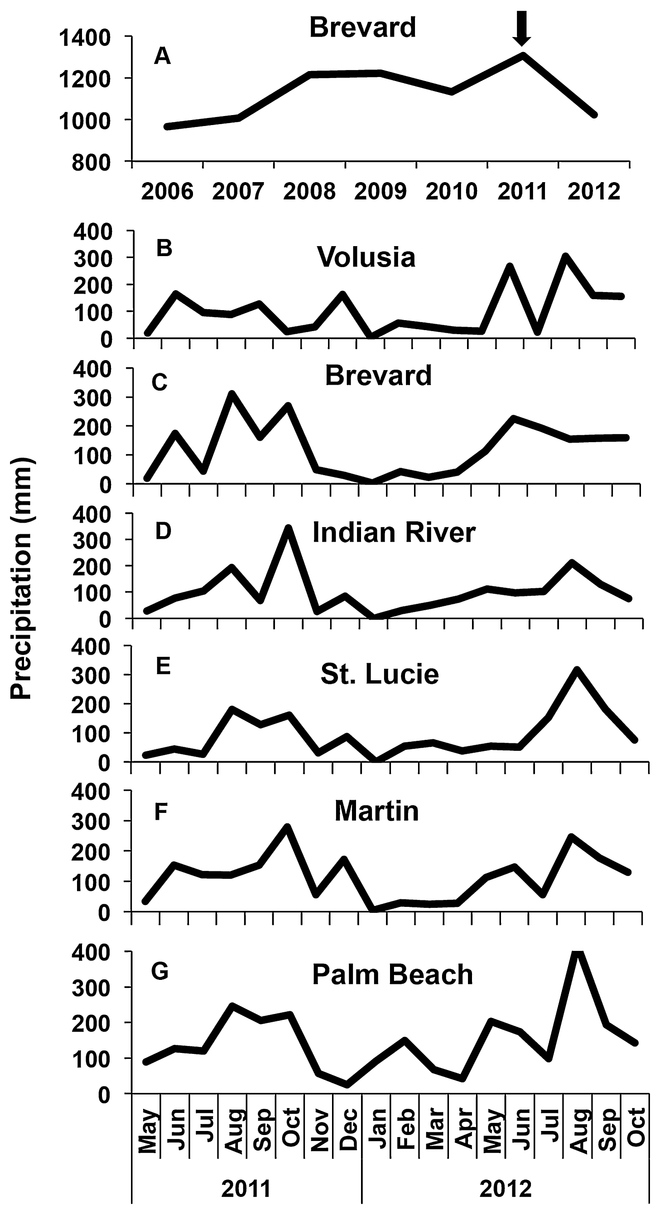

Fig. 2. Total annual precipitation in Brevard County near center of superbloom (A; arrow represents year of superbloom; Weather station GHCND: USW00092821) and monthly precipitation in each of the six counties bordering the Indian River Lagoon (B–G) during the 2011–2012 study period (NOAA National Climatic Data Center).

three successive sampling events were classified as Dry 2011 (June 2, 2011–July 13, 2011), Wet 2011 (November 4, 2011–January 11, 2012), and Wet 2012 (August 7, 2012–September 21, 2012; Fig. 2B–G).

2.1. Collection and analysis of water column nutrients and chlorophyll *a*

Seawater samples were collected in triplicate 0.25 m below the surface into acid-washed 0.5 L high-density polyethylene (HDPE) bottles and covered with ice in a dark cooler until return to the Harbor Branch Oceanographic Institute (HBOI) laboratory for processing. Sampling sites adjacent to canals and tributaries (e.g., 10, 11, 12, 13, 14, 17, and 20 on Fig. 1) were sampled during an ebbing tide. The samples were filtered (0.7 μm GF/F filters) and frozen until analysis at the Nutrient Analytical Services Laboratory, Chesapeake Biological Laboratory, Solomons, MD. The samples were analyzed on a Technicon Auto-Analyzer II (nitrate, TDN, SRP, TDP) or a Technicon TRAACS 800 (ammonium, nitrite). Detection limits were 0.21 μM for ammonium, 0.01 μM for nitrate and nitrite, 0.02 μM for SRP, 2.06 μM for TDN, and 0.05 μM for TDP. The resulting data were used to characterize ambient dissolved inorganic and total N and P concentrations, DIN:SRP ratios, and TDN:TDP ratios at the IRL sites. Calibrated YSI™ Models 63 and 85 hand-held meters were used to determine pH and salinity, conductivity, temperature, and dissolved oxygen, respectively at the time water samples were collected at each site.

For chlorophyll *a* analysis, GF/F filters were frozen until extracted. To extract, the filters were placed in a 15 mL centrifuge tube with 10 mL of 90% acetone, ground with a Telfon pestle, and allowed to extract for 2–24 h in the dark under refrigeration. After extraction, the samples were removed from refrigeration, warmed to room temperature, and centrifuged at ~2400 rpm for ~10 min. The samples were measured fluorometrically before and after acidification using 5% HCl for chlorophyll *a* and phaeopigment concentrations. Fluorescence measurements were made with a Turner Designs TD700 fluorometer equipped with a daylight white lamp, 340–500 nm excitation filter and >665 nm emission filter or with a Turner Designs Trilogy fluorometer.

2.2. δ13C, δ15N, and C:N:P analysis of HABs

Blooms of both macroalgae and phytoplankton were sampled to characterize nutritional status (C:N:P) and identify nutrient sources (δ^{13}C, δ^{15}N) supporting HAB phenomena in the IRL (Fig. 3). For macroalgae (Fig. 3D and E), triplicate samples of the two most abundant species were collected by hand, cleaned of epiphytes and debris, rinsed briefly (<5 s) in deionized water to remove excess salt, sorted into three composite replicates per species, and dried at 60 °C for 48 h in a Fisher *Isotemp*® laboratory oven. The dried algae were ground to fine powder using a Thompson Scientific Wiley Mini-Mill®, and stored in plastic screw top vials. Pre-weighed tissue samples were analyzed for δ^{13}C, δ^{15}N, total N, and total C at the University of California – Davis's Stable Isotope Facility (SIF) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Cheshire, UK). Samples were combusted at 1000 °C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650 °C). The helium carrier then flowed through a water trap (magnesium perchlorate) and an optional CO_2 trap (for N-only analyses). N_2 and CO_2 are separated on a Carbosieve GC column (65 °C, 65 mL/min) before entering the IRMS.

Sub-samples of the powdered macroalgae ($n = 3$ replicates/species/site/sampling event) were analyzed for total P contents at the Nutrient Analytical Services Laboratory, Chesapeake Biological Laboratory, University of Maryland, Solomons, MD. Tissue P was measured following the methodology of Asplia et al. (1976) using a Technicon Autoanalyzer II with an IBM-compatible, Labtronics, Inc. DP500 software data collection system (D'Elia et al., 1997). The %P tissue data, along with %C and %N from the SIF at UC Davis, were used to determine molar C:N:P ratios.

In addition to the macroalgae sampling described above, phytoplankton samples were collected during two unanticipated bloom events during the study, one in the ML and one in CIRL (specifically in the SLE). To characterize nutrient sources supporting a persistent bloom of *Resultor* sp. Ø. Moestrup (Fig. 3A) in the northern end of the IRL, nine phytoplankton samples were collected at three sites in the ML ($n = 3$ samples/site) in February 2012. At each site, seawater samples were collected in triplicate

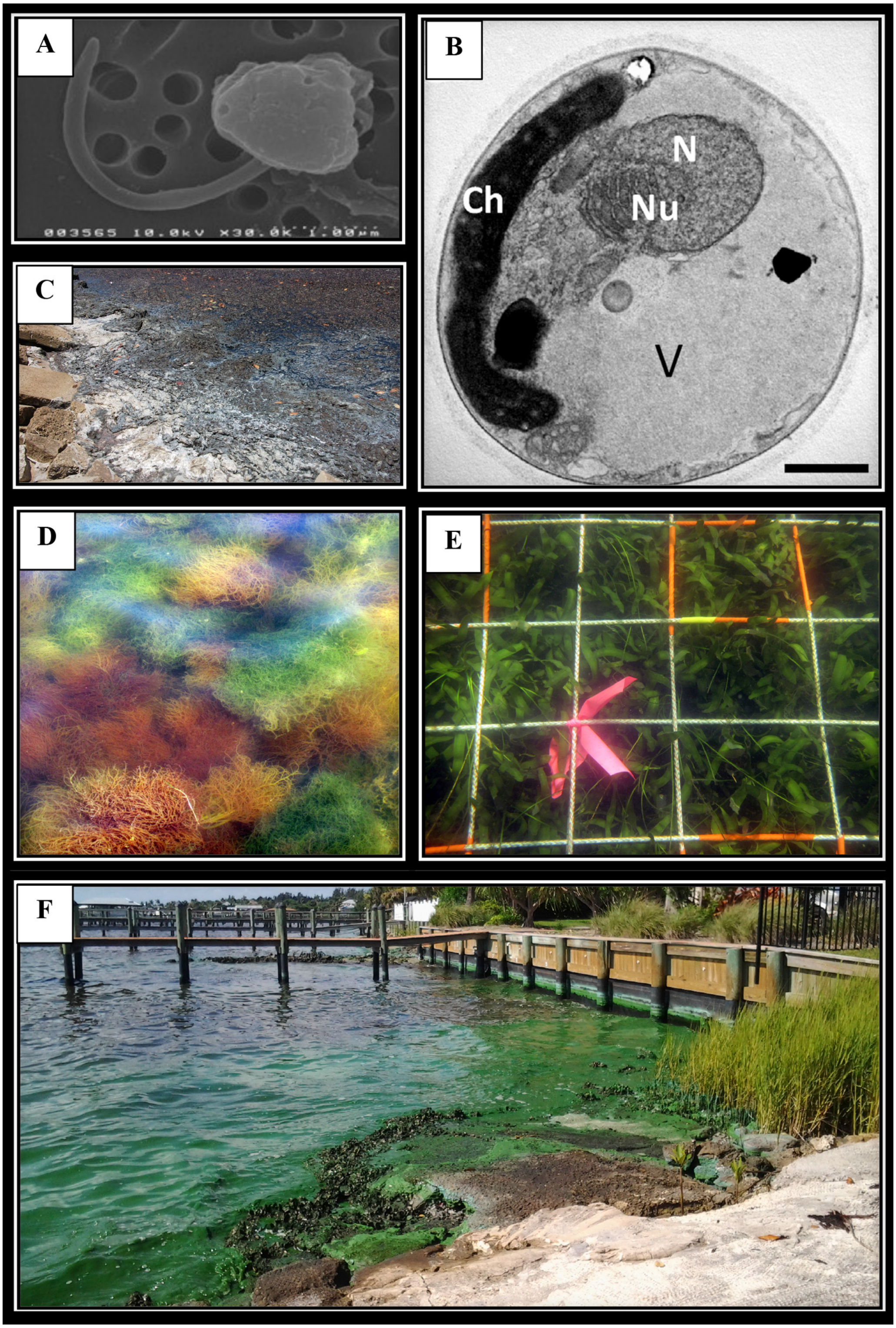

Fig. 3. Harmful algal bloom species in the Indian River Lagoon: (A) scanning electron micrograph of *Resultor* sp., (B) transmission electron microscopic micrograph of *Aureoumbra lagunensis*, (C) *Lyngbya* sp. (D) *Hydropuntia secunda* (red) and *Chaetomorpha linum* (green), (E) *Caulerpa prolifera*, and (F) *Microcystis aeruginosa* in the St. Lucie Estuary. Photo credits: (A) University of Florida Fisheries and Aquatic Sciences Program, (B) Gobler et al. (2013), (C) Carol Wilson, (D/E) St. Johns River Water Management District, (F) Mark Gavitt, Martin County.

0.25 m below the surface into clean, 1 L HDPE bottles and covered with ice in a dark cooler until return to the HBOI laboratory where they were filtered onto 0.7 μm GF/F filters, frozen, and analyzed for δ^{13}C and δ^{15}N using the methodology described above. Beginning in July 2013, during excessive fresh water releases from Lake Okeechobee, toxic *Microcystis aeruginosa* (Kützing) Kützing blooms formed in the SLE (Fig. 3F). Three samples of *M. aeruginosa* were collected in August 2013 at Sandsprit Park along Manatee Pocket in the SLE, dried and ground using the methodology described above, and analyzed for δ^{13}C, δ^{15}N, and C:N:P.

2.3. Species composition in seagrass communities

To characterize each of the 20 IRL sites, an estimate of macroalgae and seagrass abundance (percent cover) was made using five haphazardly tossed 1 m^2 quadrats divided into 100–10 cm^2 cells. Data collection was modified from protocols established by St. Johns River Water Management District (SJRWMD) for their long-term IRL-wide seagrass monitoring project (Morris et al., 2001). Parameters for each haphazard quad included depth, percent drift algae, percent attached algae, and percent seagrass by species. To calculate percent cover, each cell was counted as the parameter of interest either being present or absent. For example, a quad with 36 cells containing seagrass was reported as having 36% cover and drift macroalgae covering 27 cells was reported as 27% cover. Abundant species of macroalgae at each site were photo-documented, collected, and identified to the lowest taxonomic level using local keys (Littler et al., 2008).

2.4. Statistical analyses

Because of the non-normal distribution of data collected in this study, non-parametric tests were used to determine significance of spatial and temporal differences in measured variables. In IBM SPSS v22, the Kruskal–Wallis test was used to analyze whether or not significant ($p < 0.05$) differences in measured variables occurred among the IRL segments (ML, BR, NIRL, CIRL, SIRL, and REF) and among the three sampling events (Dry 2011, Wet 2011, and Wet 2012). The Mann–Whitney *U* test was used to identify where the significant differences occurred among the IRL segments and among the three sampling events.

3. Results

Significant temporal and spatial variation in environmental parameters, dissolved nutrients, chlorophyll *a*, stable carbon and nitrogen isotope ratios, molar concentrations of C:N:P, and percent cover of seagrasses occurred during the study.

3.1. Environmental parameters

Salinity showed both significant spatial ($\chi^2 = 70.553$; $p < 0.001$) and temporal ($\chi^2 = 55.762$; $p < 0.001$) variation over the two-year study period (Fig. 4). For all three sampling events, salinity along the IRL ranged from 6.0 to 42.9 with an overall IRL mean of 31.2 ± 0.6 and overall REF mean of 35.5 ± 0.1. The IRL-wide means decreased temporally from 35.8 ± 0.8 during the Dry 2011 sampling to 29.9 ± 0.9 and 29.9 ± 0.8 during the Wet 2011 and 2012 sampling events, respectively. This temporal variation is also reflected in four of the five IRL segment means where salinity in the dry season was significantly higher than the two wet seasons for BR ($p < 0.001$), NIRL ($p = 0.007$), CIRL ($p = 0.005$), and SIRL ($p < 0.001$). Salinity also varied spatially in the IRL with the highest mean in ML (39.9 ± 0.5) and lowest in CIRL (25.4 ± 1.6) with other segment means of 31.2 ± 1.0 (BR), 31.8 ± 1.0 (NIRL), 31.5 ± 0.7 (SIRL), and 35.5 ± 0.1 (REF). Water temperature ranged from 18.3 °C in ML to 34.7 °C in the NIRL with an IRL-wide mean of 28.5 ± 0.4 °C and a REF mean of 27.7 ± 0.6 °C during the study. The pH ranged from 6.7 in the CIRL to 8.9 in the NIRL with an IRL-wide mean of 8.0 ± 0.1 and a REF mean of 7.9 ± 0.1. Dissolved oxygen ranged from 54.7% (3.7 mg/L) to 160.0% (9.8 mg/L), where both extremes were in the NIRL. The overall IRL-wide mean was 91.3 ± 5.2% and 6.3 ± 0.4 mg/L and the REF mean was 88.5 ± 4.1% and 5.9 ± 0.5 mg/L.

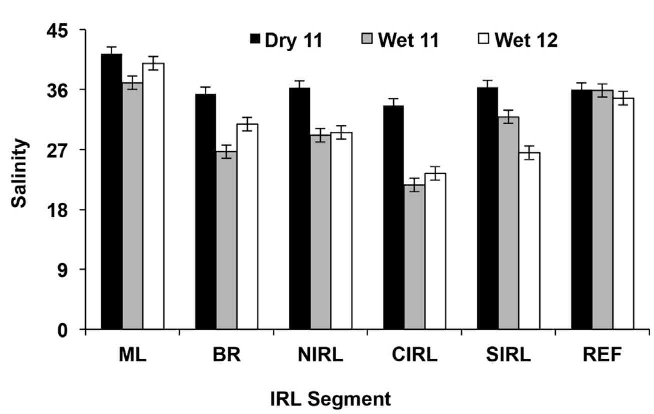

Fig. 4. Mean salinity (±S.E.) in the five IRL segments and the reference sites during the three sampling events in 2011–2012.

3.2. Dissolved nutrients

Ammonium levels showed significant temporal ($\chi^2 = 14.834$; $p = 0.001$) and spatial variation ($\chi^2 = 9.963$; $p = 0.007$) throughout the study period (Fig. 5A). Overall, values ranged from 0.19 μM in ML to 34.60 μM in the CIRL with an IRL-wide mean of 2.54 ± 0.34 μM for all sampling events and an overall REF mean of 0.75 ± 0.07 μM. The IRL-wide means varied temporally from 2.22 ± 0.75 μM (Dry 2011) to 2.44 ± 0.30 μM (Wet 2011) and then down to 2.06 μM (Wet 2012). Spatial variation was considerably different with segment means of 0.67 ± 0.07 μM (ML), 2.20 ± 0.44 μM (BR), 1.08 ± 0.23 μM (NIRL), 6.60 ± 1.17 μM (CIRL), 1.73 ± 0.30 μM (SIRL), and 0.75 ± 0.07 μM (REF). All segments, except ML and NIRL, had significantly higher ammonium values than the reference sites, with *p*-values ranging from $p < 0.001$ to $p = 0.034$.

Nitrate/nitrite values only varied spatially ($\chi^2 = 41.595$; $p < 0.001$) among IRL segments over the study period and were generally lower than the ammonium values (Fig. 5B). During the three sampling events, values ranged from a minimum of 0.04 μM to a maximum of 13.20 μM with an overall mean of 1.70 ± 0.21 μM and an overall REF mean of 1.27 ± 0.36 μM. The IRL-wide means had slight temporal variation from 0.95 ± 013 μM (Dry 2011) to 2.70 ± 0.45 μM (Wet 2011) and then back down to 1.24 ± 0.26 μM (Wet 2012). Nitrate/nitrite values showed considerable spatial variation with the highest concentrations occurring in the CIRL. Segment means for the three sampling events were 0.93 ± 0.26 μM (ML), 0.80 ± 0.16 μM (BR), 0.43 ± 0.07 μM (NIRL), 3.76 ± 0.63 μM (CIRL), 1.91 ± 0.45 μM (SIRL), and 1.27 ± 0.36 μM (REF). Nitrate/nitrite values were significantly higher in the dry season than in the wet season for ML and BR ($p < 0.001$), and NIRL ($p = 0.044$). The reverse occurred in the CIRL, SIRL, and REF, where the Wet 2011 sampling had significantly higher values than the Dry 2011 sampling ($p < 0.001$, $p = 0.006$, and $p = 0.033$, respectively).

Dissolved inorganic nitrogen (DIN) values significantly varied both temporally ($\chi^2 = 7.173$; $p = 0.028$) and spatially ($\chi^2 = 45.190$; $p < 0.001$) over the study period (Fig. 5C). During the three sampling events, values ranged from 0.26 to 34.90 μM with an overall IRL mean of 4.24 ± 0.45 μM and an overall REF mean of 2.02 ± 0.42 μM. The IRL-wide means varied temporally from 3.17 ± 0.77 μM (Dry 2011) to 5.14 ± 0.69 μM (Wet 2011) and back down to 3.30 ± 0.46 μM (Wet 2012). The CIRL also had the highest DIN concentrations and values showed considerable spatial variation with segment means measuring 1.60 ± 0.30 μM (ML), 3.00 ± 0.44 μM (BR), 1.50 ± 0.27 μM (NIRL), 9.83 ± 1.37 μM (CIRL), 3.64 ± 0.67 μM (SIRL), and 2.02 ± 0.42 μM (REF). DIN values were significantly different between dry and wet seasons in ML ($p < 0.001$), the CIRL ($p = 0.009$), the SIRL ($p = 0.004$), and the REF sites ($p = 0.017$).

Total dissolved nitrogen (TDN) values only showed significant spatial variation ($\chi^2 = 161.591$; $p < 0.001$; Fig. 5D). Over the three sampling events, TDN values ranged from 7.77 to 152.04 μM with

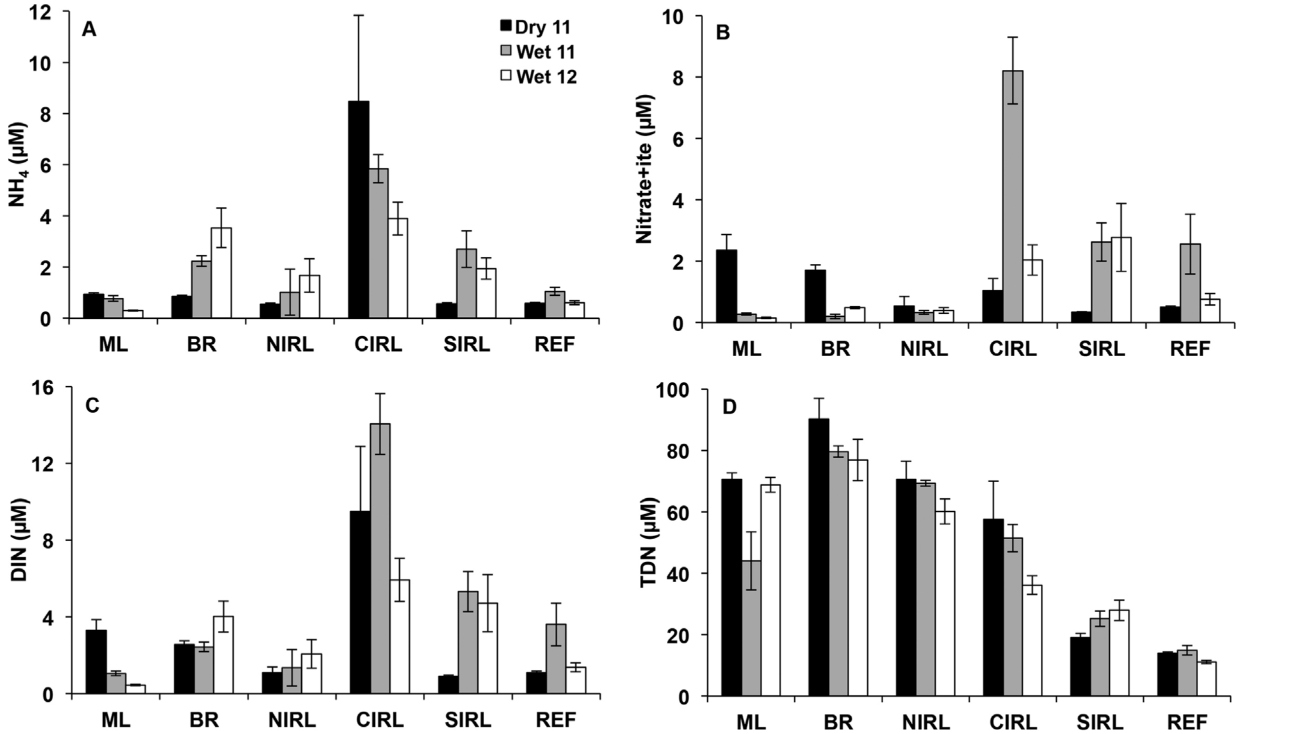

Fig. 5. Mean concentrations (±S.E.) of ammonium (A), nitrate-nitrite (B), dissolved inorganic nitrogen (DIN = ammonium + nitrate + nitrite); (C), and total dissolved nitrogen (TDN; D) in the five IRL segments and the reference sites during three sampling events in 2011–2012.

an overall IRL-wide mean of 53.49 ± 2.08 μM and an overall REF mean of 13.32 ± 0.62 μM. TDN values were similar over the three sampling events with means of 50.99 ± 4.26 μM (Dry 2011) to 46.46 ± 3.00 μM (Wet 2011) and 44.13 ± 2.96 (Wet 2012). The segment means were 61.17 ± 3.99 μM (ML), 82.30 ± 3.12 μM (BR), 66.67 ± 2.73 μM (NIRL), 48.45 ± 4.62 μM (CIRL), 24.13 ± 1.51 μM (SIRL), and 13.21 ± 0.62 (REF). All IRL segments, except ML and NIRL, varied significantly from each other ($p \leq 0.001$).

Soluble reactive phosphorus (SRP) concentrations varied significantly both temporally ($\chi^2 = 7.825$; $p = 0.020$) and spatially ($\chi^2 = 67.536$; $p < 0.019$) over the study period (Fig. 6A). Overall, values ranged from 0.08 to 4.42 μM with an IRL mean of 0.68 ± 0.06 μM and a REF mean of 0.19 ± 0.02 μM. The IRL-wide segment means varied spatially and increased from 0.44 ± 0.07 μM to 0.57 ± 0.09 μM to 0.78 ± 0.11 during the in the Dry 2011, Wet 2011, and Wet 2012 sampling events, respectively. IRL segments means for the three sampling events were 0.26 ± 0.03 μM (ML), 0.26 ± 0.02 μM (BR), 0.40 ± 0.04 μM (NIRL), 1.38 ± 0.16 μM (CIRL), 0.73 ± 0.16 (SIRL), and 0.19 ± 0.02 (REF). Although there was no significant difference between sampling events in the CIRL, the SRP concentrations were significantly higher in the CIRL when compared with all other segment means, including the reference sites ($p < 0.001$).

Total dissolved phosphorus (TDP) values only showed significant spatial variation ($\chi^2 = 108.408$; $p < 0.001$) during the study (Fig. 6B). Over the three sampling events, the TDP values ranged from 0.22 to 5.13 μM with an overall IRL-wide mean of 1.48 ± 0.07 μM and an overall REF mean of 0.49 ± 0.03 μM. IRL-wide means showed little temporal variation and measured 1.23 ± 0.10 μM (Dry 2011) to 1.27 ± 0.11 μM (Wet 2011) and 1.49 ± 0.12 μM (Wet 2012). Spatially, TDP values varied greatly with segment means of 0.98 ± 0.08 μM (ML), 1.22 ± 0.05 μM (BR), 1.51 ± 0.07 μM (NIRL), 2.20 ± 0.19 μM (CIRL), 1.19 ± 0.16 μM (SIRL), and 0.49 ± 0.03 μM (REF). All IRL segments exhibited significantly higher TDP values than the reference sites ($p < 0.001$) and the CIRL had significantly higher values when compared to all other IRL segments ($p \leq 0.007$).

The DIN:SRP ratios showed both significant temporal ($\chi^2 = 20.698$; $p < 0.001$) and spatial variation ($\chi^2 = 32.715$; $p < 0.001$) over the study period (Fig. 7A). During the study, DIN:SRP ranged from 0.45 to 51.83 with an overall IRL-wide mean of

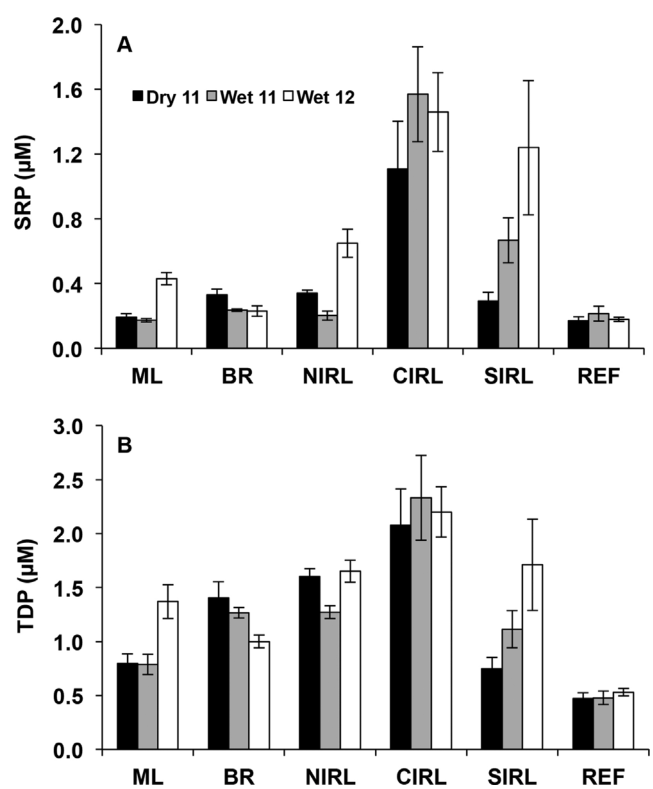

Fig. 6. Mean concentrations (±S.E.) of soluble reactive phosphorus (SRP; A) and total dissolved phosphorus (TDP; B) in the five IRL segments and the reference sites during the three sampling events in 2011–2012.

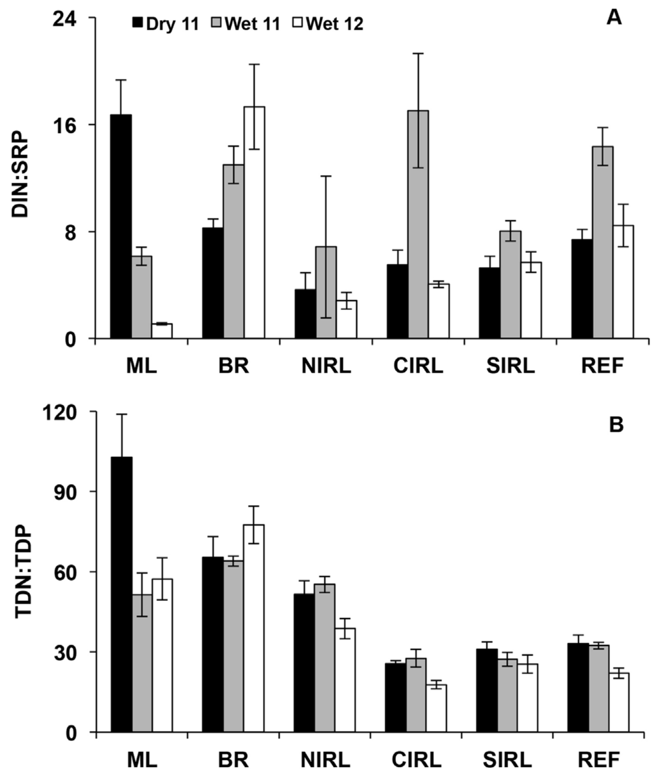

Fig. 7. Mean (±S.E.) DIN:SRP (A) and TDN:TDP (B) ratios in the five IRL segments and the reference sites during the three sampling events in 2011–2012.

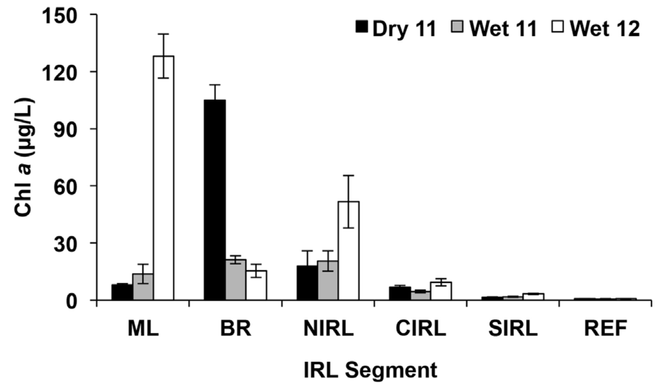

Fig. 8. Mean chlorophyll *a* concentrations (±S.E.) in the five IRL segments and the reference sites during the three sampling events in 2011–2012.

7.61 ± 0.61 and an overall REF mean of 10.06 ± 0.90. The IRL-wide means showed temporal variation with values from 7.05 ± 0.65 (Dry 2011) to 10.97 ± 1.20 (Wet 2011) and back down to 6.07 ± 0.73 (Wet 2012). DIN:SRP values showed considerable spatial variation with segment means of 7.97 ± 1.55 (ML), 12.84 ± 2.15 (BR), 4.43 ± 0.61 (NIRL), 8.86 ± 1.69 (CIRL), 6.33 ± 0.49 (SIRL), and 10.06 ± 0.90 (REF). The Wet 2011 DIN concentration was significantly higher than that recorded the Dry 2011 for the NIRL ($p = 0.023$), CIRL ($p = 0.001$), the SIRL ($p = 0.019$), and at the REF sites ($p = 0.001$).

TDN:TDP ratios in the IRL also showed significant temporal ($\chi^2 = 8.612$; $p = 0.013$) and spatial variation ($\chi^2 = 117.686$; $p < 0.001$) over the study period (Fig. 7B). During the study, TDN:TDP ranged from 10.81 to 176.86 with an IRL-wide mean of 43.71 ± 2.04 and an overall REF mean of 29.20 ± 1.55. The IRL-wide means decreased temporally with values ranging from 47.15 ± 3.82 (Dry 2011) to 41.10 ± 2.13 (Wet 2011), and 36.12 ± 2.79 (Wet 2012). TDN:TDP ratios also showed considerable spatial variation with the higher values occurring in the northern IRL segments. Segment means were recorded as 70.53 ± 7.75 (ML), 69.00 ± 3.17 (BR), 48.55 ± 3.07 (NIRL), 23.70 ± 1.41 (CIRL), 27.94 ± 1.71 (SIRL), and 29.20 (REF). TDN:TDP values were significantly different in ML ($p = 0.016$), NIRL ($p = 0.017$), CIRL ($p = 0.002$), and the REF sites ($p = 0.007$) during the study.

3.3. *Chlorophyll* a

Chlorophyll *a* concentrations showed significant spatial variation ($\chi^2 = 161.924$; $p < 0.001$) during the study (Fig. 8). During the three sampling events, chlorophyll *a* values ranged from 0.62 µg/L in the SIRL to 175.33 µg/L in ML with an IRL-wide mean of 21.94 ± 2.66 µg/L and an overall REF mean of 0.75 ± 0.06 µg/L. IRL-wide means varied temporally from 15.55 ± 3.36 µg/L (Dry 2011) to 9.70 ± 1.36 µg/L (Wet 2011) and then 30.23 ± 5.60 µg/L (Wet 2012). Spatially, chlorophyll *a* concentrations varied considerably with segment means of 49.94 ± 11.58 µg/L (ML), 39.52 ± 8.06 µg/L (BR), 30.06 ± 5.16 µg/L (NIRL), 6.91 ± 0.77 µg/L (CIRL), 2.28 ± 0.19 µg/L (SIRL) and 0.75 ± 0.06 µg/L (REF). During the Wet 2012 sampling, chlorophyll *a* concentrations were significantly higher than both the Dry 2011 sampling and the Wet 2011 sampling ($p < 0.001$). Throughout the study, all IRL segments had significantly higher chlorophyll *a* levels than that documented at the reference sites ($p < 0.001$).

3.4. *δ13C, δ15N, and C:N:P values of macroalgae and phytoplankton*

A total of 44 species of red, green and brown macroalgae were collected in the IRL and analyzed for $\delta^{13}C$, $\delta^{15}N$ and C:N:P contents. The taxa collected included 19 rhodophytes, 17 chlorophytes, and 8 phaeophytes (Table 2).

The $\delta^{13}C$ values of IRL macroalgae showed significant temporal ($X^2 = 54.87$, $p < 0.001$) and spatial variation ($X^2 = 17.96$, $p < 0.003$) over the two-year study (Fig. 9A). Overall, the $\delta^{13}C$ values ranged from −33.69‰ in the SIRL to −9.27‰ in the BR, with an IRL mean of −18.41 ± 0.22‰ and a REF mean of −19.53 ± 0.59‰. The IRL-wide $\delta^{13}C$ means varied temporally from −17.24 ± 0.30‰ (Dry 2011) to −20.22 ± 0.37‰ (Wet 2011) to −18.60 ± 0.45‰ (Wet 2012). There was also spatial variation, with overall IRL segment $\delta^{13}C$ mean values of −19.49 ± 0.35‰ (ML), −16.08 ± 0.63‰ (BR), −18.38 ± 0.38‰ (NIRL), −18.27 ± 0.29‰ (CIRL) and −18.63 ± 0.72‰ (SIRL). The ML values were significantly lighter than BR, CIRL, and SIRL values and BR values were significantly heavier than the NIRL, CIRL, and the REF values (Fig. 9A).

The $\delta^{15}N$ values of IRL macroalgae showed significant temporal ($X^2 = 8.71$, $p = 0.013$) and spatial variation ($X^2 = 28.91$, $p < 0.001$) over the two-year study (Fig. 9B). Overall, the $\delta^{15}N$ values ranged from +1.24‰ in ML to +12.37‰ in the NIRL, with an IRL mean of +6.30 ± 0.12‰ and overall REF mean of +6.30 ± 0.09‰. The IRL-wide $\delta^{15}N$ means varied temporally from +6.06 ± 0.11‰ (Dry 2011) to +6.81 ± 0.22‰ (Wet 2011) and +6.11 ± 0.18‰ (Wet 2012). Spatial variation was considerable, with overall IRL segment mean values for $\delta^{15}N$ of +5.20 ± 0.30‰ (ML), +6.5 ± 0.30‰ (BR), +6.25 ± 0.25‰ (NIRL), +7.11 ± 0.19‰ (CIRL) and +6.00 ± 0.24‰ (SIRL). ML values were significantly lower than all other segments and reference sites. Conversely, CIRL was higher than the NIRL, SIRL and REF sites (Fig. 9B). Based on regression analyses of $\delta^{15}N$ versus two forms of DIN (ammonium [NH_4] and nitrate [NO_3]), there was a trend of macroalgal $\delta^{15}N$ enrichment with increasing concentrations of both NH_4 and NO_3. Despite the potential use of both forms of DIN, macroalgae examined during this study were relatively more enriched by NH_4 compared to NO_3 (Fig. 10).

The macroalgae C:N data showed significant temporal and spatial variation in the N-limited status of these benthic algal

Table 2
Macroalgae collected during the Dry 2011, Wet 2011, and Wet 2012 sampling seasons at 20 sites in the Indian River Lagoon and 4 adjacent nearshore reef Reference sites. Sampling Event Codes: (1) May 2011, (2) October 2011, (3) October 2012.

Division	Sampling event	Sampling stations: Mosquito Lagoon			Banana River			Northern IRL				Central IRL					Southern IRL					Offshore references			
		ML1 (1)	ML2 (2)	ML3 (3)	BR1 (4)	BR2 (5)	BR3 (6)	NIRL1 (7)	NIRL2 (8)	NIRL3 (9)	NIRL4 (10)	CIRL1 (11)	CIRL2 (12)	CIRL3 (13)	CIRL4 (14)	CIRL5 (15)	SIRL1 (16)	SIRL2 (17)	SIRL3 (18)	SIRL4 (19)	SIRL5 (20)	Ambersand (21)	Pepper Park (22)	Bathtub Beach (23)	Coral Cove (24)
Rhodophyta (red algae)																									
Acanthophora muscoides	3								×																
Acanthophora spicifera	1,2,3													×	×				×						
Botryocladia occidentalis	1,2																					×			
Bryothamnion seaforthii	2,3							×														×	×	×	
Bryothamnion triquetrum	1																						×		
Ceramium sp.	1												×												
Chondria baileyana	1					×																			
Chondria sp.	2					×																			
Digenea simplex	1,2,3			×																					
Gracilaria tikvahiae	1,2,3	×	×			×	×	×		×	×	×	×	×		×									
Halymenia elongata	3																						×		
Heterosiphonia gibbesii	1																					×			
Hydropuntia secunda	1,2,3			×				×							×	×									
Hypnea musciformis	1,2,3									×								×				×			
Hypnea spinella	1,3		×						×					×							×				
Laurencia filiformis	1			×			×	×																	
Laurencia poiteaui	3																								×
Solieria filiformis	3																					×			
Spyridia filamentosa	1	×														×									
Chlorophyta (green algae)																									
Acetabularia cf. *farlowii*	2							×																	
Acetabularia schenckii	2				×	×																			
Acetabularia sp.	2,3				×	×																			
Caulerpa mexicana	1															×	×								
Caulerpa prolifera	1,2,3								×									×	×	×		×			
Caulerpa racemosa	1,2																					×		×	
Caulerpa racemosa v. peltata	1																								×
Caulerpa sertularioides	1,2,3														×		×		×	×	×				
Caulerpa verticillata	2,3																		×	×					
Chaetomorpha gracilis	1											×													
Cladophora laetevirens	2,3										×														
Cladophora prolifera	2,3							×																	
Halimeda incrassata	2																			×					
Ulva flexuosa	2	×																							×
Ulva intestinalis	1,2,3					×	×		×	×	×		×	×											
Ulva rigida	2																								×
Ulva sp.	2	2			×															×					
Phaeophyta (brown algae)																									
Dictyopteris justii	3																						×		
Dictyota cervicornis	1																				×			×	
Dictyota sp.	3																							×	
Hincksia mitchelliae	2	×																							
Hincksia sandriana	2																×	×							
Padina gymnospora	1																								×
Padina sp.	1																						×		
Sargassum filipendula	1,3																					×			

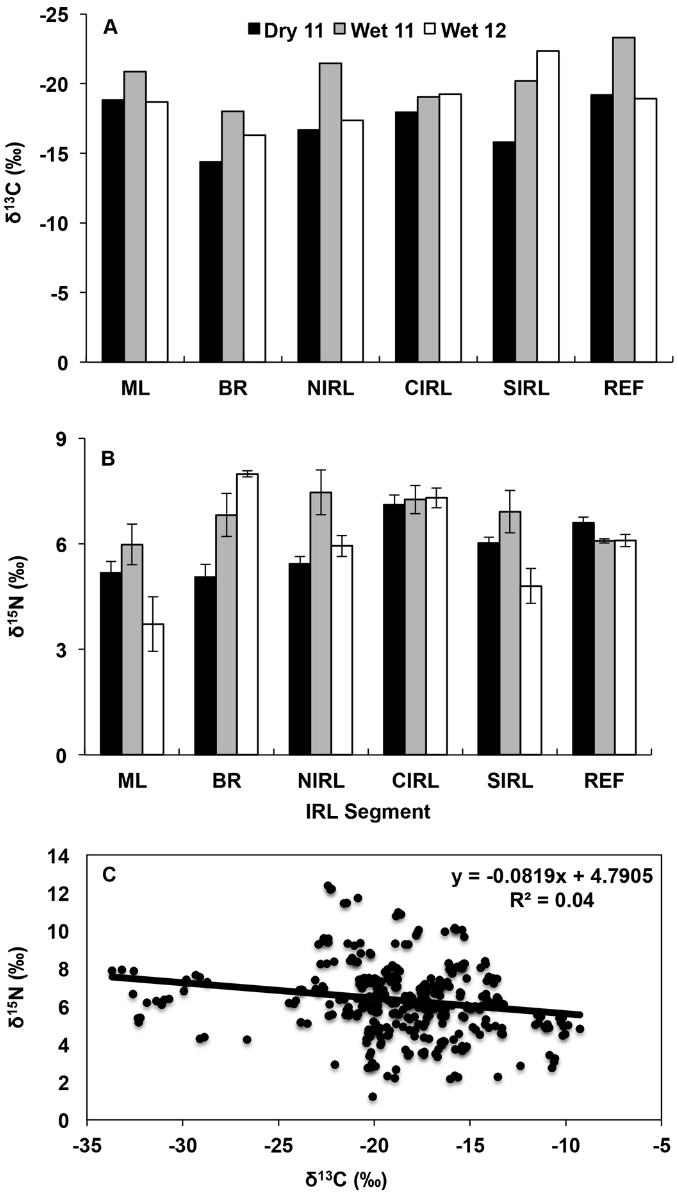

Fig. 9. Mean (±S.E.) $\delta^{15}N$ (A), $\delta^{13}C$ (B) and $\delta^{15}N/\delta^{13}C$ regression bi-plot (C) for macroalgae collected from each of the five IRL segments and the reference sites during the three sampling events in 2011–2012.

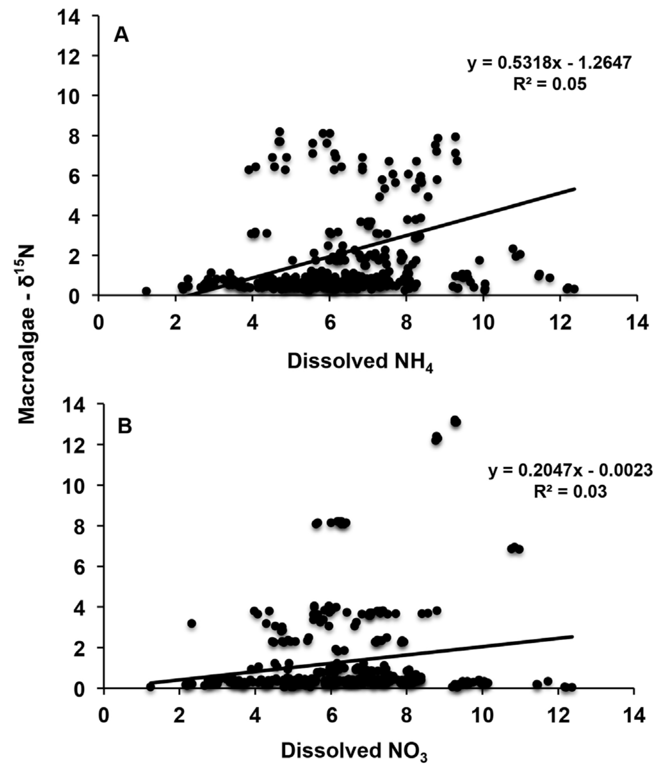

Fig. 10. Regression analysis of macroalgal $\delta^{15}N$ versus dissolved concentrations of ammonium (A) and nitrate (B) for the five IRL segments sampled in 2011–2012.

blooms during the study (Table 3). Over the three sampling events, the C:N values ranged from 9.28 ± 0.23 in the CIRL to 42.40 ± 1.50 in BR, with an overall IRL mean of 15.87 ± 0.44 and overall REF mean of 17.02 ± 0.73. The IRL-wide C:N means decreased temporally through the study from 17.74 ± 0.62 (Dry 2011) to 15.55 ± 0.78 (Wet 2011) and 14.36 ± 0.53 (Wet 2012). Spatial variation in C:N among IRL segments was considerable, with overall segment mean values ranging 10.54 ± 0.35 in the CIRL during the Wet 2011 sampling to 23.94 ± 3.76 in the BR during the Dry 2011 sampling.

The macroalgae C:P data showed significant temporal and spatial variation in the degree of P-limitation in these benthic algal blooms during the study, with generally higher values in the three northern IRL segments (Table 3). Over the three sampling events, the C:P values ranged from 102.42 ± 2.82 in the SIRL to 3281.6 ± 813.78 in ML, with an overall IRL mean of 698.9 ± 43.6 and overall REF mean of 505.96 ± 199.1. The IRL-wide C:P means varied temporally through the study from 662.7 ± 45.4 (Dry 2011) to 590.6 ± 55.0 (Wet 2011) and 737.6 ± 87.7 (Wet 2012). Spatial variation in C:P among IRL segments was also considerable, with overall segment mean values ranging from 281.8 ± 18.4 in the CIRL during the Dry 2011 sampling to 3281.6 ± 813.78 in the ML during the Wet 2012 sampling.

Similar to the C:P ratio, the N:P data showed significant temporal and spatial variation in the degree of P-limitation in benthic algal blooms during the study, with higher values in the three northern segments of the IRL (Table 3). Over the three sampling events, the N:P values ranged from 11.24 ± 0.27 in the SIRL to 151.50 ± 36.12 in ML, with an overall IRL mean of 40.62 ± 1.85 and an overall REF mean of 31.31 ± 1.21. The IRL-wide N:P means increased temporally through the study from 35.27 ± 1.65 (Dry 2011) to 35.61 ± 2.40 (Wet 2011) and 47.59 ± 3.93 (Wet 2012). Spatial variation in N:P among IRL segments was considerable, with overall segment mean values ranging from 20.53 ± 1.01 in the CIRL during the Dry 2011 sampling to 151.50 ± 36.12 in the ML during the Wet 2012 sampling.

The $\delta^{13}C$ and $\delta^{15}N$ values for phytoplankton collected throughout ML during the final stages of the "superbloom" in February 2012 averaged −22.22 ± 0.07 and +3.49 ± 0.08, respectively. The *Microcystis aeruginosa* collected from Manatee Pocket in the CIRL's SLE in August 2013 had $\delta^{13}C$ and $\delta^{15}N$ values of −24.30 ± 0.04 and +8.59 ± 0.06, respectively. The C:N, C:P and N:P values for *M. aeruginosa* were 7.39 ± 0.10, 240.85 ± 6.49, and 32.65 ± 0.62, respectively.

3.5. Species composition in seagrass communities

The percent cover of seagrasses at the 20 IRL study sites ranged from 0 to 100 percent with an overall mean of 46.67 ± 2.63. There was a significant decline in seagrass cover among the three sampling events ($X^2 = 9.001$, $p = 0.011$). Significantly more seagrass cover was documented in the Dry 2011 (57.11 ± 4.54) season when compared to the Wet 2011 (43.08 ± 4.55) and Wet 2012 (39.07 ± 4.41) seasons ($p = 0.018$ and $p = 0.006$, respectively). Significant differences were

Table 3
Mean C:N:P ratios (±S.E.) from macroalgae collected in the Dry 2011, Wet 2011, and Wet 2012 seasons at 20 sites within the Indian River Lagoon and 4 Reference sites along the adjacent nearshore reefs.

Segment	Site #	Site	Dry 2011			Wet 2011			Wet 2012		
			C:N	C:P	N:P	C:N	C:P	N:P	C:N	C:P	N:P
Mosquito Lagoon	1	ML1	11.70 ± 1.29	268.96 ± 15.72	23.79 ± 1.50	22.40 ± 1.42	341.63 ± 77.25	14.50 ± 2.56	–	–	–
	2	ML2	15.81 ± 0.45	1042.93 ± 34.46	66.23 ± 2.21	18.03 ± 1.31	595.53 ± 98.60	32.66 ± 2.96	–	–	–
	3	ML3	17.59 ± 1.44	1413.52 ± 197.17	78.97 ± 5.64	17.23 ± 3.63	1497.25 ± 628.67	80.31 ± 17.12	21.34 ± 0.86	3281.56 ± 813.78	151.50 ± 36.12
	Segment Mean ± SE		**15.03 ± 0.86**	**908.47 ± 131.63**	**56.33 ± 6.05**	**20.02 ± 1.29**	**694.01 ± 200.73**	**35.49 ± 9.00**	**21.34 ± 0.86**	**3281.56 ± 813.78**	**151.50 ± 36.12**
Banana River	4	BR1	42.40 ± 1.50	2194.70 ± 45.79	51.95 ± 1.31	12.68 ± 0.46	211.55 ± 13.89	16.69 ± 0.55	21.05 ± 0.55	1005.89 ± 38.50	47.99 ± 2.66
	5	BR2	27.29 ± 5.51	880.35 ± 106.07	36.56 ± 5.31	35.04 ± 8.03	1129.14 ± 165.35	36.58 ± 3.95	9.17 ± 0.38	418.77 ± 21.02	46.31 ± 3.48
	6	BR3	11.35 ± 0.56	344.25 ± 41.14	29.98 ± 2.32	11.50 ± 0.12	610.36 ± 36.39	53.21 ± 3.52	14.38 ± 0.14	941.94 ± 23.93	65.65 ± 1.52
	Segment Mean ± SE		**23.94 ± 3.76**	**928.78 ± 186.10**	**37.01 ± 3.07**	**23.57 ± 5.16**	**770.05 ± 140.76**	**35.76 ± 4.40**	**13.44 ± 1.49**	**696.34 ± 85.11**	**51.57 ± 3.04**
Northern IRL	7	NIRL1	29.82 ± 2.62	2036.25 ± 111.78	74.12 ± 7.25	20.79 ± 0.86	2039.54 ± 188.58	99.86 ± 9.68	17.10 ± 1.04	1411.41 ± 173.69	80.98 ± 7.00
	8	NIRL2	16.32 ± 0.11	779.85 ± 19.83	47.87 ± 0.90	19.74 ± 0.06	925.73 ± 224.74	46.99 ± 1.40	10.71 ± 0.62	648.25 ± 115.25	57.76 ± 6.88
	9	NIRL3	17.48 ± 0.34	230.42 ± 13.16	13.21 ± 0.71	10.29 ± 0.30	262.89 ± 9.40	25.73 ± 1.03	14.11 ± 1.07	305.09 ± 23.63	21.68 ± 0.40
	10	NIRL4	11.42 ± 0.67	265.56 ± 13.16	23.21 ± 0.68	10.86 ± 0.64	300.62 ± 43.05	27.06 ± 2.40	9.57 ± 0.55	233.28 ± 12.92	24.48 ± 0.43
	Segment Mean ± SE		**21.49 ± 1.90**	**1101.87 ± 173.82**	**46.35 ± 6.09**	**14.97 ± 1.03**	**937.14 ± 169.15**	**53.10 ± 7.32**	**13.75 ± 0.72**	**839.88 ± 114.32**	**55.87 ± 5.35**
Central IRL	11	CIRL1	20.65 ± 0.71	284.32 ± 26.19	13.75 ± 1.02	–	–	–	11.21 ± 0.37	199.23 ± 9.60	17.79 ± 0.26
	12	CIRL2	9.28 ± 0.23	206.07 ± 19.54	22.28 ± 2.06	11.24 ± 0.14	187.93 ± 12.63	16.79 ± 1.29	–	–	–
	13	CIRL3	11.31 ± 0.52	203.86 ± 18.99	17.82 ± 1.02	11.04 ± 0.04	229.99 ± 7.70	20.88 ± 0.64	10.03 ± 0.37	213.98 ± 15.35	21.30 ± 1.10
	14	CIRL4	15.21 ± 1.75	335.81 ± 38.70	22.30 ± 1.65	10.49 ± 0.96	298.06 ± 65.71	26.77 ± 3.82	14.21 ± 0.85	372.60 ± 47.31	25.80 ± 1.93
	15	CIRL5	14.92 ± 0.48	418.13 ± 35.29	27.87 ± 1.53	9.99 ± 0.41	239.29 ± 40.74	24.07 ± 3.91	–	–	–
	Segment Mean ± SE		**14.00 ± 0.76**	**281.84 ± 18.42**	**20.53 ± 1.01**	**10.54 ± 0.35**	**248.77 ± 26.07**	**23.22 ± 1.92**	**11.94 ± 0.62**	**274.48 ± 28.58**	**22.39 ± 1.17**
Southern IRL	16	SIRL1	14.58 ± 0.28	367.65 ± 48.92	25.34 ± 3.37	9.13 ± 0.03	102.42 ± 2.82	11.24 ± 0.27	–	–	–
	17	SIRL2	20.15 ± 1.96	352.03 ± 63.07	19.67 ± 4.91	9.72 ± 0.07	161.34 ± 17.27	16.66 ± 1.91	–	–	–
	18	SIRL3	15.10 ± 0.22	597.66 ± 38.90	39.60 ± 2.24	11.78 ± 0.35	370.71 ± 12.19	31.57 ± 0.64	10.11 ± 0.37	263.37 ± 31.65	26.50 ± 3.68
	19	SIRL4	17.19 ± 0.83	575.48 ± 70.49	34.67 ± 5.39	20.48 ± 3.59	932.70 ± 44.62	49.42 ± 7.22	10.97 ± 0.44	426.67 ± 22.67	39.72 ± 3.00
	20	SIRL5	20.28 ± 1.54	630.28 ± 50.69	31.13 ± 0.72	14.81 ± 0.35	372.46 ± 20.79	25.16 ± 0.83	–	–	–
	Segment Mean ± SE		**17.46 ± 0.67**	**504.62 ± 32.02**	**30.08 ± 2.02**	**13.34 ± 1.18**	**413.88 ± 68.57**	**28.75 ± 3.34**	**10.63 ± 0.31**	**361.35 ± 27.86**	**34.43 ± 2.83**
Reference	21	Ambersand	15.63 ± 1.38	509.80 ± 55.40	33.61 ± 2.97	10.27 ± 0.24	324.27 ± 52.99	31.75 ± 5.37	18.49 ± 3.19	396.60 ± 27.04	25.40 ± 2.98
	22	Pepper Park	15.00 ± 1.56	476.30 ± 65.44	31.24 ± 1.13	13.86 ± 1.13	315.42 ± 93.07	24.12 ± 8.04	23.10 ± 0.66	435.88 ± 27.59	18.86 ± 0.86
	23	Bathtub	16.85 ± 1.54	547.96 ± 49.99	32.76 ± 1.69	13.37 ± 0.32	480.98 ± 38.80	36.12 ± 3.01	15.51 ± 0.16	598.37 ± 61.59	38.71 ± 4.13
	24	Coral Cove	24.32 ± 2.02	852.17 ± 86.94	35.63 ± 2.92	26.15 ± 0.29	697.27 ± 29.16	26.72 ± 2.07	11.31 ± 0.37	548.56 ± 60.55	49.15 ± 3.85
	Segment Mean ± SE		**17.32 ± 0.99**	**572.90 ± 39.67**	**33.39 ± 1.47**	**15.46 ± 0.14**	**455.59 ± 32.45**	**30.63 ± 3.29**	**18.33 ± 1.59**	**458.36 ± 23.94**	**28.82 ± 2.71**
	Seasonal Mean ± SE		**17.74 ± 0.62**	**662.69 ± 45.41**	**35.27 ± 1.65**	**15.55 ± 0.78**	**590.60 ± 55.01**	**35.61 ± 2.40**	**14.36 ± 0.53**	**737.56 ± 87.74**	**47.59 ± 3.93**

also documented in the seagrass cover within the five segments ($X^2 = 5.510$, $p < 0.001$) where the mean coverage over the extent of the study was 56.09 ± 6.93 (ML), 32.70 ± 6.78 (BR), 39.15 ± 6.17 (NIRL), 34.29 ± 4.80 (CIRL), and 67.76 ± 4.45 (SIRL).

The coverage of four dominant species of seagrass (*Halodule wrightii* Ascherson, *Halophila johnsonii* N.J. Eiseman, *Syringodium filiforme* Kützing, and *Thalassia testudinum* K.D. Koenig) varied both temporally and spatially over the study period (Fig. 11). Overall, *H. wrightii*, located in all five segments of the IRL, was the most abundant seagrass species in the system with percent cover of 38.78 ± 2.57. The next most abundant seagrass species was *S. filiforme*, which was located at study sites in all IRL segments except ML and BR, and had a percent cover of 6.02 ± 1.30. The federally protected seagrass, *H. johnsonii*, and *T. testudinum* were only present at study sites in the CIRL and SIRL where they had a percent covers of 5.35 ± 1.11 and 3.00 ± 0.91, respectively. Temporally, the mean percent cover of *H. wrightii* in Dry 2011, Wet 2011, and Wet 2012 was 48.00 ± 4.50, 39.59 ± 4.52, and 28.32 ± 4.10, respectively. *S. filiforme* percent cover decreased from 6.12 ± 2.13 (Dry 2011) to 1.82 ± 1.28 (Wet 2011) then increased again to 9.88 ± 2.89 (Wet 2012). Similar to *H. wrightii*, *H. johnsonii* continuously decreased from 8.53 ± 2.38 (Dry 2011) to 5.32 ± 2.16 (Wet 2011) to 2.04 ± 0.67 (Wet 2012). *T. testudinum* percent cover was lowest in Dry 2011 (1.90 ± 1.15) then increased in Wet 2011 (5.04 ± 2.10) and then decreased again in Wet 2012 (2.23 ± 1.39). Spatially, the mean percent cover of *H. wrightii* over the study period ranged from 29.00 ± 4.53 in the CIRL to 56.09 ± 6.93 in ML. In the segments where *S. filiforme* was present (NIRL, CIRL, and SIRL), percent cover ranged from 3.63 ± 1.90 in the CIRL to 13.43 ± 3.68 in the SIRL. The mean cover of *H. johnsonii* in the CIRL and SIRL was 2.71 ± 1.26 and 18.90 ± 3.89, respectively. Similarly, the mean percent cover of *T. testudinum* in the CIRL and SIRL was 5.57 ± 2.33 and 6.26 ± 2.64, respectively.

Attached and drift macroalgae were also documented within the seagrass communities during all three samplings. The overall mean percent cover of attached and drift algae, respectively, was 27.58 ± 3.52 and 3.46 ± 1.79 (Dry 2011), 5.77 ± 1.60 and 0.88 ± 0.48 (Wet 2011), and 9.59 ± 2.40 and 6.05 ± 1.71 (Wet 2012). Spatial variation in percent cover of attached and drift algae, respectively, was observed amongst the regions with 5.40 ± 1.86 and 0.00 ± 0.00 (ML), 9.06 ± 2.99 and 0.00 ± 0.00 (BR), 17.80 ± 5.95 and 0.00 ± 0.00 (NIRL), 18.83 ± 5.81 and 2.68 ± 1.89 (CIRL), 37.08 ± 6.85 and 0.92 ± 0.28 (SIRL) during the Dry 2011 season. In the Wet 2011 season, the variation in percent cover of attached and drift macroalgae, respectively, was 1.27 ± 1.07 and 4.00 ± 2.89 (ML), 0.00 ± 0.00 and 0.00 ± 0.00 (BR), 6.05 ± 3.44 and 0.20 ± 0.20 (NIRL), 4.04 ± 1.53 and 0.04 ± 0.04 (CIRL), and 12.28 ± 4.99 and 0.76 ± 0.46 (SIRL). In the Wet 2012 season, the percent cover of attached and drift algae varied respectively, 0.00 ± 0.00 and 4.80 ± 2.48 (ML), 8.87 ± 6.02 and 1.87 ± 1.00 (BR), 14.16 ± 5.53 and 10.04 ± 3.80 (NIRL), 5.52 ± 2.24 and 0.00 ± 0.00 (CIRL), 15.75 ± 7.79 and 12.1 ± 6.37 (SIRL).

4. Discussion

4.1. Nitrogen enrichment drives phosphorus-limited phytoplankton blooms

This study represents the first IRL-wide water quality assessment that integrates water column nutrient monitoring with macroalgae "bio-observatories" and seagrass surveys to identify nutrient sources and gauge the type and degree of N versus P limitation. Macroalgae in the IRL are known to respond to increased N availability with increased growth (Lapointe and Ryther, 1979) and excessive biomass has been symptomatic of accelerating eutrophication for several decades (Bricker et al., 2007). The timing of our study was serendipitous as it coincided with the end of a multi-year drought, reduced abundance of drift macroalgae (personal observations, SJRWMD et al., 2012), and development of unprecedented phytoplankton blooms in the northern segments of the IRL. This began with the development of the "superbloom" (~130 μg/L) in the BR, NIRL and ML that was comprised predominantly of the microflagellate, *Resultor* sp., Fig. 3(A) and persisted between March 2011 and February 2012, with the highest concentrations between April and November 2011 (Phlips et al., 2014). Seagrass loss (~45% or ~31,600 acres by June 2011; SJRWMD, unpublished data) immediately following the onset of the superbloom during the first year of our study was substantial and was reflected in our Wet 2011 seagrass surveys.

The superbloom was followed by an unprecedented brown tide in July 2012 comprised of the pelagophyte *Aureoumbra lagunensis* D.A. Stockwell, DeYoe, Hargraves and P.W. Johnson in the southern ML and NIRL (Gobler et al., 2013), which caused the highest chlorophyll *a* values of our study. Coastal waters susceptible to brown tides are relatively warm, shallow, and hypersaline, with little tidal exchange (high residence times; Gobler and Sunda, 2012); however, these environmental conditions have been commonly seen in these segments of the IRL when such severe phytoplankton blooms have not occurred (Briel et al., 1973). In August 2012, we

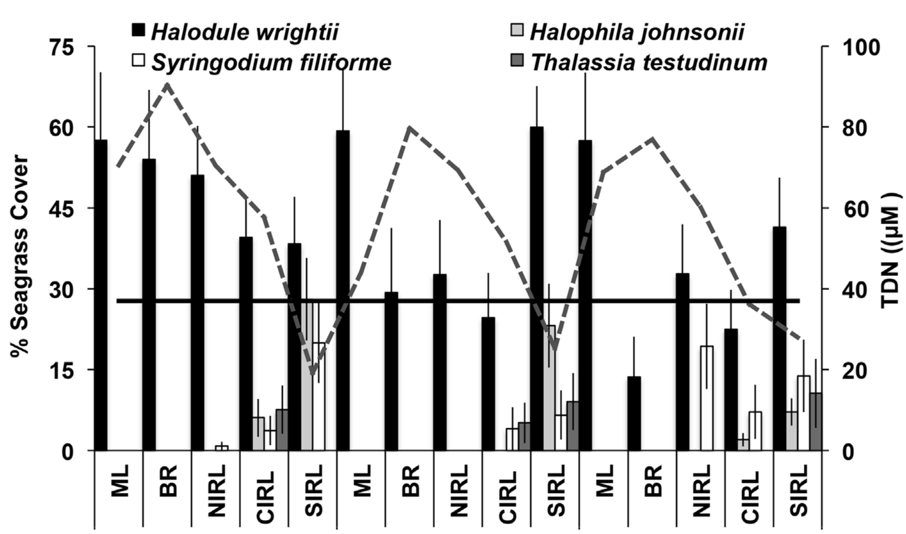

Fig. 11. Mean seagrass cover (±S.E.) and total dissolved nitrogen (TDN) by species in each of the five IRL segments during the three sampling events in 2011–2012. The dashed line denotes TDN concentrations during the study. The solid black line represents the total nitrogen threshold above which *Thalassia testudinum* becomes stressed (Lapointe et al., 1994).

observed >130 μg/L chlorophyll *a* while others reported up to 196 μg/L chlorophyll *a* and 3 × 10^6 cells/L in ML, the epicenter of the bloom (Gobler et al., 2013). A partial die-back occurred in September 2013 following the passing of tropical storm Isaac. In April 2013, a smaller *A. lagunensis* bloom re-emerged in the same area (SJRWMD, 2013). These IRL events were similar to the prolonged brown tides in the 1990s along coastal lagoons in Texas (Buskey et al., 2001; Gobler and Sunda, 2012) that similarly resulted in seagrass die-off due to reductions in light (Onuf, 1996).

Although the long residence time of water in the three northern IRL segments has long been recognized as a physical factor contributing to bloom development (Briel et al., 1973), our nutrient data indicate that these northern segments were also highly enriched in TDN (61–82 μM) at levels well above FDEP TMDL targets (~50 μM) with correspondingly high TDN:TDP ratios (49:1–71:1) during these phytoplankton bloom events. The more highly flushed CIRL and SIRL had lower TDN concentrations (24–48 μM), TDN:TDP ratios (24–29) and chlorophyll *a* concentrations and were not impacted by severe phytoplankton blooms or seagrass die-off during our study. These observations are consistent with previous studies (Sigua et al., 2000; Phlips et al., 2010), however, our interpretations of how the stoichiometry impacts the system is new. Although high N:P ratios generally indicate P-limitation, a variety of phytoplankton can have high growth rates at high N:P ratios (>70) while experiencing strong P-limitation (Terry et al., 1985). For example, *Aureoumbra lagunensis*, which formed persistent and damaging blooms in Laguna Madre, TX, is capable of growing under a wide range of N:P ratios (Liu et al., 2001) and actually formed dense blooms when the water column N:P ratio increased to high levels (~140; Rhudy et al., 1999). Similarly, the cyanobacterium *Synechococcus* spp., which formed severe and damaging blooms in Florida Bay between 1991 and 1996 following increased freshwater flows and N-loading from Everglades runoff (Brand, 2002; Lapointe and Barile, 2004), can outcompete other phytoplankton species under high N:P ratios and P-limited conditions (Lavrentyev et al., 1998; Richardson, 2009).

These data and observations contrast with the conclusions of Sigua et al. (2000) that algal blooms in the IRL are most likely to develop in the CIRL and SIRL where "a TN/TP ratio ≤10 appears to favor algal blooms, especially blue-green algae, which are capable of fixing atmospheric N." Indeed, the cyanobacterium *Microcystis aeruginosa* formed blooms in the SLE during June 2005 and July 2013 following heavy freshwater releases from Lake Okeechobee and runoff from the surrounding SLE basin (Fujimoto et al., 1997; Phlips et al., 2012; Lapointe et al., 2012). Although a decrease in the water column N:P ratio accompanied the *M. aeruginosa* blooms (Lapointe et al., 2012; Parrish, 2014), high P (relative to N) loading is not a universal "trigger" for cyanobacterial blooms (Paerl and Otten, 2013). The "P only" paradigm assumed that N_2 fixation could supply all of the required N, a viewpoint that needs revision to account for many cyanobacterial blooms that result from N-enrichment and occur at high N:P ratios (Paerl and Fulton, 2006). Toxic strains of *M. aeruginosa*, which produced the hepatotoxin microcystin, were documented in the SLE during both 2005 and 2013, resulting in numerous public warnings from the Department of Health cautioning residents and visitors to avoid recreational use of the SLE, IRL, and nearshore reefs adjacent to St. Lucie Inlet (Phlips et al., 2012; FDEP, unpublished data). Furthermore, a variety of HABs show increased toxin production under N-enrichment and increased P-limitation (Donald et al., 2011; Dolman et al., 2012; Hardison et al., 2013), which readily explains why toxins could be especially problematic in the northern segments of the IRL, where unusual mass mortality events, involving manatees, dolphins, and pelicans, developed in 2012 and 2013 following these unprecedented bloom events (SJRWMD, 2013; Wines, 2013; NOAA, 2014).

4.2. Freshwater runoff transports dissolved nutrients into the IRL

Multiple factors and nutrient sources likely worked in synergy to generate the unprecedented phytoplankton blooms in the northern segments of the IRL during our study. Prior to the blooms, the adjacent IRL watersheds experienced a multi-year drought that culminated with a "first flush" during spring and early summer 2011 (Fig. 2). These northern segments, which had high TDN:TDP ratios and were P-limited, experienced a spike in P during the spring 2011 following the end of the drought with increasing rainfall (SJRWMD et al., 2012). However, we also observed relatively little biomass of benthic macroalgae in the IRL during the study, possibly the result of reduced nutrient loading associated with the antecedent drought. In addition to the spike in nutrient loading from increased stormwater runoff, ash produced during a controlled burn in July 2011 within Merritt Island National Wildlife Refuge may also have contributed a pulse of P that would likely have fueled the bloom. Regardless of the source, the pulses of P into the northern segments of the IRL, where minimum macroalgal biomass was available to assimilate nutrients, culminated in the unprecedented phytoplankton blooms that resulted in a 60% decline in seagrass in the northern IRL (SJRWMD, 2013). Below we describe the spatial and temporal patterns among salinity, dissolved N and P concentrations, N:P ratios, chlorophyll *a*, as well as δ^{13}C and δ^{15}N values for macroalgae and phytoplankton HABs that supports our conclusions.

Overall, salinity generally decreased through the course of our two-year study, indicating that the observed algal blooms were a response to increasing land-based nutrient inputs. The lowest salinity values during our study were in the CIRL (mean 25.37), the segment of the IRL that receives considerable freshwater discharges from three drainage canals in Indian River County, as well as the St. Sebastian River, the C-54 canal, and several creeks in southern Brevard County (Fig. 1). Estimated freshwater flows into this region of the IRL are ~500,000,000 m^3 yr^{-1}, which is dominated (168,039,748 m^3 yr^{-1}) by the St. Sebastian River (Sigua and Tweedale, 2003). Not surprisingly, we found the highest concentrations of ammonium (6.60 μM), nitrate/nitrite (3.76 μM), DIN (9.83 μM), SRP (1.38 μM), and TDP (2.20 μM) associated with these low salinity values in the CIRL. These findings are in general agreement with previous IRL water quality assessments, which report the highest TN (765,211 kg N/yr) and TP (87,978 kg/yr) non-point source loadings occurred in this segment of the IRL (Sigua and Tweedale, 2003).

Despite the large freshwater inflows and high dissolved inorganic N and P concentrations in the CIRL, the phytoplankton blooms occurred in the NIRL, BR and ML where dissolved inorganic nutrients were relatively low but TDN concentrations and TDN/TDP ratios were high. We attribute this disconnect between the IRL segment with the highest dissolved inorganic nutrient concentrations and that with the most severe phytoplankton bloom development to several factors. First, the CIRL has a much shorter water residence time as a result of higher tidal flushing via the Sebastian and Ft. Pierce inlets that would limit phytoplankton bloom development despite high DIN and SRP concentrations (Lu and Gan, 2014); the longer water residence times in the northern segments would support greater development of microbial biomass and preferential scavenging of inorganic nutrient pools (Lucas et al., 1999). Second, the longer water residence time and microbial biomass in the NIRL, ML and BR allows for a higher degree of autotrophic and heterotrophic nutrient cycling, which would tend to not only deplete the more biologically available DIN and SRP, but also elevate DON and DOP concentrations over time (Bronk et al., 1998; Tyler et al., 2001). Third, urbanization has correlated with increases in macroalgae in the IRL, as is the case in many other estuaries and coastal waters (Lapointe et al., 1994;

Valiela et al., 1992). Historically, macroalgae were a minor component of seagrass communities in the IRL (Benz et al., 1979; Gilbert and Clark, 1981; Virnstein and Carbonara, 1985), but in recent years their levels have increased to three times the seagrass biomass throughout the IRL as a whole (Hall et al., 2001; Riegl et al., 2005). These macroalgal blooms are a response of the shallow estuary to nutrient enrichment and can cause reduced light, hypoxia, fragmentation and die-off of seagrasses (Lapointe et al., 1994; Valiela et al., 1997). Because macroalgae are known to assimilate DIN and release DON (Tyler et al., 2001), these benthic macroalgal blooms would also result in cumulative build-up of DON in the IRL over time.

The mean C:N:P ratios of macroalgae in the IRL indicated that growth of these shallow primary producers is potentially limited by both N and P. The overall mean C:N ratio was ~17, with higher values, up to 42, in the northern segments of the IRL. The lowest C:N ratios occurred in the CIRL where the highest DIN occurred, with the highest values in the NIRL, BR, and ML. Because C:N ratios >12 indicate increasing N-limitation (D'Elia and DeBoer, 1978; Lapointe et al., 1984), the IRL values, especially in the NIRL, BR and ML, indicate significant N-limitation and supports previous experimental enrichment studies (Lapointe and Ryther, 1979; Hanisak, 1990). As N-loading generally increased with increasing rainfall and decreasing salinity through the study, the increased N availability was reflected in decreasing C:N ratios of macroalgae. The mean C:P and N:P ratios were 495 and 31, respectively, indicating significant co-limitation by P. However, overall higher C:P and N:P ratios occurred in the northern IRL segments, indicating stronger P-limitation in this region of the IRL. These high macroalgae C:P and N:P ratios mirror the high seawater TDN/TDP ratios in this part of the IRL, and could indicate cumulative inputs of groundwater with high N:P ratios. Preferential scavenging of P by soils results in increasing N:P ratios along the flow path of OSTDS plumes in groundwaters and has been linked to high N:P ratios and P-limitation in downstream surface waters (Lapointe et al., 1990; Weiskel and Howes, 1992). Extremely high C:P and N:P ratios of 3281 and 150, respectively, were observed in summer 2012 during the brown tide in the ML when scavenging of P by the dense bloom would have caused low P availability to the macroalgae.

4.3. HABs as bio-indicators of sewage N enrichment

Measurement of stable C and N isotopes in primary producers and food webs has provided a powerful method to detect and track sewage pollution through aquatic ecosystems (Heaton, 1986; McClelland et al., 1997; Kendall et al., 2007; Risk et al., 2008; Olsen et al., 2010). The δ^{13}C data showed an overall inverse correlation with δ^{15}N, with lighter values reflecting lower salinity and lighter, terrestrial dissolved inorganic carbon (DIC) sources compared to more enriched values reflecting DIC from marine influenced sites in the IRL (Olsen et al., 2010). Macroalgae δ^{15}N data collected IRL-wide in this study showed a highly enriched overall mean value of ~+6.3‰, a value remarkably similar to those reported for macroalgae in other urbanized, sewage-polluted coastal waters (Table 1). In Sarasota Bay, the Sarasota Bay Estuary Program has used their Comprehensive Conservation and Management Plan to implement a Wastewater Treatment and Reclamation Action Plan, which includes the consolidation and removal of wastewater treatment plants, removal of septic tanks, and requires N removal as part of the wastewater treatment process. As a result of their ongoing effort, nitrogen pollution has been reduced by 64% and is reflected in macroalgae δ^{15}N values, which have been reported at the low end for sewage N (~3.8‰; Table 1), and the low abundance of drift algae and HABs in Sarasota Bay (Lapointe, unpublished data; SBEP, 2014).

The highly enriched δ^{15}N values we observed in IRL macroalgae likely reflect the widespread reliance on OSTDS on the IRL watersheds. Although direct sewage discharges (outfalls) into the IRL were largely eliminated by 1996, the intended phase-out of OSTDS in highly urbanized areas on the IRL watersheds never happened as mandated in the IRL Act of 1990. Consequently, urbanization of the IRL watersheds in recent decades has continued to rely heavily on OSTDS, which we estimate to conservatively account for at least twice the N-loading as that from municipal sewage treatment plants (Table 4). Unlike the OSTDS, the partially treated municipal wastewater is not discharged into the IRL, but rather into deep injection wells or re-used for purposes such as irrigation (Table 4). Modeling studies indicate that OSTDS account for >90% of the N in groundwaters on IRL watersheds, and that this N ultimately discharges into the IRL (Horsley and Witten, 2000; GeoHydros, 2014). Even if only 50% of the estimated OSTDS N loads to groundwaters (2,120,013 kg N yr^{-1}; Table 4) are currently reaching the IRL, this N load can more than account for the estimated total point and non-point source N loading to the IRL (893,053 kg N yr^{-1}; Sigua and Tweedale, 2004). The discrepancies between FDOH reports for installed (299,612; FDOH, 2013) vs. active (72,441; FDOH unpublished geographic information systems data as of June 2012; GeoHydros, 2014) OSTDS and recent studies (212,100; GeoHydros, 2014) also highlights the uncertainty about the number, location, and loadings from OSTDS in Florida. Recent modeling studies show that OSTDS rank as the second largest source of N contamination to Florida's surface waters, just behind agriculture (Badruzzaman et al., 2012).

Nitrogen in OSTDS effluent is primarily in the form of ammonium (Bicki et al., 1984; Lapointe et al., 1990; Valiela et al., 1997) with δ^{15}N values of ~+4–5‰ (Lapointe and Krupa, 1995; Hinkle et al., 2008; Katz et al., 2010). Ammonium containing the lighter ^{14}N isotope volatilizes preferentially, causing fractionation and enrichment of the remaining ammonium in the effluent

Table 4
Number of OSTDS and WWTPs in the five counties bordering the Indian River Lagoon. Specifically, the number of OSTDS and corresponding contributions of nitrogen to the IRL (GeoHydros, 2014) compared with number of WWTPs, flow rates, and calculated nitrogen contributions to obtain the percentage of OSTDS reliance for Volusia, Brevard, Indian River, St. Lucie, and Martin Counties (Englehardt et al., 2001; FDEP, 2014). Total % OSTDS reliance for all counties was calculated using an average of 2.45 people per OSTDS. Nitrogen input from WWTP disposal only includes facilities with a flow greater than 138,168 m^3/yr and was calculated using total nitrogen concentrations found in secondary effluent (Englehardt et al., 2001).

County	# OSTDS	TN (kg/yr)	# WWTP (>138,168 m^3/yr)	Flow (m^3/yr)	TN (kg/yr)	Class I (deep) wells	# WWTP (<138,168 m^3/yr)	Capacity (m^3/yr)*	% OSTDS reliance
Volusia	77,705	784,595	16	45,899,326	275,990	0	84	1,951,896	38
Brevard	66,720	662,742	20	53,277,484	369,709	7	37	1,211,178	30
Indian River	28,889	276,322	6	10,597,466	43,923	1	7	4,861,432	48
St. Lucie	16,837	180,959	13	20,711,346	238,878	9	16	1,590,449	16
Martin	21,949	215,395	8	10,238,230	86,672	5	17	434,952	36
Total	212,100	2,120,013	63	140,723,853	1,015,172	22	161	10,049,908	32

* Capacity of <138,168 m^3/yr WWTP should not be interpreted as actual flow.

(Böhlke et al., 2006). If there is an adequate oxic unsaturated zone (vadose zone), the remaining ammonium can be nitrified to nitrate by bacteria; however, this microbial transformation is limited on IRL watersheds by the low elevations and high water tables that are indicative of "failing" OSTDS, which results in ammonium enrichment of groundwaters (Lapointe et al., 1990; Bicki and Brown, 1990; Lapointe and Krupa, 1995a,b). Considering the importance of SGD as a pathway for OSTDS-derived N transport into the IRL (Lapointe and Krupa, 1995a,b; Martin et al., 2007), the widespread use of OSTDS in generally poor soil conditions (IRLNEP, 1996) helps explain our observed dominance of ammonium rather than nitrate throughout the IRL. Macroalgae would preferentially assimilate NH4 compared to NO3 (D'Elia and DeBoer, 1978), a phenomenon that results in δ^{15}N enrichment of these benthic mat-forming algae that are the "first responders" to SGD of wastewater N (Fig. 10). Nonetheless, δ^{15}N-NO3 values from OSTDS are commonly heavier than δ^{15}N-NH4 in septic discharges (Hinkle et al., 2008), which may reflect denitrification or reductive oxidation of ammonia and the subsequent loss of isotopically light N gas (Clark et al., 2008). However, denitrification is highly dependent on very specific environmental conditions (presence/absence of suitable aerobic/anaerobic zones in effluent flow path, adequate carbon source(s); Kendall et al., 2007), which do not generally prevail in the sandy, low organic content, porous soils and shallow groundwaters on the IRL watersheds (Bicki et al., 1984; Woodward-Clyde, 1994; Horsley and Witten, 2000).

Fertilizers represent another potential N (and P) source in urban and agricultural watersheds of the IRL. Recent nutrient management policies adopted by counties and municipalities along the IRL have focused on moderating these inputs in a variety of urban settings. However, past studies suggest that fertilizer nutrient contributions may not be as important as commonly thought. Watershed-scale studies in Waquoit Bay, Cape Cod, MA showed that fertilizer inputs from urbanized watersheds, relative to OSTDS, were negligible, because landscape vegetation was highly efficient at nutrient uptake (Valiela et al., 1992); similar conclusions were reached for N-loading studies in Narragansett Bay, RI (Thornber et al., 2008). These conclusions are supported by experimental turf studies that reported minimal environmental effects of fertilizer N leaching into groundwaters and surface waters (Petrovic, 1990; Erickson et al., 2001), and that healthy, properly fertilized turf grass helps to prevent soil erosion, especially during active growth periods, when nutrient losses are negligible (Hochmuth et al., 2011). The fact that our δ^{15}N values in IRL macroalgae so closely match δ^{15}N values in Narragansett Bay and Waquoit Bay where N-loading is dominated by wastewater strongly points to wastewater, not fertilizers, as the primary N source in the IRL. The opposite can be seen in the low δ^{15}N values (<3‰) of macroalgae in western Florida Bay that is downstream of agricultural watersheds where fertilizer and top soil N with relatively depleted δ^{15}N values dominate the N supply (Lapointe et al., 2004).

In contrast to fertilizer N applications, N derived from OSTDS in urban landscapes enters the groundwater well below the thatch and root zone and therefore have relatively little opportunity for assimilation or uptake by vegetation. The groundwater-borne N is then transported downgradient with minimal loss of N via denitrification in the sandy, porous soils on IRL watersheds (Bicki et al., 1984). Considering the substantial N-loading from OSTDS on the IRL watersheds (Table 4; GeoHydros, 2014), we conclude that the δ^{15}N enrichment in IRL macroalgae largely reflects cumulative OSTDS-derived N as it travels through the surficial groundwater and discharges into the IRL. This is a well-documented phenomenon in a wide variety of coastal settings (Lapointe et al., 1990; Valiela et al., 1992; Weiskel and Howes, 1992; Lapointe and Krupa, 1995a,b; Valiela et al., 1997; Reay, 2004; Mallin, 2013).

Highly enriched δ^{15}N values were also recently observed in the *Microcystis* blooms sampled in the SLE during the summer of 2013. Unprecedented blooms of the *Microcystis aeruginosa* in the SLE formed following massive releases of fresh water from Lake Okeechobee and the C-44 watershed in 2005 and again in 2013. Blooms of *M. aeruginosa* have been increasing in freshwater lakes and rivers worldwide as a result of increasing N and P inputs from urban, agricultural, and industrial sources (Paerl and Otten, 2013). According to Parrish (2014), *M. aeruginosa* experiences optimal growth and abundance when the N:P ratio is <44:1, as seen in the SLE during the 2005 bloom when N:P ratios decreased to ~9:1 (Lapointe et al., 2012). Once transported into the SLE with the freshwater discharges, blooms of *M. aeruginosa* are likely fueled by nutrient inputs from the surrounding watershed, especially high P inputs from the C-23 and C-24 canals (Lapointe et al., 2012). The enriched δ^{15}N value (+8.6‰) of the *M. aeruginosa* collected in August 2013 also points to wastewater N as a primary N source fueling the bloom. This highly urbanized estuary is locally impacted by thousands of OSTDS, many of which are "failing" and enrich tidal creeks and canals with high concentrations of ammonium, nitrate, and fecal bacteria (Lapointe et al., 2012). Interestingly, *M. aeruginosa* has a much higher affinity for ammonium than nitrate, further supporting the uptake of minimally-treated wastewater N in the SLE and the need for advanced wastewater treatment (N-removal) within the watershed (Parrish, 2014).

During the 2005 discharges from Lake Okeechobee to the Caloosahatchee River on Florida's west coast, similarly enriched δ^{15}N values of +11.5‰ were measured in *M. aeruginosa* in the Caloosahatchee estuary and + 7.83‰ in red tides – *Karenia brevis* – in coastal waters off Sanibel Island (Yentsch et al., 2008). Like the SLE, the Caloosahatchee River receives considerable sewage N inputs from OSTDS, but also municipal surface water sewage outfalls (Lapointe and Bedford, 2007). In comparison, the δ^{15}N value of ~+3.5‰ we measured in the superbloom in ML is at the low end of the wastewater N range, and could reflect minimal transformation/fractionation of sewage effluent as well as additional fertilizer and top soil N contributions that would have more depleted δ^{15}N values, such as those reported for macroalgal blooms in western Florida Bay (~+2‰; Lapointe et al., 2004).

4.4. Impacts of HABs on seagrasses and wildlife

With the high nitrogen concentrations and HABs during this study, also came the loss of seagrass coverage. This was reflected not only in our seagrass surveys, but also that of SJRWMD researchers. Between 2009 and 2012, over the course of the entire two-year study, seagrass coverage between Ponce and Ft. Pierce inlets decreased by about 60% with a loss of 47,000 acres (SJRWMD, 2013). Not only did overall seagrass coverage decrease within the IRL, but the northern extent of *Thalassia testudinum* also receded (SJRWMD, unpublished data). During the study period, *T. testudinum* was absent at the northern CIRL sites, including Sebastian Inlet, which was the previously known northern limit (Fig. 11; SJRWMD, unpublished data). In the ML, BR, and NIRL, where TDN values exceed 37 μM, there was no *T. testudinum* present. However, in the SIRL (Hobe Sound National Wildlife Refuge Nature Center to Jupiter Inlet), where *T. testudinum* abundance increases, the TDN values were well below this TN threshold (Fig. 11). The same pattern of decreased *T. testudinum* abundance with elevated TN concentrations was first reported for the Florida Keys, where only *Halodule wrightii* was present at transects in urbanized areas with dense OSTDS and eutrophic conditions (mean TN ~37 μM); in comparison, *T. testudinum* was the dominant species of seagrass at other mesotrophic and oligotrophic locations (Lapointe et al., 1994). Although the exact

TDN threshold where *T. testudinum* can grow may vary with location, higher TDN concentrations correlates with lower *T. testudium* and higher *H. wrightii* cover in a variety of Florida estuaries as well. In Florida Bay, for example, *H. wrightii* began to colonize and outcompete *T. testudinum* when TN levels increased (Fourqurean et al., 1995). This pattern is also illustrated in areas of Tampa Bay, where only sparse patches of *H. wrightii* are found in areas that receive high nitrogen loads (Hillsborough Bay) and *T. testudinum* dominates in areas with comparatively small nitrogen loads (Lower Tampa Bay; Coastal Resources Group Inc., 2009; Pribble et al., 2001; Yarbro and Carlson, 2013). Although there are many factors that can affect seagrass abundance and distribution, it appears that N levels can be a useful indicator of ecosystem health and species composition and that a threshold for sustained *T. testudinum* growth may exist. These dynamics between seagrass abundance, distribution and composition, and nutrient enrichment need to be better understood for proper management of seagrass habitats and the wildlife they support.

Following the unprecedented HABs and seagrass loss in 2011–2012, loss of food sources resulted in a trophic cascade and unusual mortality of wildlife in the northern IRL. Between 2012–2013, an unprecedented die-off of Atlantic bottlenose dolphins (*Tursiops truncatus*; ~100), West Indian manatees (*Trichechus manatus* Linnaeus; ~335), and brown pelicans (*Pelecanus occidentalis* Linnaeus; ~250) occurred in the NIRL and BR segments (Florida Fish and Wildlife Conservation Commission, unpublished data; Hubbs SeaWorld, unpublished data; Wines, 2013) prompting the National Oceanic and Atmospheric Administration (NOAA) to declare an Unusual Mortality Event in August 2013. IRL dolphins that stranded during this time were mal-nourished (NOAA, 2014). Petersen et al. (2013) links loss of seagrass to reduction in the number of pigfish (*Orthopristis chrysoptera* Linnaeus) and subsequently its predator the spotted seatrout (*Cynoscion nebulosus* Cuvier); the preferred food choice of the IRL dolphins. With the dramatic decline in seagrass cover manatees have had to supplement their diet with increased amounts of drift macroalgae, which continues to grow well in the shallow, hypereutrophic waters of the northern IRL segments. Ongoing research suggests that *Gracilaria tikvahiae* McLachlan, an abundant member of the drift algal community that is widely available as food to manatees, is producing N-containing cyanogenic glycosides (Peter Moeller et al., unpublished data) that are known to cause severe health problems, and even death, in humans (Seigler, 1991; Halstead and Haddock, 1992; Noguchi et al., 1994). In addition to the potential lethal effects of these macroalgal toxins, other more common HAB toxins, including brevetoxin, saxitoxin, and okadaic acid, are known to have sublethal effects on federally endangered manatees and green sea turtles in Florida (Capper et al., 2013). Because the eutrophication problem in the IRL appears to be driven largely by N from wastewater, there is a critical and urgent need for improved sewage collection and treatment – specifically involving Advanced Wastewater Treatment (AWT) and nutrient removal. Diversion of the N loading associated with sewage from the IRL would help restore the water quality conditions necessary for a healthy, seagrass-based ecosystem, a pre-requisite for sustaining populations of state and federally protected species.

4.5. Economic impacts to IRL

As of 2007, the estimated annual economic value of the IRL was approximately \$3.7 billion (Hazen and Sawyer, 2008). The primary recreational uses of the lagoon are fin fishing (38%), swimming or wading (20%), and power boating (13%). Both commercial and recreational fishing activities are tied to the health of IRL seagrass beds. Based on this relationship, Hazen and Sawyer (2008) estimated IRL seagrass habitat (~72,400 acres in 2005) to be worth approximately \$4,600 per acre per year or ~\$227,000 per acre over the next 100 years. With a 60% loss of seagrass in the northern segments of the IRL (SJRWMD, 2013), the estimated economic impacts of the superbloom in 2011 and brown tides in 2012 and 2013 were substantial (~\$197 M loss/year).

5. Conclusions

Although benthic macroalgal blooms have been common in seagrass habitats in the IRL for many decades (Benz et al., 1979), unprecedented phytoplankton blooms and seagrass die-off in the IRL during 2011–2012 indicates an ecosystem "tipping point" occurred during our study. The dominance of seagrass species (e.g., *Halodule wrightii*), with higher N tolerance (Fourqurean et al., 1995; Lapointe et al., 1994) combined with widespread macroalgal blooms and seagrass epiphyte loads in the NIRL is consistent with the classic model of nutrient enrichment and eutrophication in shallow, seagrass-dominated estuaries (Valiela et al., 1992; Bricker et al., 2007; Burkholder et al., 2007). The unprecedented phytoplankton "superbloom" we observed in 2011 followed a prolonged, 5-year drought and began following the onset of increased rainfall in spring 2011 (Fig. 2A). Such "first flush" stormwater events are well known to transport peak loads of nutrients from both urban stormwater runoff (Wanielista and Yousef, 1993) as well as OSTDS-contaminated groundwater discharges (Lapointe et al., 1990; Bicki and Brown, 1990). These rainfall-related increases in land-based nutrient inputs, especially P, help explain the high biomass phytoplankton blooms in the northern IRL segments in 2011–2012 where high TDN concentrations and TDN:TDP ratios resulted in strong P-limited conditions. The brown tide organism *Aureoumbra lagunensis* is well known to compete favorably with other phytoplankton under high DON concentrations (Gobler et al., 2013) and N:P ratios (Liu et al., 2001), an environmental condition that occurred in the ML and NIRL during our study.

Other researchers have suggested that hypersalinity in the ML as a result of the prolonged drought may have also contributed to the brown tide (Gobler et al., 2013). While hypersalinity (>40 psu) did occur in the Laguna Madre, TX during formation of the *Aureoumbra lagunensis* bloom, this organism has a broad tolerance to salinity and can grown between 10 and 90 psu (Buskey et al., 1998). The ML has had a long history of hypersaline conditions (>40) without the occurrence of brown tides, and hypersalinity has long been recognized as indicative of poor flushing and long residence times that are also conducive to bloom development (Briel et al., 1973; Buskey et al., 1998). Because freshwater inputs to the IRL are also the major source of N and P driving eutrophication, the issue of hypersalinity raises a note of caution to water managers considering a "quick fix." For example, similar phytoplankton blooms and seagrass die-off occurred in Florida Bay in the 1980s following diversion of N-rich agricultural runoff from Lake Okeechobee south to Everglades National Park and Florida Bay, which resulted in high N concentrations and N:P ratios similar to that in the northern IRL segments (Brand, 2002; Lapointe and Barile, 2004). Following a short drought in 1989–1990, Florida Bay researchers and water managers suggested that the phytoplankton blooms resulted from hypersalinity (baywide average ~41), which they claimed caused die-off of *T. testudinum*, subsequent internal nutrient enrichment and development of phytoplankton blooms. Water managers responded by further increasing flows of N-rich waters to both Shark River and Taylor Slough between 1991 and 1995, exacerbating nitrogen enrichment and phytoplankton blooms including red tide, *Karenia brevis*, causing more seagrass die-off, sponge die-off, regional-scale eutrophication, and unprecedented coral diseases and die-off in the Florida Keys National Marine Sanctuary (Lapointe and Barile, 2004). The decision to increase freshwater flows into Florida Bay was a political decision, as no scientific evidence occurred at that time, or presently, that the hypersalinity in Florida Bay was either a primary or secondary factor in the die-off of *T. testudinum*. Hence, adding more freshwater to the ML and NIRL could similarly cause a worsening of the eutrophication problem in the IRL, as was the case in Florida Bay.

The common thread between eutrophication of the IRL and Florida Bay is the role of excess nitrogen in driving HABs and the loss of seagrasses and biodiversity, the latter being the biggest problem facing our planet (NRC, 2000; Rockström et al., 2009). In the case of the urbanized IRL, our results indicate that wastewater, and OSTDS in particular, is the major N source, compared to agricultural fertilizers and top soil N in Florida Bay (Brand, 2002; Glibert et al., 2004; Lapointe et al., 2004). These findings are consistent with recent modeling efforts showing that sewage is the primary source of N pollution in urban areas, compared to fertilizers in agricultural areas (McCrackin et al., 2013). Despite this knowledge, FDEP has emphasized WWTPs and stormwater in multiple TMDL and Basin Management Action Plans (BMAP) recently drafted for the IRL and several of its tributaries. Considering that point-source sewage inputs to the IRL were largely eliminated by the mid-1990s and that N loads from OSTDS are substantial (Table 4), we conclude that greater emphasis should instead be placed on quantifying non-point source nutrient pollution from the ~300,000 OSTDS in the IRL watershed; a major and growing N source to the system. This is particularly evident in the NIRL, ML, and BR where high TDN concentrations and TDN:TDP ratios reflect cumulative buildup and cycling of nitrogen-rich groundwater inputs. The high estimated N loadings from OSTDS suggest that previous nutrient loading models for the IRL have greatly underestimated the contribution of OSTDS as an N source to the IRL (GeoHydros, 2014). Thus, N loading from OSTDS and inadequate sewage treatment facilities should be at the forefront in planning documents, such as BMAPs, aimed at nutrient reduction to the IRL and its tributaries. Currently, any gains from nutrient-reducing BMAP projects are likely offset by the installation of additional OSTDS in newly developed areas.

Acknowledgements

The authors thank Ian MacLeod, Jeff Beal (Florida Fish and Wildlife Conservation Commission), Karen Holloway-Adkins and Doug Scheidt (Innovative Health Applications – Kennedy Space Center), and Gabrielle Barbarite (HBOI) for field and logistical support, James Nelson (HBOI) for boat support, and James Lyon and Mike Legare (U.S. Fish and Wildlife Service – Merritt Island National Wildlife Refuge) for permitting support. Lori Morris, Lauren Hall, Joel Steward, and Bob Chamberlain (St. Johns River Water Management District) provided information on seagrasses and photographs of macroalgae in the IRL. We also acknowledge Mike Bechtold (Florida Department of Environmental Protection) for providing access to wastewater data for the IRL. The study was funded through the Save Our Seas specialty license plate fund administered through the Harbor Branch Oceanographic Institute (HBOI) Foundation and supported through the work of Coastal Ocean Association of Science and Technology (COAST). This is contribution #1942 of the Harbor Branch Oceanographic Institute at Florida Atlantic University. [SS]

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