



# Nutrient over-enrichment and light limitation of seagrass communities in the Indian River Lagoon, an urbanized subtropical estuary

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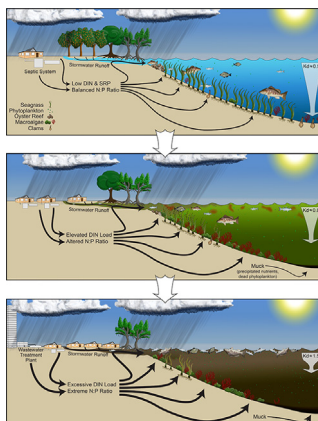
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## HIGHLIGHTS

- Nutrient enrichment drives seagrass loss through light limitation.
- $K_d$  measurements showed the importance of light limitation from algal blooms.
- Light limitation is most severe in the poorly-flushed and highly urbanized northern segments.
- Human wastewater is the primary N source fueling eutrophication.
- The Indian River Lagoon needs a master wastewater plan.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Historically, extensive seagrass meadows were common throughout the Indian River Lagoon (IRL) in east-central Florida, USA. Between 2011 and 2017, widespread catastrophic seagrass losses (~95%) occurred in the IRL following unprecedented harmful algal blooms (HABs), including persistent brown tides (*Aureoumbra lagunensis*). Little is known about how dissolved nutrients and chlorophyll *a* are related to light limitation or how biochemical factors, such as the elemental composition (C:N:P) and stable isotope signatures ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ), of seagrasses within the IRL relate to coverage. Accordingly, we conducted a survey from 2013 to 2015 at 20 sites to better understand these relationships. Results showed a negative correlation between DIN and salinity, indicating freshwater inputs as a DIN source. Seawater N:P ratios and chlorophyll *a* concentrations were higher in the urbanized, poorly-flushed northern IRL segments.  $K_d$  values were higher in the wet season and often exceeded seagrass light requirements ( $0.8 \text{ m}^{-1}$ ) for restoration, demonstrating light limitation. Species distribution varied by location. *Halodule wrightii* was ubiquitous, whereas *Syringodium filiforme* was not found in the northernmost segments. *Thalassia testudinum* was only present in the two southernmost segments that had the lowest TDN and highest light availability ( $K_d$ ). Blade %N and %P also frequently exceeded critical values of 1.8% and 0.2%, respectively, especially in the northern segments. Further,  $\delta^{15}\text{N}$  was positively correlated with ammonium, suggesting wastewater as a major N source. The  $\delta^{13}\text{C}$  values indicated a trend of increasing light limitation from south to north, which helps explain the recent catastrophic loss of seagrasses in the northern IRL. Overall, elemental composition reflected high N-availability and seagrass species distributions were relatable to spatial trends in N and light limitation. For effective restoration, resource managers must reduce N-loading to the IRL to diminish HABs and increase light availability. Regular biochemical monitoring of seagrass tissue should also be implemented during restoration efforts.

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## 1. Introduction

Seagrasses are submerged marine angiosperms commonly found in shallow tropical, sub-tropical, and temperate coastal waters worldwide (Den Hartog, 1970). The ecological importance of healthy seagrass communities includes primary production, nutrient cycling, and sediment stabilization. Seagrasses also provide habitat and nutrition for invertebrates, fishes, and birds, as well as federally protected sea turtles and manatees (Stoner, 1980, 1983; Dawes, 1981; Capper et al., 2013). Expanding human activities on watersheds of coastal ecosystems have resulted in the degradation of water quality and large-scale losses of seagrasses are being reported globally (Orth et al., 2006; Waycott et al., 2009). Phenomena contributing to these unprecedented seagrass losses include increases in nutrient pollution, sediment runoff, harmful algal blooms (HABs), physical disturbance, and climate change.

In east-central Florida, USA, much of the historical seagrass coverage has been lost in the Indian River Lagoon system (IRL). The IRL is a shallow (mean depth 0.8 m), well-mixed, bar-built estuary extending 251 km from Jupiter Inlet to Ponce Inlet (Steward and VanArman, 1987; Fig. 1), and includes the Mosquito Lagoon and Banana River in the northern region. This estuary comprises a transition zone between temperate and tropical biomes, and therefore is considered a regional-scale ecotone and one of the most species diverse estuaries in North America (Swain et al., 1995). All six tropical seagrass species known from the western hemisphere (*Halophila decipiens*, *Halophila engelmannii*, *Halodule wrightii*, *Ruppia maritima*, *Syringodium filiforme*, and *Thalassia testudinum*), as well as the endogenous, federally threatened *Halophila johnsonii*, are found in the IRL and help support the high biodiversity and fisheries found within (Dawes et al., 1995). Long residence times and limited flushing of the system (Briel et al., 1973; Smith, 1993; Philips et al., 2015), combined with the sensitivity of seagrasses to nutrient enrichment, eutrophication, and light limitation (Lapointe et al., 1994; Steward et al., 2005; Tewfik et al., 2005; Burkholder et al., 2007), has made water quality a key concern for scientists and managers attempting to protect the biodiversity and marine resources of the IRL.

Growing concern about the health of the IRL seagrass communities has been spurred by the dramatic expansion and changes in land-use of adjacent watersheds 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) 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 ha to >566,560 ha (SJRWMD, 2007). These changes greatly altered the hydrology of the IRL and also increased stormwater inflow. Further, the population in the IRL watershed has increased from ~250,000 in 1960 to ~2 million today (US Census Bureau, 2014). Consequently, land-use changes in the IRL watershed have been dramatic with an overall trend of decreasing agricultural coverage and increasing coverage of residential and urban areas (Bricker et al., 2008; Duncan et al., 2004).

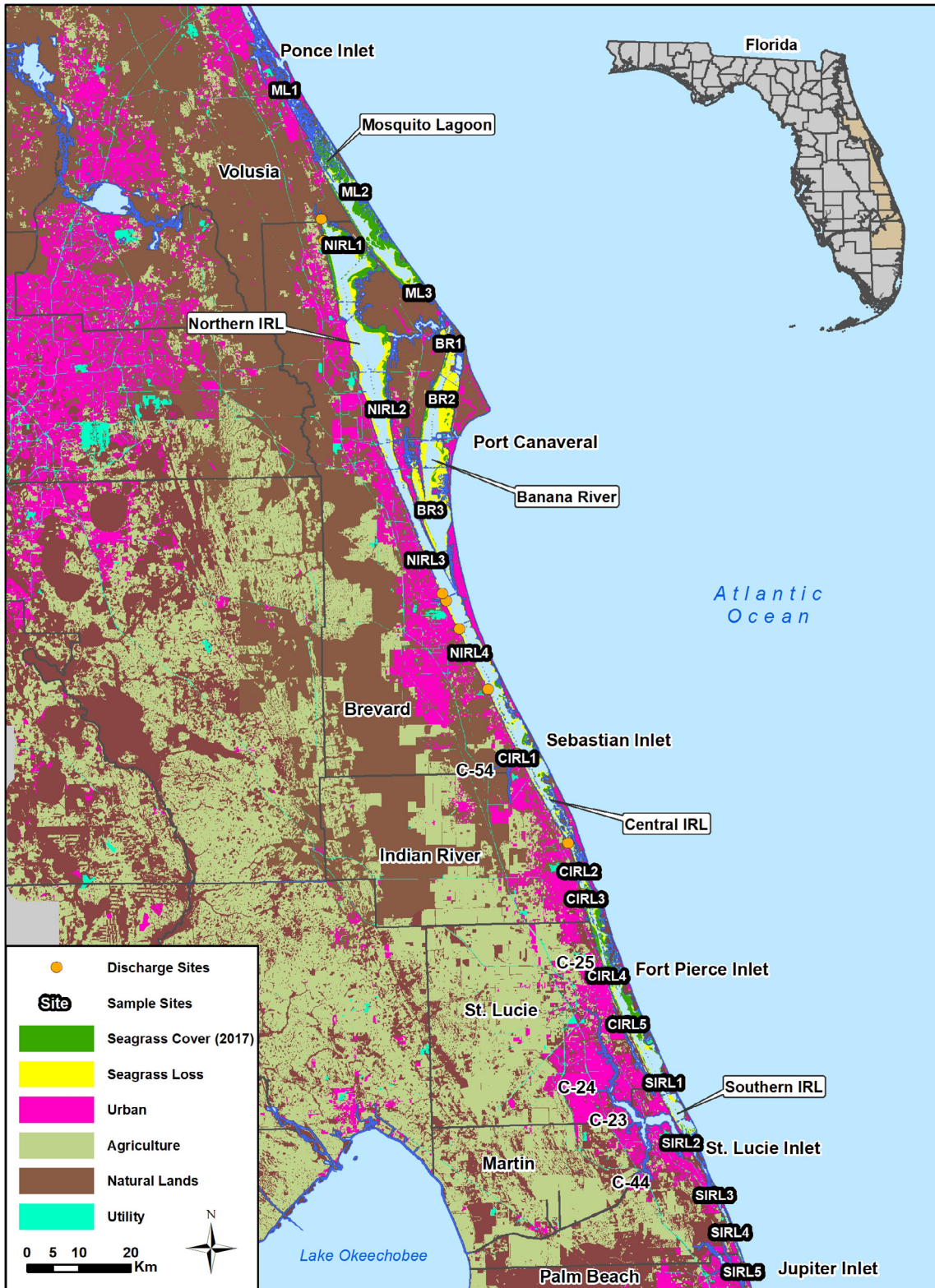
Eutrophication of the IRL resulting from widespread urbanization and population growth has long been a concern of scientists and resource managers. In the mid-1970s, Gibson et al. (1975) predicted that the poor sewage infrastructure along the IRL would lead to a phytoplankton dominated system in ten to twenty years. These 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 01 July 1994. The IRL

Act also required municipalities to identify areas where package sewage treatment plants and septic systems (on-site sewage treatment and disposal systems) posed threats to the IRL and implementation plans to provide centralized sewage treatment to these areas by 01 July 1995. By 1996, point source sewage discharges (outfalls) into surface waters of the IRL were largely eliminated (IRLNEP, 2008). Unfortunately, nonpoint source sewage pollution from septic systems was not adequately addressed and has become a significant environmental and human health concern throughout the IRL (Lapointe et al., 2015, 2017; Barile, 2018). Further, worsening eutrophication of the IRL has led to persistent HABs involving both macroalgae (Lapointe et al., 2015, 2018) and phytoplankton (Philips et al., 2010, 2012; Gobler et al., 2013; Philips et al., 2015).

Seagrass protection in the IRL has been a mutual goal of both state (Steward et al., 2003) and federal (IRLNEP, 1996) programs since 1990 (IRL Act; Chapter 90-262, Laws of Florida). Seagrass health in the system has been assessed by monitoring temporal and spatial trends in hectares of coverage. In the mid-1990s, Fletcher and Fletcher (1995) reported a 50% decrease in maximum depth of seagrasses relative to 1943 depths. This loss included a 16% reduction between 1986 and 1992, most of which occurred in urbanized areas north of Vero Beach, FL (Fletcher and Fletcher, 1995). In 1999, a survey of seagrass coverage showed a high loss of acreage, up to 60%, in a 56-mile stretch from Cocoa Beach to Palm Bay (Steward et al., 2003). Despite these documented losses, there were still 26,101 ha of seagrass reported between Vero Beach and Ponce Inlet in 2005 (Virmstein et al., 2007), with a subsequent peak in coverage reported following several drought years in 2009 (Morris et al., 2018).

Unfortunately, high phytoplankton biomass in the IRL has recently decreased light penetration for long enough intervals to become detrimental to seagrass communities (Lapointe et al., 2015; Philips et al., 2015). For example, in 2011 chlorophyll *a* concentrations in northern segments of the IRL spiked from <10 µg/l to >100 µg/l during an unprecedented phytoplankton “superbloom” that was primarily *Resultor* sp. (Gobler et al., 2013; Lapointe et al., 2015; Philips et al., 2015). The “superbloom” was followed in 2012 by an unprecedented brown tide (*Aureoumbra lagunensis*; Gobler et al., 2013; Lapointe et al., 2015; Philips et al., 2015) that caused further light reduction. These HABs resulted in catastrophic losses of seagrasses in mapped areas (60%; 47,000 acres) spanning from Ponce Inlet to Ft. Pierce Inlet (SJRWMD, 2013; Lapointe et al., 2015). After the re-emergence of the brown tide in 2016, additional seagrass losses were reported in long-term monitoring transects (up to 95% since 2009 levels) between Ponce Inlet and Ft. Pierce Inlet (see Fig. 1; Morris et al., 2018). Similarly, in the Laguna Madre, Texas, USA, light limitation by brown tide initially decreased seagrass (*H. wrightii*) biomass and ultimately affected its distribution (Onuf, 1996). The subsequent seagrass losses following these HABs can further contribute to greater light reduction through a positive feedback loop wherein resuspension of sediments increases due to the loss of stabilization previously provided by the seagrass (de Boer, 2007). Accordingly, most seagrass loss in the IRL has historically been attributed to light limitation (Steward et al., 2003, 2005), which is especially critical at the deep edge of seagrass beds (Dennison, 1987; Duarte, 1991).

Photosynthetically Active Radiation (PAR) represents solar wavelengths from 400 to 700 nm and light limitation can be estimated by measuring the decrease in these photons as they downwell through the water column, as represented by the diffuse attenuation coefficient ( $K_d$  PAR; Brito et al., 2013). In Chesapeake Bay, MD, USA, Dennison et al. (1993) used the distribution of seagrasses to define their light requirements and concluded that chlorophyll *a* concentrations <15µg/l and median  $K_d$  PAR



**Fig. 1.** Indian River Lagoon (IRL), Florida, USA showing associated counties, land-use, tidal inlets, and major freshwater discharge sites (orange dots), as well as seagrass cover (dark green shading) and loss (as of 2017, yellow shading) with the 20 sampling sites labeled by segment, including Mosquito Lagoon (ML 1 - 3), Banana River (BR 1 - 3), the Northern IRL (NIRL 1 - 4), Central IRL (CIRL 1 - 5), and Southern IRL (SIRL 1 - 5). Note: major freshwater discharge sites are also located at NIRL4, CIRL1, CIRL2, CIRL3, CIRL4, SIRL2, and SIRL5. Seagrass cover and land-use data for Volusia, Brevard, and Indian River counties from St. Johns Water Management District Geospatial Open Data (<http://data-floridaswater.opendata.arcgis.com>) and land-use data for St. Lucie, Martin, and Palm Beach counties from South Florida Water Management District Open Data Portal (<https://geo-sfwmd.opendata.arcgis.com/>). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)



values  $< 1.5\text{m}^{-1}$  were required to sustain seagrass beds to 1.0 m depth or greater. They also estimated that recovery of seagrass beds to 2.0 m depths would require  $K_d$  values  $< 0.8\text{m}^{-1}$ . Thus, an understanding of relative light attenuation is critical for understanding patterns in seagrass coverage and loss in the IRL.

Despite the concerns of scientists regarding the threat of sewage pollution, eutrophication, HABs, and seagrass loss in the IRL, no direct assessments of seagrass status using elemental composition (carbon, nitrogen, phosphorus; C:N:P) or stable C ( $\delta^{13}\text{C}$ ) and N ( $\delta^{15}\text{N}$ ) isotopes were made during the past three decades. In a review of global seagrass data, Duarte (1990) was the first to use elemental composition of seagrass tissue to quantify critical levels of N and P, indicating oligotrophic vs. nutrient-enriched or eutrophic conditions. The median values of 1.8% N and 0.2% P discriminated between seagrass beds that responded to nutrient enrichment (oligotrophic) and those that did not (nutrient saturated, eutrophic; Duarte, 1990). Moreover, spatial analyses of elemental composition combined with stable C and N isotopes can provide insight into the contribution of nutrient sources to seagrasses (Johnson et al., 2006). Stable C isotopes can also provide information on environmental conditions related to seagrass health. For example,  $\delta^{13}\text{C}$  values have been used to understand light limitation in seagrasses because the tissue becomes more enriched as irradiance increases, due to photosynthesis being carbon-limited for a larger portion of the day, as opposed to light-limited (Durako and Hall, 1992; Hemminga and Mateo, 1996; Hu et al., 2012). Finally, the  $\delta^{15}\text{N}$  value of seagrasses can serve as an indicator of N sources contributing to eutrophication (McClelland and Valiela, 1998; Fourqurean et al., 2015). Therefore, monitoring the elemental composition and stable C and N isotopes of seagrasses can provide insight into their health and distribution.

Due to an active IRL-wide water quality monitoring program that was ongoing in 2013 (Lapointe et al., 2015), an opportunity existed to focus on the health of the remaining seagrasses to address the interrelated issues of nutrient enrichment and light limitation during the period of widespread seagrass die-off. Seagrasses require nutrients for growth and can initially respond positively to nutrient enrichment (Williams, 1987; Short et al., 1993; Cabaço et al., 2013). Excessive nutrient loading, however, can lead to advanced symptoms of eutrophication, including blooms of macroalgae, epiphytes, and phytoplankton, which ultimately cause light limitation, hypoxia/anoxia, and seagrass die-off (Kemp et al., 1983; Williams, 1987; Tomasko and Lapointe, 1991; Lapointe et al., 1994; Duarte, 1995; Valiela et al., 1997; Lapointe et al., 2004; Burkholder et al., 2007). To assess the status of nutrient enrichment and light limitation of seagrass communities during a die-off period, we performed a multi-year study (2013–2015) that included the following: 1) dissolved nutrient and chlorophyll *a* analyses to gauge the level of nutrient enrichment and phytoplankton biomass, 2) measurement of water column optical properties ( $K_d$  PAR) to assess the potential for light limitation, and 3) seagrass tissue analyses for C:N:P to determine the type and degree of nutrient limitation (N vs. P), as well as stable C and N isotope signatures to identify the degree of light limitation and potential sources of N-enrichment. These quantitative measurements were compared to values reported for seagrass communities in other oligotrophic and eutrophic systems to gauge the overall degree of nutrient enrichment and light limitation among different segments of the IRL.

## 2. Materials and methods

Twenty long-term monitoring sites spanning the IRL from Ponce to Jupiter inlets, reported previously by Lapointe et al. (2015), were used in this study (Fig. 1). The lagoon was categorized

into five segments: Mosquito Lagoon (ML), Banana River (BR), the Northern IRL (NIRL), Central IRL (CIRL), and Southern IRL (SIRL). The number of sites varied by segment: ML ( $n = 3$ ), BR ( $n = 3$ ), NIRL ( $n = 4$ ), CIRL ( $n = 5$ ), and the SIRL ( $n = 5$ ; Fig. 1).

### 2.1. Environmental parameters and light attenuation

Sampling was conducted seasonally to capture effects of increased rainfall. Therefore timing of seasonal sampling events (wet or dry season) was determined by precipitation. Rainfall data for the duration of the project (01 January 2013–31 December 2015) was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (<https://www.ncdc.noaa.gov/>).

A combination of calibrated YSI™ Models 63, 85, 1030, and ProODO handheld meters were used to determine salinity, conductivity, temperature, and dissolved oxygen at the time and depth (0.25 m below surface) that water samples were collected at each site. The 3-point calibrated YSI 1030 was also used to measure pH during the 2015 sampling events.

At each sampling site, three sensors were used to determine the amount of light ( $K_d$ ) reaching the seagrass beds; two LiCOR® Underwater Quantum Sensors (LI-192) and one LiCOR® Quantum Sensor (LI-190). The LI-190 Quantum Sensor was placed on the surface of the boat, while one of the LI-192 sensors was placed approximately two cm beneath the surface of the water and the other at the top of the seagrass canopy. Measurements were recorded with an LI-1400 Datalogger every second and reported as a mean every 15 s for at least three minutes. From these data the diffuse attenuation coefficient ( $K_d$  PAR,  $\text{m}^{-1}$ ) was then calculated for each 15-s interval using the following formula,

$$K_d = \frac{(\ln E_{z_1} - \ln E_{z_2})}{(z_2 - z_1)}$$

where  $E_{z_1}$  = water surface irradiance (two cm depth),  $E_{z_2}$  = irradiance at the top of the canopy,  $z_2$  = depth to top of the canopy, and  $z_1$  = depth of sensor beneath water surface (0.02 m). All  $K_d$  values were then averaged by site. There are no  $K_d$  values available for ML in the wet season due to equipment failure.

### 2.2. Dissolved nutrient and chlorophyll *a* concentrations

Water samples were collected for analysis of dissolved nutrient concentrations in three seasons: the 2013 wet season, the 2015 dry season, and the 2015 wet season. Sampling sites adjacent to canals, tributaries, and inlets (e.g., NIRL4, CIRL1, CIRL2, CIRL3, CIRL4, SIRL2, and SIRL5 in Fig. 1) were sampled during an ebbing tide to account for tidal pumping of groundwater (Lapointe et al., 1990). At all sites, seawater samples were collected in triplicate 0.25 m below the surface into acid-washed 500 ml high-density polyethylene (HDPE) bottles and covered with ice in a dark cooler until returned to the Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) laboratory for processing. The samples were filtered through 0.7- $\mu\text{m}$  GF/F filters and frozen until analysis. Filters were retained and frozen for estimation of chlorophyll *a* concentrations. Filtered water samples were analyzed to determine dissolved nutrient concentrations by the Nutrient Analytical Services at Chesapeake Biological Laboratory (NAS-CBL). Detection limits were 0.21  $\mu\text{M}$  for ammonium, 0.01  $\mu\text{M}$  for nitrate + nitrite (combined as “nitrate” hereafter), 0.02  $\mu\text{M}$  for soluble reactive phosphorus (SRP), 2.06  $\mu\text{M}$  for total dissolved N (TDN), and 0.05  $\mu\text{M}$  for total dissolved P (TDP). At NAS-CBL, nitrate, TDN, SRP, and TDP were analyzed using a Technicon Auto-Analyzer II, while ammonium and nitrite were analyzed with a Technicon

TRAACS 800. At NAS-CBL, the filtered chlorophyll *a* samples were placed in a 15 ml centrifuge tube with 10 ml of 90% acetone, ground with a pestle, and allowed to extract in the dark under refrigeration. After extraction, the samples were removed from refrigeration, warmed to room temperature, and centrifuged. The samples were then measured fluorometrically for chlorophyll *a* and phaeopigment concentrations before and after acidification with 5% HCl. 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. The resulting data were used to characterize ambient dissolved inorganic nitrogen (DIN = ammonium + nitrate) and chlorophyll *a* concentrations, as well as DIN:SRP and TDN:TDP ratios at the 20 IRL sites. As a point of reference, TDN, TDP, and chlorophyll *a* were compared to the Florida Department of Environmental Protection Numeric Nutrient Criteria for the Indian River Lagoon from the St. Lucie Estuary to the southern border of Indian River County (Florida Administrative Code 62-302.532). While not applicable to the entire IRL, these criteria were chosen as they represented the largest segment of our study area with numerically defined target concentrations.

### 2.3. Seagrass tissue elemental composition, molar ratios, and stable isotopes

Seagrass tissue samples were collected during the 2013 wet season and the 2015 dry season in water depths ranging from 1.0 to 1.5 m to characterize elemental composition (%C, %N, and %P), molar ratios (C:N:P), and stable carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) isotopes. Triplicate samples of three seagrass species, *Halodule wrightii*, *Syringodium filiforme*, and/or *Thalassia testudinum*, were collected when present at sites by cutting at the base of the blade with scissors. Samples were cleaned of epiphytes and debris, rinsed briefly (<5 s) in deionized water, and dried at 60 °C for 48 h in a Fisher Isotemp® laboratory oven. The dried seagrasses were ground to fine powder using a Thompson Scientific Wiley Mini-Mill® and stored in plastic screw top vials. Samples were shipped to University of Georgia's Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL) for analysis. At UGA-SIEL, samples were split. One half was analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , as well as %C and %N on a Thermo Delta V IRMS coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo ConFlo III Interface (see <http://sisbl.uga.edu/ratio.html#top>; Thermo Scientific, 2007). National Institute of Standards and Technology reference materials 8549, 8558, 8568, and 8569 were used to routinely calibrate working standards prepared in the laboratory. QA/QC results were incorporated into the raw data reports received by UGA-SIEL. The other half of each sample was analyzed for %P, where approximately 2 mg of dried tissue was weighed into crucibles, ashed at 500 °C for four hours, and extracted with 0.2 mL of Aqua Regia acid (Allen et al., 1974; Jones Jr. et al., 1990; UGA-SIEL, 2015). The acid extracts were then diluted 41:1 with deionized water for TP (as  $\text{PO}_4\text{-P}$ ) analysis on an Alpkem 300 series analyzer. The resulting C:N:P data of the seagrasses were used to make inferences regarding nutrient availability (Atkinson and Smith, 1983; Duarte, 1990; Lapointe et al., 1994, 2015; Yang et al., 2018).

### 2.4. Statistical analyses

Data were analyzed in SPSS25 for Mac. Shapiro-Wilk normality test and Levene's test of equality of error variances were used to assess data conformation to parametric assumptions. As parametric assumptions were not met, the non-parametric Kruskal-Wallis (3 or more groups) and Mann-Whitney U (2 groups) tests were used on non-transformed data. Variation in environmental parameters and dissolved nutrient concentrations were com-

pared between IRL segment (ML, BR, NIRL, CIRL, and SIRL) and sampling event (wet season 2013, dry season 2015, and wet season 2015). Seagrass elemental composition, molar ratios, and stable carbon and nitrogen isotopes were compared by species, due to expected interspecific differences (Duarte, 1990; Fourqurean et al., 2015). Then, tissue percent elemental composition, nutrient ratios, and stable isotope values were compared between IRL segments and seasons (wet season 2013 and dry season 2015). For all analyses, differences were considered statistically significant at  $p \leq 0.05$ .

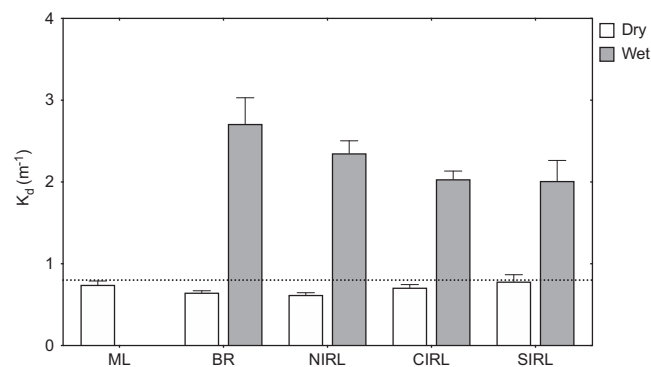
## 3. Results

### 3.1. Environmental parameters and light attenuation

Rainfall varied over the study period and generally similar patterns were observed for all counties along the IRL (Supplemental Fig. 1). The 2013 wet season sampling was conducted in September–October, following heavy rainfall. The 2015 dry season sampling took place in April–May, when precipitation had been low for multiple months, and the 2015 wet season sampling occurred in September–October, following heavy summer rainfall.

Environmental parameters of surface waters varied both spatially and seasonally (Supplemental Table 1). IRL-wide mean salinity was  $26.5 \pm 0.9$  psu. Mean salinity varied by lagoon segment ( $H = 35.3$ ,  $df = 4$ ,  $p < 0.01$ ). For example, mean salinity within the CIRL ( $20.9 \pm 1.8$  psu) was statistically similar to the NIRL ( $24.5 \pm 2.0$  psu) and SIRL ( $25.3 \pm 1.6$  psu), but significantly lower than ML ( $36.0 \pm 1.2$  psu) and BR ( $29.1 \pm 1.3$  psu). Salinity in ML was significantly higher than all other segments. The segment with the greatest range in salinity was the CIRL, which ranged from brackish at CIRL4 (Taylor Creek) during the 2013 wet season (5.7 psu) to marine (31.1 psu) at the same site during the 2015 dry season.

$K_d$  was measured in the 2015 wet and dry seasons and values are presented in Fig. 2. The IRL-wide mean  $K_d$  was  $1.32 \pm 0.1 \text{ m}^{-1}$ .  $K_d$  varied by season ( $H = 196$ ,  $n = 1$ ,  $p < 0.01$ ), with the wet season having a wider spatial range and higher mean ( $2.29 \pm 0.1 \text{ m}^{-1}$ ) than the dry season ( $0.71 \pm 0.1 \text{ m}^{-1}$ ). All segments approached the  $K_d$  threshold for minimum seagrass light requirements ( $0.8 \text{ m}^{-1}$ , Dennison et al., 1993) during the dry season, but exceeded this threshold in the wet season (Fig. 2).  $K_d$  did not vary spatially in the dry season ( $H = 3.93$ ,  $df = 4$ ,  $p = 0.42$ ) or the wet season ( $H = 1.34$ ,  $df = 3$ ,  $p = 0.72$ ).



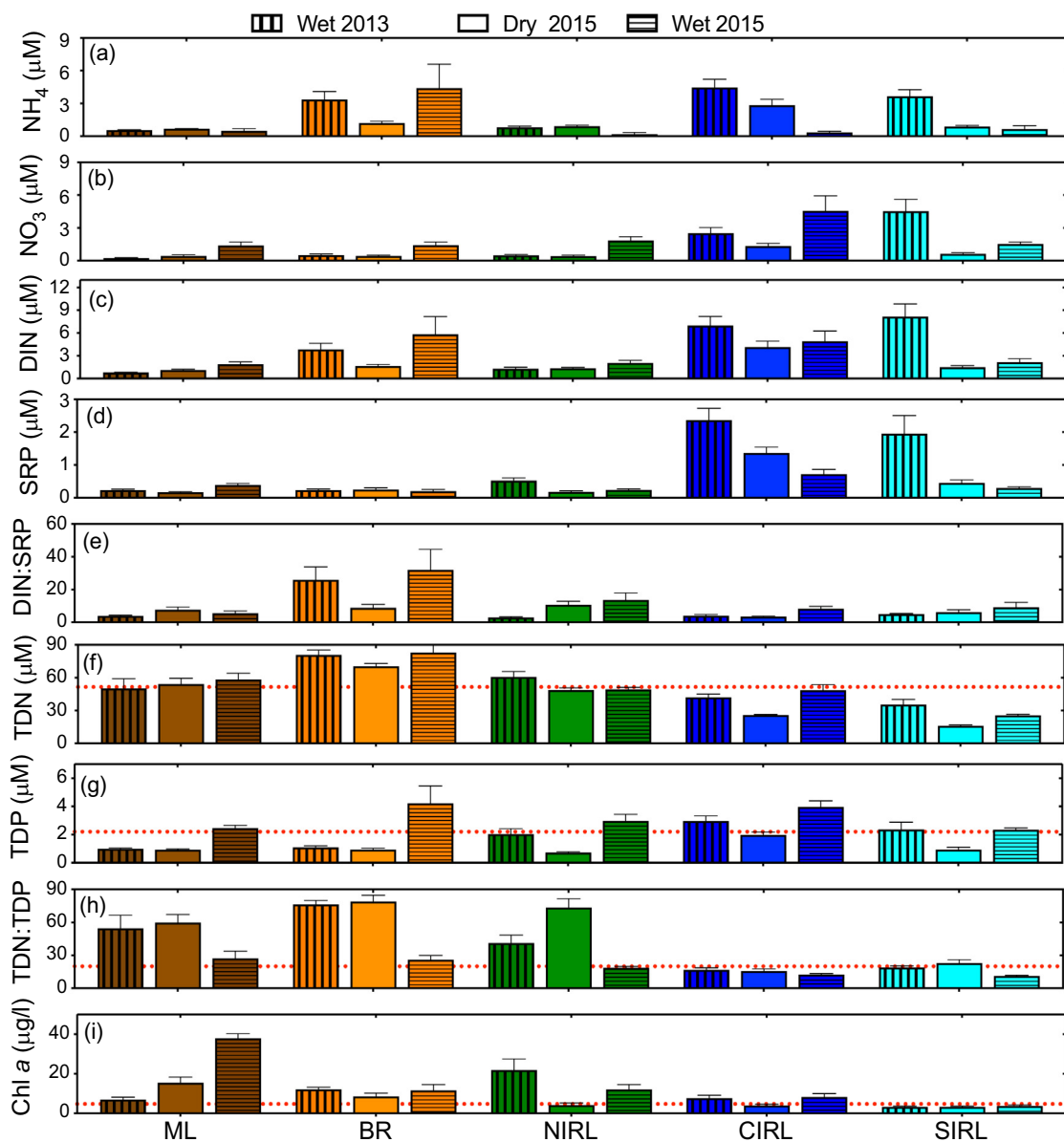
**Fig. 2.**  $K_d$  values (mean  $\pm$  SE  $\text{m}^{-1}$ ) by season (wet and dry seasons 2015) for segments of the Indian River Lagoon, Florida, USA, including Mosquito Lagoon (ML), Banana River (BR), the northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL); the dashed line indicates the maximum  $K_d$  threshold ( $0.8 \text{ m}^{-1}$ ) required for seagrass survival in restoration programs (Dennison et al., 1993).

### 3.2. Dissolved nutrient and chlorophyll a concentrations

The IRL-wide mean ammonium concentration was  $1.72 \pm 0.2 \mu\text{M}$  (Supplemental Table 2). Ammonium varied spatially ( $H = 15.4$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonally ( $H = 53.3$ ,  $df = 2$ ,  $p < 0.01$ ; Fig. 3a). IRL segment means were especially elevated for ammonium within BR ( $3.00 \pm 0.8 \mu\text{M}$ ), the CIRL ( $2.55 \pm 0.4 \mu\text{M}$ ), and SIRL ( $1.73 \pm 0.3 \mu\text{M}$ ; Fig. 3a). In particular, high ammonium was measured at sites BR2–3 (middle to southern BR), CIRL2–4 (Vero Main Relief Canal, a bird rookery spoil island adjacent to Vero South Relief Canal, and Taylor Creek, respectively), and SIRL1–2 (Nettles Island and St. Lucie River, respectively). By sampling event, ammonium was significantly higher in the wet season 2013 ( $2.68 \pm 0.3 \mu\text{M}$ ) and the dry season 2015 ( $1.40 \pm 0.2 \mu\text{M}$ ) than in the wet season 2015 ( $1.02 \pm 0.2 \mu\text{M}$ ; Fig. 3a).

The overall mean nitrate concentration in the IRL was  $1.63 \pm 0.2 \mu\text{M}$  (Supplemental Table 2). Nitrate varied spatially ( $H = 32.1$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonally ( $H = 28.0$ ,  $df = 2$ ,  $p < 0.01$ ; Fig. 3b). By lagoon segment, nitrate was significantly lower in ML ( $0.64 \pm 0.1 \mu\text{M}$ ) and BR ( $0.78 \pm 0.1 \mu\text{M}$ ) than the CIRL ( $2.80 \pm 0.5 \mu\text{M}$ ) and SIRL ( $2.23 \pm 0.4 \mu\text{M}$ ). Seasonally, nitrate was significantly higher in the wet season 2015 ( $2.31 \pm 0.4 \mu\text{M}$ ) than in the wet season 2013 ( $1.88 \pm 0.4 \mu\text{M}$ ) or the dry season 2015 ( $0.70 \pm 0.1 \mu\text{M}$ ; Fig. 3b).

The IRL-wide mean DIN concentration was  $3.35 \pm 0.3 \mu\text{M}$  (Supplemental Table 2). DIN varied spatially ( $H = 29.6$ ,  $df = 4$ ,  $p < 0.01$ ), but not seasonally ( $H = 2.21$ ,  $df = 2$ ,  $p < 0.33$ ; Fig. 3c). DIN was significantly lower within ML ( $1.22 \pm 0.1 \mu\text{M}$ ) and the NIRL ( $1.55 \pm 0.2 \mu\text{M}$ ) than within the CIRL ( $5.35 \pm 0.7 \mu\text{M}$ ). ML was also significantly lower than the SIRL ( $3.96 \pm 0.7 \mu\text{M}$ ). Seasonally, the



**Fig. 3.** Dissolved nutrient concentrations by segment of the Indian River Lagoon (IRL), Florida, USA arranged left to right from north to south for Mosquito Lagoon (ML), Banana River (BR), the northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL), including (a) ammonium ( $\text{NH}_4$ ), (b) nitrate ( $\text{NO}_3$ ), (c) dissolved inorganic nitrogen (DIN), (d) soluble reactive phosphorus (SRP), (e) DIN:SRP ratio, (f) total dissolved nitrogen (TDN) and (g) total dissolved phosphorus (TDP) showing IRL-targets for both as dotted horizontal lines, as well as (h) the ratio of TDN:TDP indicating N-limitation with a dotted horizontal line at 20 and (i) chlorophyll a concentrations, also showing an IRL-target at  $4.7 \mu\text{g/l}$ . IRL-targets shown are Florida Department of Environmental Protection Numeric Nutrient Criteria for the Indian River Lagoon from the St. Lucie Estuary to the southern border of Indian River County (Florida Administrative Code 62–302.532).

highest mean DIN was in the wet season 2013 within the SIRL ( $8.20 \pm 1.7 \mu\text{M}$ ) and the lowest (also in the wet season 2013) was in ML ( $0.79 \pm 0.04 \mu\text{M}$ ; Fig. 3c). There was a significant negative linear relationship observed between DIN and salinity (Linear Regression,  $r^2 = 0.21$ ,  $p < 0.01$ ; Fig. 4). The highest DIN was associated with the lowest salinities within the CIRL and SIRL, while the lowest DIN was associated with the highest salinities within ML (Fig. 4).

The IRL-wide mean SRP concentration was  $0.72 \pm 0.08 \mu\text{M}$  (Supplemental Table 2). SRP varied spatially ( $H = 45.2$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonally ( $H = 12.6$ ,  $df = 2$ ,  $p < 0.01$ ; Fig. 3d). By lagoon segment, SRP was significantly higher in the CIRL ( $1.48 \pm 0.17 \mu\text{M}$ ) than in the BR ( $0.23 \pm 0.02 \mu\text{M}$ ), ML ( $0.26 \pm 0.02 \mu\text{M}$ ), or NIRL ( $0.31 \pm 0.04 \mu\text{M}$ ). The SIRL ( $0.90 \pm 0.2 \mu\text{M}$ ) was also significantly higher than BR. These higher concentrations were driven by CIRL1-4 (IRL from Sebastian River to Taylor Creek), with lower values observed at CIRL5 (no major external freshwater influence). The higher SRP observed in the SIRL was driven by SIRL1-2 (Nettles Island and St. Lucie River) during the wet season 2013 (Supplemental Table 3). Seasonally, SRP was significantly higher in the wet season 2013 ( $1.21 \pm 0.02 \mu\text{M}$ ) than in the wet season 2015 ( $0.39 \pm 0.05 \mu\text{M}$ ) or the dry season 2015 ( $0.55 \pm 0.08 \mu\text{M}$ , Fig. 3d).

Overall mean DIN:SRP for the IRL was  $8.96 \pm 1.0$  (Supplemental Table 2). High DIN:SRP ratios were observed in the BR in both wet seasons (2013 =  $26.0 \pm 7.9$  and 2015 =  $31.9 \pm 12.6$ ; Fig. 3e; Supplemental Table 3). DIN:SRP varied spatially ( $H = 14.0$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonally ( $H = 8.98$ ,  $df = 2$ ,  $p = 0.01$ ; Fig. 3e). By lagoon segment, DIN:SRP in BR ( $22.3 \pm 5.2$ ) was significantly higher than in the CIRL ( $5.28 \pm 0.6$ ). Seasonally, IRL-wide DIN:SRP was significantly lower during the wet season 2013 ( $7.19 \pm 1.5$ ) than in the wet season 2015 ( $12.7 \pm 2.4$ ), while the dry season 2015 was statistically similar to both wet seasons (Fig. 3e).

The IRL-wide mean TDN concentration was  $46.6 \pm 1.72 \mu\text{M}$  (Supplemental Table 2). TDN was elevated lagoon-wide and mean concentrations exceeded an IRL-target of  $\sim 51 \mu\text{M}$  in the three northern lagoon segments (Fig. 3f). There was spatial ( $H = 96.2$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonal ( $H = 10.6$ ,  $df = 2$ ,  $p < 0.01$ ) variation observed in TDN. Spatially, SIRL ( $25.7 \pm 2.0 \mu\text{M}$ ) had significantly lower TDN than all other lagoon segments. The mean TDN in the BR ( $78.1 \pm 3.0 \mu\text{M}$ ) was significantly higher than all other lagoon segments (Fig. 3f). Seasonally, the IRL-wide mean for the wet sea-

son 2013 was significantly higher ( $51.1 \pm 3.0 \mu\text{M}$ ) than in the dry season 2015 ( $38.9 \pm 2.7 \mu\text{M}$ ) or the wet season 2015 ( $49.6 \pm 3.0 \mu\text{M}$ ; Fig. 3f).

The overall IRL mean TDP concentration was  $2.11 \pm 0.12 \mu\text{M}$  (Supplemental Table 2). The segment TDP means within CIRL exceeded an IRL-target of  $\sim 2.2 \mu\text{M}$  during all sampling events (Fig. 3g). The NIRL and SIRL exceeded the IRL-target for TDP in both wet seasons, and ML and BR exceeded the target in the wet season 2015 (Fig. 3b). Generally, there was higher TDP within the southern segments of the lagoon. Spatial ( $H = 30.5$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonal ( $H = 72.9$ ,  $df = 2$ ,  $p < 0.01$ ) variation was also observed in TDP (Fig. 3g). Mean TDP within CIRL ( $2.97 \pm 0.2 \mu\text{M}$ ) was significantly higher than all other segments, which were all similar (Fig. 3g). The IRL-wide TDP means were all significantly different by season.

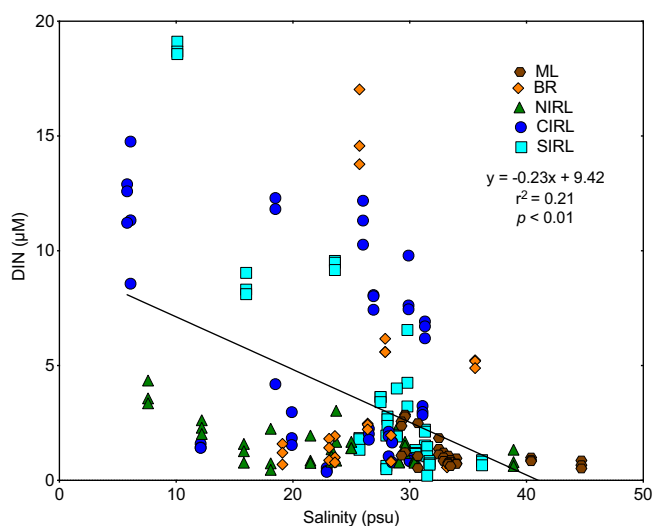
The overall mean TDN:TDP was  $33.6 \pm 2.1$  (Supplemental Table 2). TDN:TDP varied spatially ( $H = 80.5$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonally ( $H = 30.4$ ,  $df = 2$ ,  $p < 0.01$ ; Fig. 3h). The CIRL ( $14.9 \pm 1.0$ ) and SIRL ( $17.7 \pm 1.3$ ), which were statistically similar, had significantly lower TDN:TDP than ML ( $48.1 \pm 5.9$ ), the NIRL ( $44.5 \pm 5.2$ ), and BR ( $60.5 \pm 5.4$ ). Segment mean TDN:TDP exceeded the Redfield ratio (16:1) within ML (48.1), BR (60.5), the NIRL (44.5), and SIRL (17.7; Fig. 3h). Seasonal TDN:TDP was significantly lower in the wet season 2015 ( $17.6 \pm 2.4$ ) than the wet season 2013 ( $37.7 \pm 3.8$ ) or the dry season 2015 ( $45.2 \pm 4.1$ ; Supplemental Table 3).

The IRL mean chlorophyll *a* was  $9.63 \pm 0.8 \mu\text{g/l}$  (Supplemental Table 2), exceeding an IRL-target of  $4.7 \mu\text{g/l}$  (Fig. 3i). As such, this target was also exceeded in many segments, including ML ( $18.8 \pm 2.7 \mu\text{g/l}$ ), BR ( $10.7 \pm 1.2 \mu\text{g/l}$ ), and NIRL ( $12.7 \pm 2.4 \mu\text{g/l}$ ; Fig. 3i). SIRL was the only segment with consistently low chlorophyll *a* ( $3.39 \pm 0.3 \mu\text{g/l}$ ). Spatial ( $H = 49.8$ ,  $df = 4$ ,  $p < 0.01$ ) and seasonal ( $H = 11.6$ ,  $df = 2$ ,  $p < 0.01$ ) variation was observed for chlorophyll *a*. Chlorophyll *a* within the SIRL was statistically similar to the CIRL ( $6.61 \pm 0.8 \mu\text{g/l}$ ) but was significantly lower than all other segments; the CIRL was also significantly lower than ML. Seasonal means in the wet season 2015 ( $12.8 \pm 1.7 \mu\text{g/l}$ ) were significantly higher than the wet season 2013 ( $9.83 \pm 1.4 \mu\text{g/l}$ ) or the dry season 2015 ( $6.23 \pm 0.8 \mu\text{g/l}$ ; Supplemental Table 3).

### 3.3. Seagrass tissue elemental composition, molar ratios, and stable isotopes

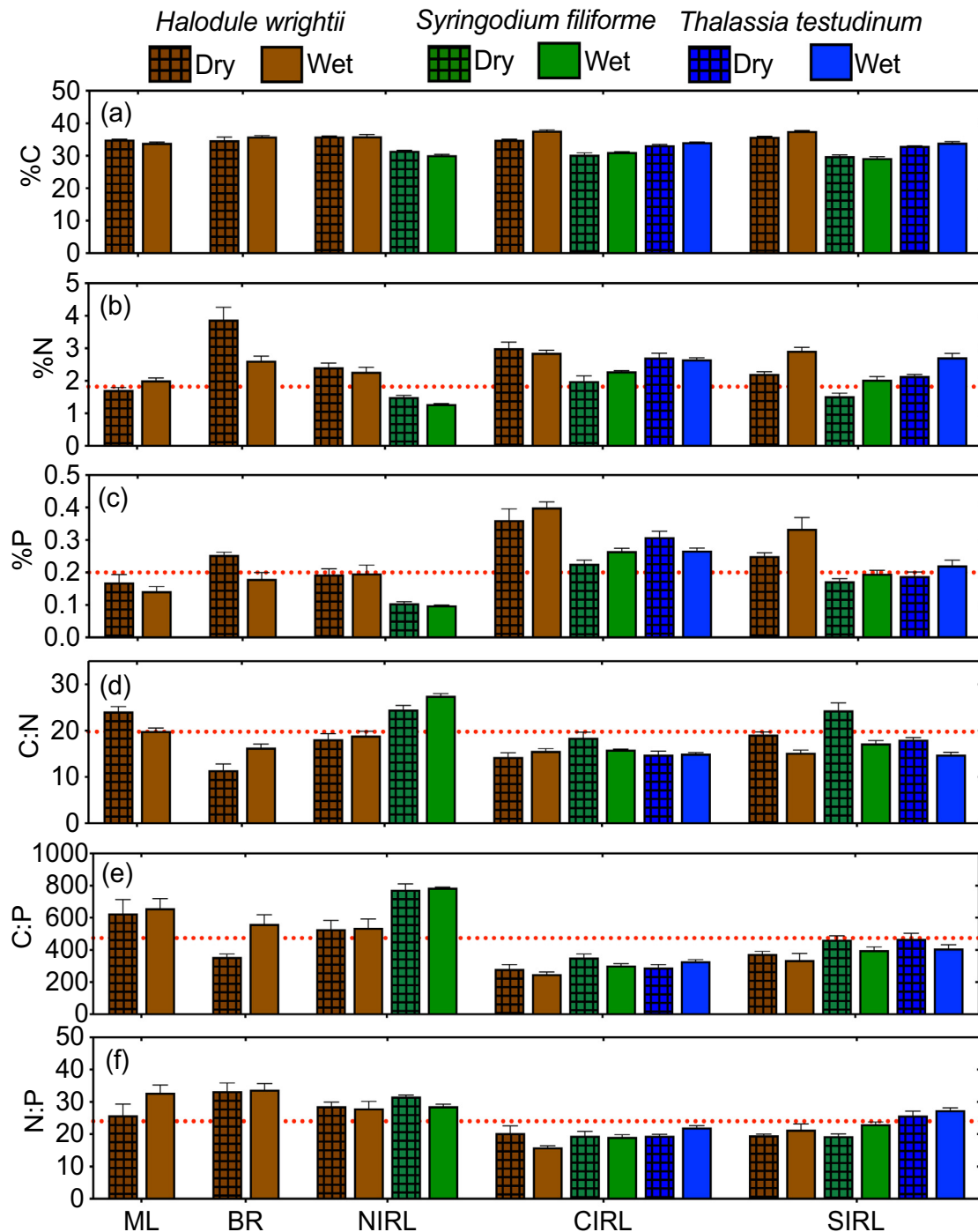
Seagrass species composition varied by sampling location (Supplemental Table 4). *H. wrightii* was collected in all segments; *S. filiforme* in the NIRL, CIRL, and SIRL; and *T. testudinum* in the CIRL and SIRL. In total, 204 seagrass blade samples were analyzed for C:N:P,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$ ; 117 *H. wrightii*, 51 *S. filiforme*, and 36 *T. testudinum*. Due to the uneven species distribution and physiological variability between species, each seagrass species was analyzed separately for spatial and seasonal differences.

The IRL-wide means for elemental composition of *H. wrightii* were %C =  $35.9 \pm 0.15$ , %N =  $2.61 \pm 0.07$ , and %P =  $0.26 \pm 0.01$ . Lagoon-wide, %C varied spatially ( $H = 30.1$ ,  $df = 4$ ,  $p < 0.01$ ) with ML ( $34.5 \pm 0.16$ ) significantly lower than all other segments (Fig. 5a). Seasonal means for %C were also significantly higher ( $H = 13.1$ ,  $n = 1$ ,  $p < 0.01$ ) in the wet season ( $36.5 \pm 0.23$ ) than the dry season ( $35.3 \pm 0.18$ ; Supplemental Table 5). %N of *H. wrightii* varied spatially ( $H = 45.9$ ,  $df = 4$ ,  $p < 0.01$ ). The highest mean %N of the study was found in the BR ( $3.88 \pm 0.38$ ), while the lowest %N was in ML, both in the dry season ( $1.72 \pm 0.07$ ; Fig. 5b). Finally, there was spatial variability in the %P of *H. wrightii* ( $H = 57.2$ ,  $n = 4$ ,  $p < 0.01$ ). For example, the CIRL ( $0.38 \pm 0.02$ ) had significantly higher %P than all other segments, except the SIRL ( $0.29 \pm 0.02$ ). The SIRL was also significantly higher in %P than ML ( $0.16 \pm 0.01$ ) or the NIRL ( $0.20 \pm 0.01$ ; Fig. 5c).



**Fig. 4.** The relationship between dissolved inorganic nitrogen (DIN) and salinity with linear regression line for the Indian River Lagoon, Florida, USA, including Mosquito Lagoon (ML), Banana River (BR), the northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL).





**Fig. 5.** Elemental composition and molar ratios (mean  $\pm$  SE) for seagrass tissue collected in the Indian River Lagoon (IRL) showing critical values of eutrophication for seagrass (Duarte, 1990), including (a) %C, (b) %N, (c) %P, (d) C:N, (e) C:P, and (f) N:P for Mosquito Lagoon (ML), Banana River (BR), the northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL).

Differences were observed in *H. wrightii* molar ratios (Supplemental Table 4). For example, *H. wrightii* tissue varied spatially for C:N ( $H = 43.9$ ,  $df = 4$ ,  $p < 0.01$ ). The highest overall C:N was in ML ( $22.0 \pm 0.8$ ), followed by the NIRL ( $18.5 \pm 0.7$ ), and SIRL ( $17.3 \pm 0.5$ ), with lower ratios in the CIRL ( $15.0 \pm 0.5$ ) and BR ( $13.9 \pm 1.0$ ; Fig. 5d). *H. wrightii* C:P also varied spatially ( $H = 56.9$ ,  $df = 4$ ,  $p < 0.01$ ), with the highest ratios found within ML (overall mean  $644 \pm 51$ ), followed by the NIRL (overall mean  $534 \pm 38$ ). These highest segments were significantly different from the low-

est segments, the CIRL ( $267 \pm 15$ ) and SIRL ( $358 \pm 20$ ; Fig. 5e). Further, the N:P of *H. wrightii* was also spatially variable ( $H = 58.9$ ,  $df = 4$ ,  $p < 0.01$ ). Specifically, the northernmost IRL segments, BR ( $33.6 \pm 1.5$ ), ML ( $29.4 \pm 2.2$ ), and the NIRL ( $28.4 \pm 1.2$ ), all had significantly higher N:P ratios than the more southern segments, the CIRL ( $18.2 \pm 1.2$ ) and SIRL ( $20.5 \pm 0.8$  Fig. 5f).

The elemental composition of *S. filiforme* was also variable throughout the IRL (Supplemental Table 4). *S. filiforme* varied spatially for %C ( $H = 8.23$ ,  $df = 2$ ,  $p = 0.02$ ). %C was highest within the



NIRL ( $30.9 \pm 0.3$ ), followed by the CIRL ( $30.7 \pm 0.3$ ) and SIRL ( $29.6 \pm 0.3$ ), which were significantly different from each other (Fig. 5a). Additionally, *S. filiforme* %N varied spatially ( $H = 13.2$ ,  $df = 2$ ,  $p < 0.01$ ) and was highest within the CIRL ( $2.12 \pm 0.10$ ), followed by the SIRL ( $1.77 \pm 0.08$ ), and NIRL ( $1.39 \pm 0.05$ ; Fig. 5b). %N of *S. filiforme* was also variable by season ( $U = 483$ ,  $p < 0.01$ ) and was significantly higher in the wet season ( $2.02 \pm 0.08$ ) than the dry season ( $1.68 \pm 0.09$ ; Supplemental Table 5). The %P of *S. filiforme* was spatially variable ( $H = 29.2$ ,  $df = 2$ ,  $p < 0.01$ ), with the highest values found within the CIRL ( $0.24 \pm 0.01$ ), followed by the SIRL ( $0.18 \pm 0.01$ ), and NIRL ( $0.10 \pm 0.01$ ; Fig. 5c). The %P of all segments were significantly different from each other. Seasonal variability was not observed in *S. filiforme* %P ( $U = 402$ ,  $p = 0.14$ ).

Spatial and seasonal differences were also observed in *S. filiforme* molar ratios (Supplemental Table 4). In particular, *S. filiforme* varied spatially for C:N ( $H = 12.1$ ,  $df = 2$ ,  $p < 0.01$ ). Specifically, mean C:N was significantly higher within the NIRL ( $26.1 \pm 0.8$ ) than the CIRL ( $17.4 \pm 0.7$ ), while the SIRL was statistically similar to both (Fig. 5d). C:N varied seasonally ( $U = 152$ ,  $p < 0.01$ ) and was significantly higher in the dry season ( $22.4 \pm 1.1$ ), than the wet season ( $18.1 \pm 0.8$ ; Supplemental Table 5). All lagoon segments were significantly different from each other for C:P ( $H = 26.5$ ,  $df = 2$ ,  $p < 0.01$ ). The highest *S. filiforme* C:P was found within the NIRL ( $782 \pm 16$ ), followed by the SIRL ( $432 \pm 17$ ) and CIRL ( $331 \pm 17$ ; Fig. 5e). C:P did not vary seasonally ( $U = 249$ ,  $p = 0.16$ ). Spatial variation was observed in the N:P ratio of *S. filiforme* ( $H = 18.9$ ,  $df = 2$ ,  $p < 0.01$ ). For example, N:P within the NIRL ( $30.2 \pm 0.7$ ) was significantly higher than within the CIRL ( $19.4 \pm 0.8$ ) and SIRL ( $21.2 \pm 0.6$ ). N:P also varied seasonally ( $U = 435$ ,  $p = 0.04$ ) with the lowest ratios found in the dry season ( $20.8 \pm 0.9$ ) and the highest in the wet season ( $22.7 \pm 0.7$ ; Fig. 5f).

There were also spatial and seasonal differences observed in the elemental composition of *T. testudinum* (Supplemental Table 4). Overall mean %C of *T. testudinum* was  $34.0 \pm 0.2$  and it did not vary spatially ( $U = 107$ ,  $p = 0.33$ ). %C varied seasonally ( $U = 203$ ,  $p < 0.01$ ) and was significantly higher in the wet season ( $34.1 \pm 0.2$ ) versus the dry season ( $33.2 \pm 0.2$ ; Fig. 5a). The overall mean %N of *T. testudinum* was  $2.57 \pm 0.08$ . *T. testudinum* %N varied seasonally ( $U = 189$ ,  $p = 0.02$ ) and was significantly higher in the wet season ( $2.71 \pm 0.09$ ) than the dry season ( $2.50 \pm 0.10$ ; Fig. 5b). %P varied spatially ( $U = 32.0$ ,  $p < 0.01$ ) and was significantly higher within the CIRL ( $0.30 \pm 0.01$ ) than the SIRL ( $0.21 \pm 0.01$ ; Fig. 5c).

Finally, there were also significant differences observed in the molar ratios of *T. testudinum* (Supplemental Table 4). Mean C:N was highest in the SIRL ( $16.5 \pm 0.5$ ) and lowest within the CIRL ( $14.9 \pm 0.6$ ; Fig. 5d). The C:P of *T. testudinum* was spatially variable

( $U = 240$ ,  $p < 0.01$ ) with SIRL ( $439 \pm 22$ ) being significantly higher than the CIRL ( $297 \pm 15$ ; Fig. 5e). *T. testudinum* N:P was spatially variable ( $U = 266$ ,  $p < 0.01$ ) and significantly higher within the SIRL ( $26.6 \pm 0.8$ ) compared to the CIRL ( $20.0 \pm 0.4$ ). Seasonal variation was also observed in *T. testudinum* N:P ( $U = 198$ ,  $p < 0.01$ ) with the wet season ( $26.1 \pm 0.9$ ) having higher ratios than the dry season ( $26.6 \pm 0.9$ ; Fig. 5f).

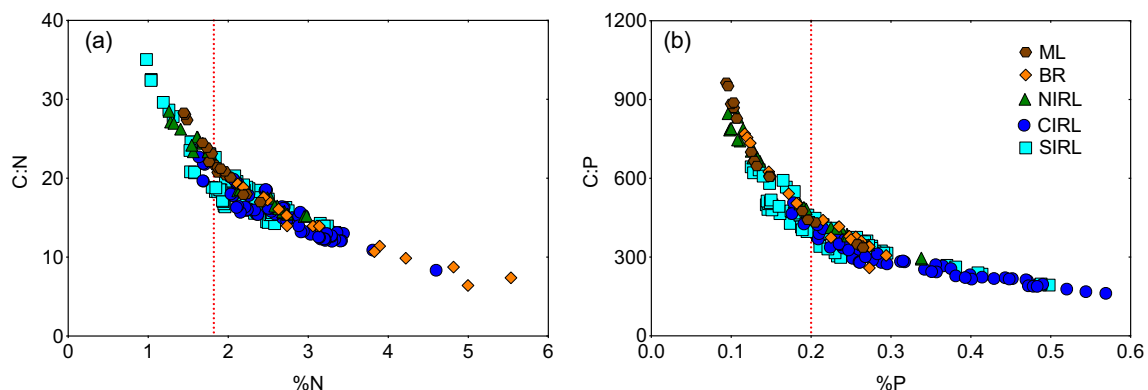
### 3.4. Relationships of variables

Relationships were observed between seagrass tissue elemental composition and molar ratios. For example, the relationship between seagrass tissue %N and C:N varied by lagoon segment (Fig. 6a). For C:N, 163 out of 201 individual samples (81%) exceeded the %N threshold value of 1.8 proposed by Duarte (1990). The relationship of C:P to %P also varied spatially, with 131 individual samples (65%) in exceedance of the 0.2 threshold (Duarte, 1990; Fig. 6b).

Stable isotope analyses were also conducted simultaneously on the same 201 seagrass tissue samples. The overall mean  $\delta^{13}\text{C}$  was  $-11.3 \pm 0.2\text{‰}$  (Supplemental Table 4). The highest mean  $\delta^{13}\text{C}$  was found within the SIRL ( $-10.7 \pm 0.36\text{‰}$ ), followed by the NIRL ( $-11.0 \pm 0.59\text{‰}$ ), CIRL ( $-11.3 \pm 0.36\text{‰}$ ), ML ( $-12.9 \pm 0.33\text{‰}$ ), and BR ( $-13.3 \pm 0.14\text{‰}$ ). The overall mean  $\delta^{15}\text{N}$  was  $+2.23 \pm 0.11\text{‰}$ . The most enriched  $\delta^{15}\text{N}$  values were observed within the CIRL ( $+3.31 \pm 0.18\text{‰}$ ) and BR ( $+3.05 \pm 0.35\text{‰}$ ), with lower values found within the SIRL ( $+1.83 \pm 0.17\text{‰}$ ), ML ( $+1.31 \pm 0.11\text{‰}$ ) and the NIRL ( $+0.95 \pm 0.19\text{‰}$ ). As with elemental composition, statistical analyses of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were conducted by species.

*Halodule wrightii*  $\delta^{13}\text{C}$  did not vary by segment ( $H = 2.81$ ,  $n = 4$ ,  $p = 0.59$ ) or season ( $H = 0.45$ ,  $n = 1$ ,  $p = 0.51$ ). The most enriched  $\delta^{13}\text{C}$  values were observed within the NIRL ( $-12.4 \pm 0.39\text{‰}$ ), followed by the CIRL and SIRL ( $-12.7 \pm 0.45\text{‰}$  and  $-12.7 \pm 0.60\text{‰}$  respectively; Supplemental Fig. 2). Slightly more depleted values were found within ML ( $-12.9 \pm 0.33\text{‰}$ ) and BR ( $-13.3 \pm 0.14\text{‰}$ ; Supplemental Table 4). *H. wrightii*  $\delta^{15}\text{N}$  varied spatially ( $H = 42.1$ ,  $n = 4$ ,  $p < 0.01$ ), but not seasonally ( $H = 0.25$ ,  $n = 1$ ,  $p = 0.62$ ). The highest mean *H. wrightii*  $\delta^{15}\text{N}$  values were observed within the CIRL ( $+3.34 \pm 0.28\text{‰}$ ), which was statistically similar to BR ( $+3.05 \pm 0.35\text{‰}$ ), but significantly enriched above the SIRL ( $+2.07 \pm 0.28\text{‰}$ ), ML ( $+1.31 \pm 0.11\text{‰}$ ), and the NIRL ( $+1.00 \pm 0.23\text{‰}$ ).

*Syringodium filiforme*  $\delta^{13}\text{C}$  varied spatially ( $H = 10.9$ ,  $df = 2$ ,  $p < 0.01$ ), but not seasonally ( $U = 272$ ,  $p = 0.33$ ). Similar to *H. wrightii*, the most enriched  $\delta^{13}\text{C}$  values were also found within the NIRL ( $-5.82 \pm 0.38\text{‰}$ ), followed by the CIRL ( $-8.10 \pm 0.48\text{‰}$ ),



**Fig. 6.** Elemental composition and molar ratios of Indian River Lagoon seagrass (*Halodule wrightii*, *Syringodium filiforme*, and *Thalassia testudinum*) tissue samples by segment, including Mosquito Lagoon (ML), Banana River (BR), the northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL), showing (a) carbon to nitrogen ratio (C:N) compared to %N and (b) carbon to phosphorus ratio (C:P) compared to %P, both showing critical median values of %N and %P indicating eutrophic conditions (Duarte, 1990).

and SIRL ( $-9.37 \pm 0.57\text{‰}$ ; Supplemental Fig. 2). NIRL *S. filiforme*  $\delta^{13}\text{C}$  values were significantly more enriched than within the SIRL. *S. filiforme*  $\delta^{15}\text{N}$  varied spatially ( $H = 11.3$ ,  $df = 2$ ,  $p = 0.02$ ) and seasonally ( $U = 203$ ,  $p < 0.01$ ). The overall mean  $\delta^{15}\text{N}$  value of *S. filiforme* was higher in the dry season ( $+2.51 \pm 0.18\text{‰}$ ) than the wet season ( $+1.68 \pm 0.23\text{‰}$ ; Supplemental Table 5). Mean *S. filiforme*  $\delta^{15}\text{N}$  was highest within the CIRL ( $+2.62 \pm 0.29\text{‰}$ ) and SIRL ( $+2.12 \pm 0.18\text{‰}$ ), which were significantly enriched above the NIRL ( $+0.76 \pm 0.13$ ; Supplemental Table 4).

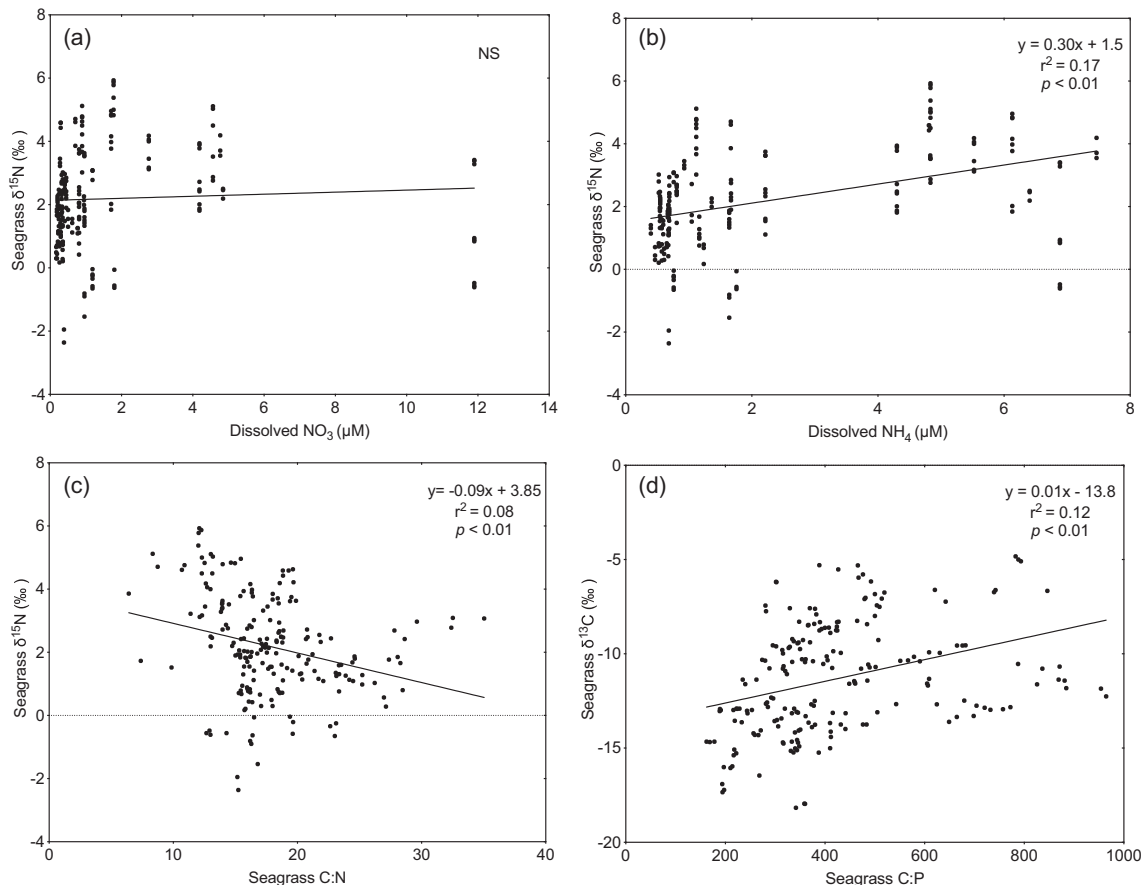
*Thalassia testudinum*  $\delta^{13}\text{C}$  values also varied spatially ( $U = 192$ ,  $p < 0.01$ ), but not seasonally ( $U = 114$ ,  $p = 0.67$ ). The most enriched  $\delta^{13}\text{C}$  values were observed within the SIRL ( $-9.69 \pm 0.23\text{‰}$ ) with more depleted values found within the CIRL ( $-11.6 \pm 0.44\text{‰}$ ; Supplemental Fig. 2). *T. testudinum*  $\delta^{15}\text{N}$  varied spatially ( $U = 42$ ,  $p < 0.01$ ), but not seasonally ( $U = 150$ ,  $p = 0.39$ ). The highest  $\delta^{15}\text{N}$  values were found within the CIRL ( $+3.89 \pm 0.33\text{‰}$ ) with significantly lower values observed within the SIRL ( $+0.98 \pm 0.43\text{‰}$ ; Supplemental Table 4).

The relationship between seagrass blade  $\delta^{15}\text{N}$  and dissolved nitrate was not significant (Linear Regression,  $r^2 < 0.01$ ,  $p = 0.46$ ; Fig. 7a), however there was a significant positive linear relationship between seagrass blade  $\delta^{15}\text{N}$  and dissolved ammonium (Linear Regression,  $r^2 = 0.17$ ,  $p < 0.01$ ; Fig. 7b). Seagrass tissue  $\delta^{15}\text{N}$  had a weaker negative relationship with seagrass tissue C:N (Linear Regression,  $r^2 = 0.08$ ,  $p < 0.01$ ; Fig. 7c), while seagrass tissue  $\delta^{13}\text{C}$  also had a weaker positive relationship with seagrass tissue C:P (Linear Regression,  $r^2 = 0.12$ ,  $p < 0.01$ ; Fig. 7d).

#### 4. Discussion

Past monitoring programs in the IRL have relied largely on areal coverage as an indicator of seagrass health. Such an approach does not inform resource managers of intrinsic ecological or biochemical factors that could otherwise quantify the status of seagrass health with respect to understanding and managing nutrient pollution. The present research represents the first IRL-wide assessment of seagrass status through critical examination of dissolved nutrients and optical properties of the water column, combined with nutrient analysis of seagrass tissue. Spatial patterns in light attenuation, TDN, TDN:TDP ratios, and chlorophyll *a* among the IRL segments revealed a greater degree of nutrient enrichment and altered stoichiometry in the northern segments of the IRL. This observation helps explain the worsening eutrophication (Lapointe et al., 2015), HABs (Lapointe et al., 2015; Philips et al., 2015; Lapointe et al., 2018), and catastrophic seagrass loss (up to 95%; Morris et al., 2018) that these areas have recently experienced (Figs. 1, 8). Though variable by location, some segments of the IRL have undergone nutrient-mediated, biotic phase-shifts that have resulted in alterations of primary producer biomass (Fig. 8). In some of these locations this has resulted in a biotic shift from a climax seagrass community to a phytoplankton dominated, eutrophic system.

The northern and central regions of the IRL are particularly susceptible to eutrophication because of the high degree of urbanization and lower flushing rates that increase the residence time of



**Fig. 7.** Seagrass blade stable nitrogen ( $\delta^{15}\text{N}$ ) isotope values compared to (a) dissolved nitrate ( $\text{NO}_3$ ), (b) dissolved ammonium ( $\text{NH}_4$ ), and (c) seagrass tissue C:N, as well as (d) stable carbon isotope values ( $\delta^{13}\text{C}$ ) compared to seagrass tissue C:P, showing the results of linear regressions between variables; NS = not significant.

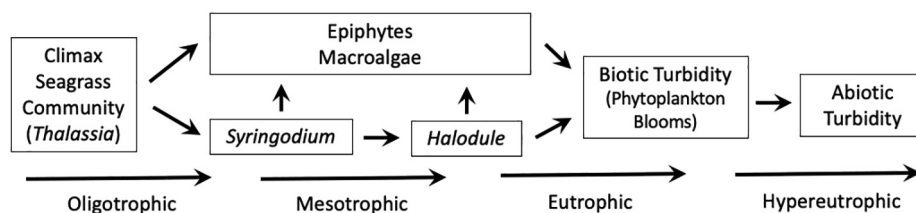


Fig. 8. Nutrient-mediated, biotic phase shift model for the Indian River Lagoon (adapted from Lapointe et al., 2002).

water in these segments (Smith, 1993; Bricker et al., 2008; Philips et al., 2015). The high residence times of these segments and relatively efficient recycling of N, results in buildup of high TDN concentrations, TDN:TDP ratios, and tendency towards P-limitation (Lapointe et al., 2015). Further, the loss of benthic primary producers, such as seagrass and macroalgae, frees up bound nutrients and also reduces competition with phytoplankton for water column nutrients (Sfriso et al., 1992; Philips et al., 2015). As such, in the highly urbanized northern lagoon segments (ML and NIRL), the lowest concentrations of ammonium, nitrate, DIN, and SRP co-occurred with higher TDN:TDP ratios and chlorophyll *a*. In contrast, the better flushed CIRL and SIRL segments had higher concentrations of reactive nutrients that co-occurred with lower TDN:TDP ratios and chlorophyll *a*. The relatively low concentrations of ammonium observed in the Mosquito Lagoon and northern IRL segments would be expected from scavenging of these nutrient species by the high biomass of the brown tide (Kang et al., 2015; Lapointe et al., 2015; Philips et al., 2015). During our study in the Banana River, high ammonium concentrations (3.0  $\mu\text{M}$ ) and TDN:TDP ratio (60.5) co-occurred with relatively low chlorophyll *a* (10.7  $\mu\text{g/l}$ ). This is characteristic of an area heavily influenced by wastewater in a “non-bloom” period (Barile, 2018). The following year in 2016, the Banana River experienced a brown tide bloom and during that occurrence the ammonium concentration was much lower (0.52  $\mu\text{M}$ ) and the chlorophyll *a* much higher (112  $\mu\text{g/l}$ ) than in this study (Lapointe, unpublished data). Similar environmental conditions were also noted during the 2012 brown tide bloom in the Mosquito Lagoon (Philips et al., 2015).

#### 4.1. Light limitation

The role of increasing light limitation to seagrass loss in the IRL was evidenced by the high phytoplankton biomass and  $K_d$  values measured in this study. This increase in turbidity supports the concept of a nutrient-mediated, biotic phase shift in the northern IRL (Fig. 8). For example, the chlorophyll *a* concentrations in the northern segments, which ranged up to 40  $\mu\text{g/l}$ , were much higher than historical values that were generally <10  $\mu\text{g/l}$  (Gibson et al., 1975). These high chlorophyll *a* levels associated with the escalating eutrophication in the IRL have greatly diminished submarine light availability to levels below that needed for seagrass growth and future seagrass restoration. For comparison, chlorophyll *a* concentrations in the ML, BR, and NIRL segments during this study in 2013 and 2015 averaged 14.1  $\mu\text{g/l}$ . Further, much higher chlorophyll *a* concentrations (>100  $\mu\text{g/l}$ ) were typical of the brown tides that affected these segments (Gobler et al., 2013; Lapointe et al., 2015). Chlorophyll *a* values during our study were not at “bloom-level” concentrations for all sites. Despite this,  $K_d$  values were >1.5 during the wet season and close to or above the critical 0.8 value for seagrass recovery (Dennison et al., 1993) during the dry season (Fig. 2). Other sources of light limitation to IRL seagrasses include sediment resuspension due to seagrass loss (de Boer, 2007) and heavy epiphyte growth on the seagrass blades, which can be enhanced by anthropogenic nutrient enrichment (Lapointe et al., 1994, 2004). Confounding effects may also occur

wherein decreased light availability can reduce the ability of seagrasses to uptake DIN (Kraemer and Hanisak, 2000). While the effects of light limitation on seagrasses are species-specific (Statton et al., 2018), current conditions in the IRL indicate widespread light limitation of seagrass growth.

#### 4.2. Dissolved nutrient and chlorophyll *a* concentrations

The significant negative relationship between DIN and salinity indicates that ammonium and nitrate enrichment of the IRL is associated with freshwater inputs. These inputs include surficial stormwater runoff from rivers, canals, and tributaries, as well as submarine groundwater discharges (baseflow). Ammonium, the preferred N source for many algal blooms, including the red macroalga *Gracilaria tikvahiae* (Lapointe and Ryther, 1979; D’Elia and DeBoer, 1978) and the brown tide (*Aureoumbra lagunensis*; Kang et al., 2015; Lapointe et al., 2015), was similar to or higher than nitrate (averaging 1.7  $\mu\text{M}$  vs 1.6  $\mu\text{M}$ , respectively) throughout the IRL. Detailed hydrogeological studies using groundwater monitoring wells, flow meters, stable isotopes, and human wastewater tracers (sucralose, acetaminophen) indicate that the widespread use of septic systems (~300,000 IRL-wide) relates to the elevated ammonium and nitrate in the IRL (Lapointe and Krupa, 1995; Lapointe et al., 2017). In addition, aging municipal wastewater infrastructure in urban areas, combined with inadequate levels of N-removal at most wastewater treatment plants (WWTPs) along the IRL, likely also contribute to the high ammonium and nitrate concentrations. Further, multiple WWTPs located on the IRL periodically release “wet weather discharges” of raw sewage into the IRL (IRLNEP, 2008; Barile, 2018), especially during extreme rain events, such as Tropical Storm Faye (2008), Hurricane Matthew (2016), and Hurricane Irma (Waymer, 2018). Recent research has suggested this influx of poorly treated wastewater on the east-central Atlantic coast of Florida is driving the escalating eutrophication and HABs observed in the IRL (Lapointe et al., 2015, 2017; Barile, 2018). Other external sources of dissolved nutrients to the IRL include agricultural and urban stormwater runoff and atmospheric deposition (Sigua and Tweedale, 2003; Lapointe et al., 2015). Internal nutrient sources include natural  $\text{N}_2$  fixation (Patriquin and Knowles, 1972; Capone and Taylor, 1980; Peterson and Fry, 1987) and sediment flux from IRL “muck” (Fox and Trefry, 2018).

The distribution of seagrass species within the IRL aligns with the spatial trends in water quality observed in this study. The species best adapted for eutrophic environments, *H. wrightii* (Lapointe et al., 1994; Fourqurean et al., 1995), was the most ubiquitous species throughout the IRL. This species is known to replace seagrass species that are more sensitive to nutrient loading, such as *Syringodium filiforme* and *Thalassia testudinum*, in coastal waters experiencing eutrophication (Lapointe et al., 1994; Durako et al., 2002; Fig. 8). For example, during this study *S. filiforme* was not found within BR or ML, where dissolved nutrients and chlorophyll *a* were highest and light availability lowest. Furthermore, the most sensitive species to nutrient enrichment, *T. testudinum*, (Lapointe et al.,



1994) was only found in the two southernmost segments, CIRL and SIRL, which had the lowest TDN, chlorophyll *a*, and  $K_d$  values.

Although the Sebastian Inlet area has been considered the northernmost extent of *T. testudinum* on Florida's east coast (Phillips, 1960), this species grows abundantly at higher latitudes, such as the northern Gulf of Mexico (Iverson and Bittaker, 1986; Johnson et al., 2006) and Bermuda (Den Hartog, 1970). Therefore, we cannot preclude that *T. testudinum* did not occur in the more northern IRL segments before the period of record. While some assume that low temperature sets the northernmost distributional limit of *T. testudinum* in the IRL, our data suggest that N-enrichment may be another contributing factor. The CIRL sites around Sebastian Inlet where *T. testudinum* was historically abundant were revisited by the authors in June 2018 and none could be found. This suggests this species may no longer be able to survive in that location, despite a trend of warming water temperatures. In the Florida Keys, Lapointe et al. (1994) found that shoot productivity of *T. testudinum* was very low in coastal waters enriched above 40  $\mu\text{M}$  TDN. The TDN concentrations in the CIRL and SIRL during this study, where *T. testudinum* still grows, were below this dissolved nitrogen threshold. In the NIRL, BR, and ML where *T. testudinum* does not grow, TDN concentrations were elevated well above this threshold value. Similarly, experimental mesocosm experiments indicated that nitrate enrichment of 3.5–7.0  $\mu\text{M}$ , combined with high temperatures, led to decline in shoot density of the temperate seagrass *Zostera marina* in sandy sediments (Burkholder et al., 2007). The high seawater TDN and TDN:TDP ratios in the northern segments also correlated with high N:P ratios (>30:1) in seagrass tissue, suggesting a greater degree of P-limitation in the IRL region experiencing seagrass loss. We suggest that more research is needed to further elucidate the direct and indirect effects of N-enrichment on health of *T. testudinum* to better understand the physiological mechanism(s) through which N-enrichment exacerbates P-limitation and stresses growth of this ubiquitous and important seagrass species.

#### 4.3. Seagrass elemental composition, molar ratios, and stable isotope values

The tissue nutrient contents of seagrasses in our study further indicated nutrient-over enrichment of the IRL, as they were greatly elevated above values reported for healthy meadows in oligotrophic environments. The mean %C, %N, and %P contents in Duarte (1990) were 33.6% C, 1.92% N, and 0.32% P. In comparison, the overall mean values for the IRL seagrasses were 34.1%, 2.41%, and 0.24%, respectively, suggesting N over-enrichment of IRL seagrasses by some 26%. Further, median %P values in the IRL (0.2% P) were identical to those in Duarte's (1990) survey, while median %N values were higher (2.4% N). In the IRL, 81% of the seagrasses exceeded the median N threshold of 1.8% N and 65% exceeded the median P threshold of 0.2% P. Furthermore, the overall mean C:N ratio of IRL seagrasses was 17.9, and that of *T. testudinum* was 16.5; both these values are lower than Duarte's mean C:N of 19.8, indicating N-enrichment. Interestingly, the median %N value of *T. testudinum* in Florida Bay was 2% in the late 1980s, higher than the N-enrichment threshold (Duarte, 1990). This supports longstanding contentions of a primary role of N-enrichment in the widespread algal blooms, eutrophication, and seagrass die-off that has occurred in Florida Bay since the 1980s (Lapointe et al., 2002; Lapointe and Barile, 2004).

Stable carbon isotope values indicated light limitation of seagrasses throughout the IRL. The spatial trend in *T. testudinum*  $\delta^{13}\text{C}$  values in the IRL suggest increasing light limitation from south to north. This finding is supported by the higher  $K_d$  values observed in the wet season at the more northern segments (NIRL and BR) compared to the more southern segments (CIRL and SIRL). Accord-

ingly, seagrass  $\delta^{13}\text{C}$  values in the IRL may provide a useful tool for monitoring light availability to seagrasses, as suggested by Hu et al. (2012). The depleted *T. testudinum* carbon isotope values ( $-10.5\text{‰}$ ) indicate seagrasses in the IRL are exposed to greater light limitation compared to more enriched  $\delta^{13}\text{C}$  values in shallow waters (2 m) of the Bahamas ( $-4.5\text{‰}$ ) and Florida Bay ( $-7.0\text{‰}$ ; Hu et al., 2012; Campbell and Fourqurean, 2009). In fact, the  $\delta^{13}\text{C}$  values of *H. wrightii* observed in this study ( $-12.8\text{‰}$ ) are slightly depleted compared to those from Mississippi Sound ( $-12.2\text{‰}$ ), a more temperate location that is heavily influenced by runoff from the Biloxi Back Bay, as well as the Biloxi and Pascagoula Rivers (Moncreiff and Sullivan, 2001).

A relationship between geographic latitude and seagrass  $\delta^{13}\text{C}$  values indicates that seagrasses become more depleted from tropical to subtropical latitudes (Hemminga and Mateo, 1996). Interestingly, the IRL encompasses this important biogeographic transition zone and, as temperatures warm, tropical species may experience a broadening of their niche space in the IRL. However, as IRL waters are warming, the distribution of the most tropical seagrass species in our study, *T. testudinum*, appears to be shifting south from its northern distribution limit at Sebastian Inlet. Therefore, despite the tropical niche space increasing northward, *T. testudinum* is losing previously suitable habitat in the IRL. Multiple lines of evidence, including depleted  $\delta^{13}\text{C}$  values, elevated %N, low C:N ratios, and high  $K_d$  values, suggest this is likely attributable to decreased light availability from increasing N-loading, eutrophication, and increased phytoplankton biomass.

The stable nitrogen isotope values of seagrasses in the IRL were relatively low (overall mean  $+2.19\text{‰}$ ), compared to values reported for macroalgae in this system. For example, in 2011–2012 IRL macroalgae at these same sites had an overall mean  $+6.30\text{‰}$  (Lapointe et al., 2015). Another study using a greater number of sites in 2013–2014 found a mean of  $>+3\text{‰}$  lagoon-wide with values of  $+7\text{‰}$  to  $+10\text{‰}$  in tidally restricted, urban areas (Barile, 2018). These differences in  $\delta^{15}\text{N}$  values between macroalgae and seagrasses reflect how seagrasses rely, to a large extent, on  $\text{N}_2$  fixation in the rhizosphere rather than direct uptake by blades from the water column (Patriquin and Knowles, 1972; Capone and Taylor, 1980; Peterson and Fry, 1987). This makes the  $\delta^{15}\text{N}$  values of some seagrass species less ideal as an indicator for ambient water quality and more informative regarding conditions of sediment pore water (Lepoint et al., 2004). However, the relatively long lifespan of seagrass, as opposed to phytoplankton and macroalgae, allows them to serve as an indicator of environmental quality over time, with more enriched plants being suggestive of human wastewater influence (Fourqurean et al., 2015). In this study, lagoon segments with the most enriched  $\delta^{15}\text{N}$  values ( $>+3\text{‰}$ ) were CIRL and BR, suggesting a larger wastewater influence in these areas. This wastewater influence was reinforced by the positive linear relationship observed between increasing  $\delta^{15}\text{N}$  values and water column ammonium concentrations in the present study.

#### 4.4. Implications for seagrass monitoring, management, and restoration

An understanding of the driving role of land-based nutrient inputs, especially wastewater, was key to the successful restoration of seagrasses in urbanized areas in southwest Florida. Following citizens demand for action in Tampa Bay, reduction in wastewater nutrient loading by 90% in the late 1970s lowered external nitrogen loading by 50% over the following three years (Greening et al., 2014). This management action initiated the recovery of Tampa Bay from a turbid, phytoplankton-based system to a clear, seagrass-based system that today resembles the relatively pre-disturbance period of Tampa Bay in the 1950s (Greening et al., 2014). Similar trends of seagrass recovery have

occurred in Sarasota Bay, St. Josephs Sound, Clearwater Harbor, and Charlotte Harbor in recent decades through reductions in nitrogen loading from point source sewage inputs (Tomasko et al., 2018). This underscores the need to prioritize water quality improvement before seagrass restoration is viable (Dennison et al., 1993). As management actions are taken to reduce wastewater nutrient loads, water quality monitoring efforts should focus on documenting water quality improvements necessary to reduce nutrient concentrations and increase light availability needed to support seagrass restoration. Further, the identification of ecological thresholds in seagrass systems may allow for improved management and adaptive monitoring (Connell et al., 2017). These findings in the IRL are relatable to urbanized estuaries globally that are threatened by population growth, anthropogenic N-enrichment, climate change, and seagrass decline.

## 5. Conclusions

The results of this study suggest basin management action plans (BMAPs) need to prioritize strategies for N-loading reductions to support recovery of water quality and seagrass growth. In particular, the more reactive, inorganic forms of nitrogen, especially ammonium and nitrate, must be reduced. The negative relationship between salinity and DIN in this study indicates that the driving force for nutrient enrichment, eutrophication, and HABS in the IRL is freshwater inputs that deliver land-based nutrients into the IRL. The strong climatic linkage between increased rainfall, stormwater runoff, nutrient enrichment, and development of HABS has been well-documented in the IRL system (Phlips et al., 2010, 2012; Lapointe et al., 2015; Phlips et al., 2015; Lapointe et al., 2017; Kramer et al., 2018) and underscores this fundamental understanding of watershed nutrient pollution and coastal ecosystem health (NRC, 2000).

## Declaration of Competing Interest

There is no conflict of interest.

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## Appendix A. Supplementary data

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