



Comparative ecophysiology of bloom-forming macroalgae in the Indian River Lagoon, Florida: *Ulva lactuca*, *Hypnea musciformis*, and *Gracilaria tikvahiae*



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ABSTRACT

Macroalgal blooms are ecological responses to nutrient enrichment in shallow seagrass-dominated estuaries. For decades the Indian River Lagoon (IRL) a biodiverse estuary in east-central Florida, has experienced persistent blooms of red drift macroalgae, including *Gracilaria* and *Hypnea* spp. Since 2013, extensive blooms of green macroalgae, such as *Chaetomorpha* and *Ulva* spp., have developed. To better understand IRL nutrient effects on bloom-forming macroalgae, field and laboratory studies (2012) assessed nitrogen (N) versus phosphorus (P) limitation and morphological/physiological characteristics in relatively urbanized (Titusville, FL) versus rural (Fort Pierce, FL) IRL segments. Field studies indicated *Ulva lactuca*, *Hypnea musciformis*, and *Gracilaria tikvahiae* all grew fastest in Titusville (average \pm SD; 0.49 ± 0.07 , 0.35 ± 0.03 , and 0.14 ± 0.05 doublings d^{-1} , respectively). However, *U. lactuca* had the most rapid biomass doubling time (2 days). Laboratory nutrient enrichment assays revealed 3-fold increases in rapid light curve (RLC) maximum values and 2-fold faster growth at high concentrations of N and P for *U. lactuca*. This superior growth and photosynthesis was attributed to higher surface area:volume ratios averaging (\pm coefficients of variation, %) $565.2 \pm 2.15 \text{ cm}^2 \text{ g dry wt.}^{-1}$ compared to lower ratios for *H. musciformis* ($110.7 \pm 3.97 \text{ cm}^2 \text{ g dry wt.}^{-1}$) and *G. tikvahiae* ($91.1 \pm 1.81 \text{ cm}^2 \text{ g dry wt.}^{-1}$). Finely- and coarsely-branched *H. musciformis* and *G. tikvahiae* were similar photosynthetically but not morphologically based on a functional/form model. These data provide a physiological basis explaining bloom distributions and the recent success of green macroalgae in the increasingly eutrophic IRL.

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1. Introduction

Macroalgal blooms are primary symptoms of eutrophication in shallow coastal ecosystems experiencing increased nitrogen (N) and phosphorus (P) loadings (Bricker et al., 2007; Lapointe et al., 1994; Valiela et al., 1997). The increased nutrients fuel the growth of opportunistic macroalgae and phytoplankton until light reduction has compromised submerged aquatic vegetation (SAV) growth, like seagrasses (Burkholder et al., 2007; Lapointe et al., 2004; Morris and Virnstein, 2004; Smith et al., 1999). High biomass macroalgae accumulations that result from excess nutrients are considered harmful algal blooms (HABs; Lapointe and Bedford, 2007), which can provide major nutrient sinks during bloom formation and sources of recycled nutrients after decomposition, eventually affecting phytoplankton blooms. Unlike phytoplankton HABs, macroalgal HABs are usually non-toxic, but can cause major ecosystem impacts such as habitat destruction, oxygen depletion, and nutrient/biogeochemical cycling alterations (Lapointe and Bedford, 2007; Lapointe et al., 1994; McGlathery, 2001; Valiela et al., 1997),

thereby reducing biodiversity (Burkholder et al., 2007; Howarth et al., 2000; McGlathery, 2001).

The Indian River Lagoon (IRL) in east central Florida is a biologically diverse estuary (Gilmore, 1977) that has been affected by nutrient enrichment (Sigua et al., 2000) and macroalgal blooms (Virnstein and Carbonara, 1985; Bricker et al., 2007, 2008) for decades. Eutrophication in the IRL is exacerbated by limited water circulation through six widely spaced inlets to the Atlantic Ocean with longer residence times in the northern section (Phlips et al., 2002, 2004; Sigua and Tweedale, 2003; Smith, 1993). Changes in land use have decreased water quality through nutrient pollution from stormwater, wastewater discharges including septic tanks (Barile, 2004; Lapointe et al., 2012; Sigua and Tweedale, 2003; Lapointe et al., 2015), and atmospheric N deposition (Howarth, 2008). The IRL is highly susceptible to increased N loads from the combination of high input and low tidal flushing with a eutrophic condition from 1999 to 2004 of moderate with no change between assessments (NEEA, Bricker et al., 2007, 2008). More recently, the northern IRL experienced an unprecedented “super bloom” of *Resultor* spp. in 2011 that was followed by a brown tide of *Aureoumbra lagunensis* in 2012 (DeYoe et al., 1997; Gobler et al., 2013). Light attenuation from these phytoplankton blooms led to a 60% loss of seagrasses in the northern IRL between 2009 and 2012 (SJRWMD, 2014). The IRL SWIM Plan

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(SFWM and SJRWMD, 2002; Steward et al., 2003) has included specific strategies and nutrient criteria (section 62–302.531, F.A.C.) to protect seagrass beds (Fletcher and Fletcher, 1995; Steward et al., 2005). In order to reach this endpoint, environmental factors, specifically nutrient dynamics regulating macroalgal blooms (Lapointe et al., 2015) and associated nutrient cycling (Zimmerman and Montgomery, 1984) require more understanding.

Macroalgae are commonly used in ecological studies of nutrient pollution because they are sessile and respond to nutrient enrichment, providing an integrated assessment of nutrient availability (Dailer et al., 2010; Fong et al., 2001; Jones et al., 1996; Lapointe and Ryther, 1978). For instance, *Ulva* spp. readily assimilate all forms of dissolved inorganic nitrogen (DIN: NO_3^- and NH_4^+) while rhodophytes such as, *Gracilaria tikvahiae* and *Hypnea musciformis*, preferentially assimilate NH_4^+ (D'Elia and DeBoer, 1978; Rosenberg and Ramus, 1982, 1984; Teichberg et al., 2007, 2008). Accordingly, whereas *Ulva lactuca* would be more competitive in a high N environment, *G. tikvahiae* (Teichberg et al., 2008) and *H. musciformis* (Dawes et al., 1976; Guist et al., 1982) are better adapted for growth in more N-limited areas due to broad tolerances to temperatures, light levels, and nitrogen storage in the form of non-photosynthetic accessory pigments, especially N-rich phycobiliprotein complexes (Dawes et al., 1984; Lapointe, 1981; Lapointe et al., 1984b; Lapointe and Ryther, 1978).

Indicator species of rhodophytes and chlorophytes have formed extensive blooms in coastal waters impacted by nutrient pollution. The rhodophytes, *H. musciformis* and *G. tikvahiae* have been common bloom-formers in the IRL for decades (Benz et al., 1979). Massive drift rhodophyte blooms which were composed mainly of *H. musciformis*, *Hypnea spinella*, and some *G. tikvahiae*, developed in Lee County in 2003 and 2004 following nutrient-rich discharges from Lake Okeechobee (Lapointe and Bedford, 2007). The chlorophyte *U. lactuca* and non-native *H. musciformis* have also become problematic in shallow coastal waters impacted by sewage pollution Maui, Hawai'i (Dailer et al., 2012). Blooms of *Ulva* spp. typically occur in coastal waters chronically impacted by nutrient enrichment, such as the Venice lagoon (Sfriso et al., 1992; Teichberg et al., 2010) and Po River Delta, Italy (Naldi and Viaroli, 2002) due to a combination of sewage, agricultural runoff, and limited tidal flushing similar to the IRL. *Ulva* spp. bloom in a wide variety of nutrient-rich environments in both temperate (Thornber et al., 2008) and tropical waters (Lapointe et al., 2010), including Boston Harbor, USA (Sawyer, 1965), Mondego Estuary, Portugal, Waquoit Bay, Massachusetts, San Antonio Bay, Argentina, Urias Estuary, Mexico, Jobos Bay, Puerto Rico, Flamengo Sound, Brazil, (Teichberg et al., 2010) and in the Yellow and East China seas (Hu et al., 2010).

Expanding human activities have caused macroalgae to increasingly compete for space in nutrient enriched coastal waters. Algal dominance is related to physiological profiles, morphological characteristics (i.e., the functional/form hypothesis; Littler, 1980), and nutrient uptake kinetics. Thin sheet-like forms of macroalgae, such as *U. lactuca*, have optimum photosynthetic productivity and growth strategies lending the ability to outcompete other morphological forms of algae in environments with elevated nutrients (Carpenter, 1990; Littler, 1980; Rosenberg and Ramus, 1984). Quantification of these morphological characteristics can easily be determined with 3-D scanning technology which can offer insight into nutrient uptake and growth patterns in varying macroalgae due to a functional form (Taylor et al., 1999). In addition, some macroalgae, such as *Gracilaria* spp., have greater capacity for "luxury consumption" and nutrient storage, allowing them to more effectively compete in highly dynamic coastal waters receiving "pulsed" nutrient inputs (Lapointe, 1981). Therefore, respective algal morphologies play a critical role in bloom temporal and spatial dynamics due to differences among taxa.

Historically, photosynthesis irradiance curves (PI-curves; Lapointe, 1997) and oxygen evolution have been used to assess the physiological effects of nutrient enrichment on macroalgae. Recently, pulse amplitude modulated (PAM) fluorometry has been used to assess physiological

stress through nutrient enrichment within the photosynthetic apparatus of photosystem II (PSII) and rapid light curves (RLCs; Haan et al., 2013; Necchi, 2004; Ralph and Gademann, 2005; White and Critchley, 1999) providing easy in situ data collection. For instance, Teichberg et al. (2013) used RLCs and photosynthetic quantum yield (Y_{II} , $\Delta F/F_m$) to demonstrate increased photosynthetic capacities with nutrient enrichment of the green alga, *Halimeda opuntia*. Other studies have found close correlations between oxygen evolution and PAM methods in *Ulva* spp. (Beer et al., 2000; Franklin and Badger, 2001). Thus, the use of RLCs may provide important information on nutrient-induced stress limitations on growth rates of bloom-forming macroalgae.

This study addressed some gaps in knowledge concerning macroalgal bloom prediction, composition, and possible control methods using comparative ecophysiologicals as a function of nutrient availability of two rhodophytes, *H. musciformis* and *G. tikvahiae*, and the chlorophyte *U. lactuca*. Our objectives were to: 1) use an established nutrient gradient to compare algal growth rates of macroalgae in nutrient-rich waters at urbanized Titusville, FL with lower nutrient waters of rural Fort Pierce, FL at the Harbor Branch Oceanographic Institute (HBOI) in the IRL, 2) determine if inherent morphological and physiological advantages of the opportunistic *U. lactuca* facilitate faster growth rates and photosynthesis than *H. musciformis* and *G. tikvahiae* in the nutrient-rich Titusville compared to Fort Pierce, and 3) use laboratory nutrient enrichment studies to see if higher nutrient concentrations affect macroalgal photosynthesis and growth more than N:P ratios.

2. Methods

2.1. Study sites and rationale

Field caging experiments with *U. lactuca*, *G. tikvahiae*, and *H. musciformis* were conducted during November and June 2012 at Titusville in the northern IRL (NIRL; $28^\circ 36' 43.52''$, $-80^\circ 48' 17.05''$) and at the HBOI ($27^\circ 32' 10.57''$, $-80^\circ 20' 58.40''$; Fig. 1) in Fort Pierce in the central IRL (CIRL). Macroalgal growth rates were taken from field experiments. Dissolved oxygen, salinity, conductivity, and temperature were measured each week during November and June 2012 sampling using a calibrated YSI Model 85 salinity/conductivity/DO sensor to describe site conditions. Surface area:volume (SA:V) ratios were calculated using a NextEngine 3-D scanner for *U. lactuca*, *G. tikvahiae*, and *H. musciformis* to quantify morphological differences. To quantify

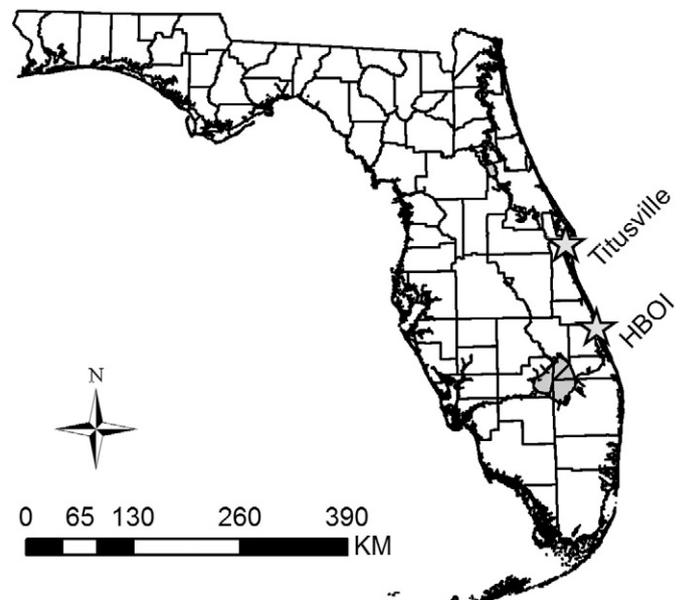


Fig. 1. Map of the sites where bloom-forming macroalgae occur in the IRL (Titusville and Harbor Branch (HBOI)) used in this study.

photophysiological differences, RLCs (Walz Diving-PAM) in the lab were conducted as well. Finally, RLCs and specific growth rates in a laboratory nutrient enrichment experiment of *U. lactuca* were conducted in the HBOI laboratory.

IRL-wide water quality analyses in 2011/2012 (Lapointe *pers. comm.*) were used as a proxy for nutrient data of our Titusville and HBOI sites. The NIRL data from the sample site entitled NIRL 2 and CIRL data from the sample site CIRL 5 were used to establish similar nutrient conditions in Titusville and HBOI during field algal growth experiments (Table 1).

2.2. Field experimental design

Growth treatments ($n = 4$) were placed haphazardly at each site in the NIRL and CIRL (Titusville and Fort Pierce) of *U. lactuca*, *H. musciformis*, and *G. tikvahiae* in a randomized complete block design. Twelve cylindrical (24 cm long; 9 cm in diameter; 1526.8 cm³) cages constructed from small-sized mesh VEXAR (5 × 6 mm) and were arranged horizontally in clusters of 3 on PVC poles 2.3 m in length buried 0.8 m deep with cages suspended 0.3 m above the sediment. Cages were staggered on each PVC pole by 120° so light attenuation was not compromised by shading.

2.3. Specific growth rate calculations

Initial and final wet weights of caged specimens were measured to quantify growth rates. Growth data were collected after 14 days (for field cages) and 2.5 days (for laboratory nutrient enrichment) for *U. lactuca*, *H. musciformis*, and *G. tikvahiae*. Specific growth rates (μ) were calculated in doublings d⁻¹ as:

$$\mu = \frac{\log_2\left(\frac{N}{N_0}\right)}{\Delta t}$$

where N_0 and N are the initial and final biomass, respectively, and time (t) is calculated in days (Lapointe, 1981; Lapointe et al., 1984a). This calculation for macroalgal growth is not density-dependent and accurately describes biomass accumulation over time in days (Lapointe and Tenore, 1981). The inverse of μ corresponds to biomass doubling time in days.

2.4. Diving-PAM methods

Walz Diving-PAM fluorometry measurements for all macroalgal species were conducted using the universal sample holder (USH) with the optical fiber set at a 90° angle approximately 5 mm away. The external PAR sensor was turned off to conduct an RLC based off the calibrated internal actinic table of PAR values. For this study, the typical 10 s between collections and the highest actinic light setting available were utilized to calculate RLCs for light-adapted macroalgae in the laboratory and field at PAR irradiances of 0, 192, 287, 390, 572, 768, 1151, 1645, and 2398 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The factory absorption factor (AF) of 0.84 for rETR calculations was utilized for all macroalgal species (Beer and Axelsson, 2004; Longstaff et al., 2002; Saroussi and Beer, 2007a,b; Silva et al., 1998).

2.5. RLC curve-fitting model

All RLCs were curve-fitted using the following modified equation in the absence of photoinhibition ($\beta = 0$) derived from Platt et al.'s (1980) original curve fitting model:

$$P = P_m \left[1 - e^{-\left(\frac{\alpha E_d}{P_m}\right)} \right]$$

where P_m is the photosynthetic capacity at saturating PAR, α is the initial slope of the first 3 rETR values of the RLC, and E_d is the downwelling irradiance for the curve-fitting parameter, P (Ralph and Gademann, 2005). Hereafter, P from this equation is denoted as P_{RLC} for this study.

2.6. Laboratory nutrient enrichment experiment

Nutrient enrichment experiments were conducted in the laboratory at HBOI in incubators (Percival Intellus) to calculate specific algal growth rates and RLCs of *U. lactuca* in similar field temperatures and light levels for comparison to nutrient-rich Titusville. Due to limitations of the Diving-PAM on quantifying the photosynthetic apparatus of highly branched rhodophytes (Beer and Axelsson, 2004; Saroussi and Beer, 2007a,b), only *U. lactuca* was used for laboratory nutrient enrichment. Algal samples were collected from mass culture at HBOI in an N-limited environment. A 3 × 3 factorial design consisted of 0, 20, and 40 $\mu\text{M NH}_4^+$; 0, 10, and 20 $\mu\text{M NO}_3^-$; and 0, 1, and 2 μM soluble reactive phosphorus (SRP) with 3 replicates per treatment. Both NO_3^- and NH_4^+ were used as N sources to match IRL site water quality conditions and compare data to in situ *U. lactuca* growth rates. Specimens were kept in the incubators at 20 °C, immersed in an Instant Ocean synthetic seawater solution made at ~35‰ in 1-L Wheaton borosilicate wide-mouth containers for a total of 60 h (2.5 days). Algae were kept at diurnal rhythms where the light intensity was incrementally increased each hour from 0 to 500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 12 h and incrementally decreased each hour 12 h. All RLCs in the lab were taken with plants acclimated to 250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in incubators.

2.7. Surface area:volume analysis

A NextEngine 3-D Scanner was used to calculate the relative surface areas of *U. lactuca*, *H. musciformis*, and *G. tikvahiae*. Approximately 2 g wet weight of each sample was dried and painted with floral paint (Design Master Colortool Spray; Colors 676 Basil and 710 Burgandy). The algae (4 replicates of each species) were sprayed with floral paint to preserve structure and to enhance laser capture. These data allow assessment of quantified growth strategies of three morphologically different species by comparing SA:V ratios in cm² g dry wt.⁻¹.

2.8. Interspecies physiological comparisons

Laboratory RLCs of *U. lactuca*, *G. tikvahiae*, and *H. musciformis* were measured to establish instantaneous inherent physiological differences among macroalgae. Algae were removed from outside cultures at HBOI and were immediately brought back to the lab for analysis. Specimens were kept in incubators (Percival Intellus) at 20 °C, immersed in collection water in 1-L Wheaton borosilicate wide-mouth containers for

Table 1

Water quality data from the IRL in 2011/2012 (Lapointe *pers. comm.*; sites NIRL 2; CIRL 5) from northern and central sites that are close to sites used in this study.

Date	Site ID	NH ₄ ⁺ (μM)	NO ₃ ⁻ (μM)	DIN (μM)	SRP (μM)	DIN:SRP	TDN (μM)	TDP (μM)	TDN:TDP	f-ratio
6/22/11	NIRL2	0.5 ± 0.1	0.4 ± 0.05	0.8 ± 0.1	0.5 ± 0.2	1.7 ± 0.3	103.7 ± 16.9	2.4 ± 0.4	44 ± 6.4	0.5 ± 0.1
12/20/11	NIRL2	0.3 ± 0.04	0.05 ± 0.01	0.4 ± 0.03	0.2 ± 0.01	1.9 ± 0.1	76.8 ± 0.5	1.3 ± 0.1	60.7 ± 4.2	0.1 ± 0.03
8/8/12	NIRL2	0.7 ± 0.2	0.2 ± 0.04	0.9 ± 0.2	0.3 ± 0.04	3.1 ± 0.4	76.6 ± 3.7	1.4 ± 0.1	56.7 ± 4.6	0.3 ± 0.1
6/7/11	CIRL5	0.4 ± 0.02	0.3 ± 0.0	0.7 ± 0.0	0.3 ± 0.0	2.2 ± 0.02	25.0 ± 0.4	1.02 ± 1.1	24.7 ± 2.5	0.5 ± 0.03
11/10/11	CIRL5	3.1 ± 0.01	3.7 ± 0.1	6.8 ± 0.1	0.1 ± 0.01	48.5 ± 3.2	37.0 ± 1.7	0.7 ± 0.1	51.0 ± 2.5	0.6 ± 0.01
9/12/12	CIRL5	0.6 ± 0.2	0.3 ± 0.1	0.9 ± 0.1	0.2 ± 0.01	4.8 ± 0.4	30.0 ± 3.7	1.1 ± 0.1	28.5 ± 3.6	0.3 ± 0.1

1 hour acclimated to $250 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ prior to RLC measurements. Acclimation was done to establish photosynthetic saturation baselines.

2.9. Statistical analyses

MANOVA analyses were performed for dissolved nutrients NH_4^+ , NO_3^- , DIN, TDN, SRP, and TDP (Table 1; R 3.1.3), algal growth rate calculations (μ) in the field (SAS 9.3) between sites and season in Titusville (NIRL2) and HBOI, (CIRL2), and on laboratory nutrient enrichment experiment on *U. lactuca* growth and photosynthesis (μ and P_{RLC} ; R 3.1.3). Nutrient data were non-normal for site (Shapiro Wilk's $p < 0.010$) and season (Shapiro Wilk's $p < 0.017$), but normal for site and season interactions (Shapiro Wilk's $p < 0.117$). Field growth rate data were non-normal (Shapiro Wilk's $p < 0.005$). Laboratory nutrient enrichment pairwise comparisons were made for treatments 5 and 9 with a two-fold increase in nutrient concentration and constant DIN:SRP ratios at 30:1. Residuals of the data were normal.

One-way ANOVAs were conducted for SA:V ratios ($\text{cm}^2 \text{g dry wt.}^{-1}$) and laboratory RLCs of *U. lactuca*, *G. tikvahiae*, and *H. musciformis* (SAS 9.3). Residuals of the SA:V data were normal (Shapiro–Wilk's $p < 0.925$) and homogeneous (Levene's test). Residuals of the natural log transformed P_{RLC} data were normal (Shapiro–Wilk's $p < 0.053$) but not homogeneous (Levene's test; ($F_{2, 71} = 4.16, p < 0.020$)).

3. Results

3.1. Dissolved nutrient comparisons among sites

Dissolved nutrient concentrations (NH_4^+ , NO_3^- , DIN, SRP, TDN, and TDP) from the water quality data (Table 1) were analyzed to see if there were differences between Titusville and HBOI sites in the IRL. The overall MANOVA was significant for all nutrients between sites: NH_4^+ ($F_{1, 14} = 5.36, p < 0.036$), NO_3^- ($F_{1, 14} = 5.01, p < 0.042$), DIN ($F_{1, 14} = 5.28, p < 0.037$), SRP ($F_{1, 14} = 15.90, p < 0.001$), TDN ($F_{1, 14} = 270.84, p < 0.001$), and TDP ($F_{1, 14} = 70.83, p < 0.001$). Despite P-limitation in the NIRL shown by total dissolved nitrogen (TDN):TDP ratios $> 30:1$, SRP and TDP concentrations are still significantly higher due to increased urbanization. There was no seasonality significance in nutrient concentrations except for SRP ($F_{1, 14} = 42.61, p < 0.001$), TDP ($F_{1, 14} = 41.60, p < 0.001$) and TDN ($F_{1, 14} = 6.80, p < 0.021$). No site and season combined interactions were significant except for TDN ($F_{1, 14} = 24.99, p < 0.001$) and TDP ($F_{1, 14} = 25.55, p < 0.001$).

3.2. Field specific growth rates

Data shown in Fig. 2 corroborate a physiological difference among *U. lactuca*, *G. tikvahiae*, and *H. musciformis* relating to growth rates, site locations, and seasonality. Contrasts reveal *U. lactuca* had significantly higher growth rates than rhodophytes at Titusville and HBOI in November and June 2012 ($F_{1, 36} = 81.40, p < 0.001$). Average growth rates \pm SD in June 2012 of *U. lactuca* were 0.27 ± 0.03 doublings d^{-1} in Titusville and 0.12 ± 0.02 doublings d^{-1} at HBOI compared to November 2012 growth rates in Titusville of 0.49 ± 0.07 doublings d^{-1} and 0.26 ± 0.05 doublings d^{-1} at HBOI. These growth rates for *U. lactuca* correspond to a biomass doubling time of $\sim 3.7, 8.3, 2.0$, and 3.9 days, respectively. Both highly branched rhodophytes had significantly different specific growth rates between sites and seasons ($F_{1, 36} = 10.73, p < 0.002$). In particular, *H. musciformis* had an average growth rate of 0.35 ± 0.03 doublings d^{-1} compared to 0.14 ± 0.05 doublings d^{-1} for *G. tikvahiae*. These growth rates correspond to a biomass doubling time of 2.9 days for *H. musciformis*, which is close to the fastest doubling time of 2 days for *U. lactuca* at the same site and season.

MANOVA analysis reveals a three-way interaction among sites, seasons, and algae ($F_{2, 36} = 7.33$, Wilk's Lambda $p < 0.002$, $R^2 = 0.29$) and a two-way interaction between algal species and season

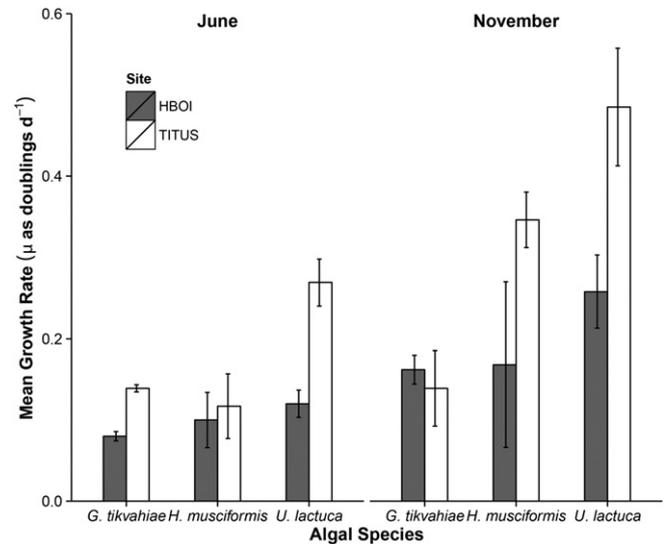


Fig. 2. Graph of growth rates (μ) of *G. tikvahiae*, *H. musciformis*, and *U. lactuca* at HBOI and Titusville (TITUS) expressed as doublings d^{-1} ($n = 4$). Data represent means \pm SD over 14 days in June 2012 and November 2012. Analyses for site ($F_{1, 36} = 58.49, p < 0.001$), season ($F_{1, 36} = 85.09, p < 0.001$), and algae, site, and season interactions ($F_{2, 36} = 7.33, p < 0.002$) were all significant. Contrasts such as *U. lactuca* versus rhodophytes ($F_{1, 36} = 81.40, p < 0.001$), and *H. musciformis* versus *G. tikvahiae* ($F_{1, 36} = 38.14, p < 0.001$) were also significant.

sampling ($F_{2, 36} = 9.71$, Wilk's Lambda $p < 0.004$, $R^2 = 0.35$) on algal growth rates. These results indicate species specific preferences to high nutrients and seasonality. Temporal differences existed between field experiments in June and November 2012 ($F_{1, 36} = 85.09$, Wilk's Lambda $p < 0.001$, $R^2 = 0.70$) and all macroalgae grew faster in nutrient-rich Titusville compared to HBOI ($F_{1, 36} = 58.49$, Wilk's Lambda $p < 0.001$, $R^2 = 0.62$).

3.3. Nutrient enrichment experiment

MANOVA was significant for DIN ($F_{1, 23} = 17.21, p < 0.001$), but not SRP ($F_{1, 23} = 3.69, p < 0.087$) and the interaction was not significant ($F_{1, 23} = 3.50, p < 0.245$). The combinations of NO_3^- and NH_4^+ (DIN) had the greatest effect on RLCs ($F_{1, 23} = 24.81, p < 0.001$) compared to PO_4^{3-} (SRP), which was not significant ($F_{1, 23} = 3.15, p < 0.089$). The DIN and SRP interaction was also not significant ($F_{1, 23} = 1.12, p < 0.301$). Overall, DIN alone increased RLCs of *U. lactuca* three-fold. A *posteriori* contrasts of RLCs in the high nutrient treatment (9) compared to its lower nutrient counterpart (treatment 5) with 30:1 N:P ratio were significant ($F_{1, 23} = 14.02, p < 0.020$) revealing that nutrient concentrations, not N:P ratios, regulate photosynthesis and growth (Fig. 3).

DIN and SRP similarly affected growth rates of *U. lactuca* ($F_{1, 23} = 84.06, p < 0.001$; $F_{1, 23} = 20.05, p < 0.001$), respectively. The combined nutrient interaction was not significant ($F_{1, 23} = 11.79, p < 0.110$). High nutrient concentrations similarly had a greater effect on growth rates than N:P ratios from contrasts ($F_{1, 23} = 46.00, p < 0.002$). Additionally, the doubling time for biomass decreased from ~ 9.1 days (average $\mu \pm$ SD; $\mu = 0.11 \pm 0.06$) to 2.5 days ($\mu = 0.40 \pm 0.11$) with increasing nutrient concentrations (treatment 1 compared to treatment 9). Specifically, biomass doubling time decreased two-fold from 4.5 days ($\mu = 0.22 \pm 0.11$) to 2.5 days ($\mu = 0.40 \pm 0.11$) with increasing nutrient concentrations and constant N:P ratios (30:1) for treatments 5 and 9, respectively (Fig. 4).

3.4. Surface area:volume analysis

SA:V ratios of macroalgae to quantify morphological differences among species measured as average (\pm coefficients of variation, %) for

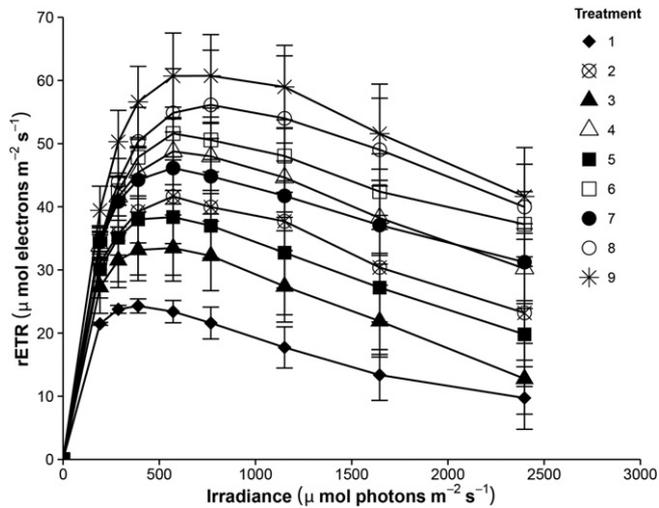


Fig. 3. Final RLC graphs from the nutrient pulsing experiment after 60 hour incubations at 20 °C on *U. lactuca*. Treatment 1 is the control (0 μM SRP and DIN). Treatment 2 (1 μM SRP, 0 μM DIN). Treatment 3 (2 μM SRP, 0 μM DIN). Treatment 4 (0 μM SRP, 30 μM DIN). Treatment 5 (1 μM SRP, 30 μM DIN). Treatment 6 (2 μM SRP, 30 μM DIN). Treatment 7 (0 μM SRP, 60 μM DIN). Treatment 8 (1 μM SRP, 60 μM DIN). Treatment 9 (2 μM SRP, 60 μM DIN). Data represent means ± SE. Contrasts between treatments 5 and 9 had similar RLCs ($F_{1,23} = 14.02$, $p < 0.020$).

U. lactuca, *G. tikvahiae*, *H. musciformis*, and were 565.2 ± 2.15 , 91.1 ± 1.81 , and 110.7 ± 3.97 cm² g dry wt.⁻¹, respectively. Overall, SA:V ratios ($F_{2,9} = 2277.86$, $p < 0.001$, $R^2 = 0.998$; one-way ANOVA) and contrasts between *U. lactuca* and rhodophytes ($F_{1,9} = 4549.69$, $p < 0.001$) were significant. In addition, contrasts revealed higher SA:V ratios in *H. musciformis* than *G. tikvahiae* ($F_{1,9} = 6.03$, $p < 0.036$).

3.5. Interspecies physiological differences

Laboratory RLCs of *U. lactuca*, *G. tikvahiae*, and *H. musciformis* were measured to establish distinct physiological characteristics among macroalgae. P_{RLC} differed significantly among species ($F_{2,71} = 33.04$, $p < 0.001$, $R^2 = 0.48$; one-way ANOVA, Fig. 5). *U. lactuca* had a significantly higher photosynthetic response (~2-fold increase) compared to

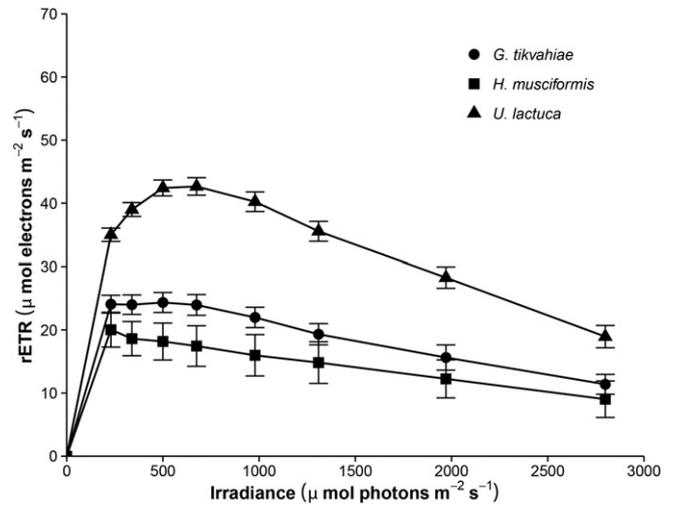


Fig. 5. RLC graph for *U. lactuca* ($n = 27$), *G. tikvahiae* ($n = 24$), and *H. musciformis* ($n = 21$) conducted in the lab. RLCs between *U. lactuca* and rhodophytes were significantly different ($F_{1,71} = 62.62$, $p < 0.001$). Contrasts between rhodophytes revealed similarities in photosynthesis ($F_{1,71} = 3.10$, $p < 0.083$). Data represent means ± SE.

rhodophytes ($F_{1,71} = 62.62$, $p < 0.001$). In contrast, P_{RLC} of the rhodophytes, *G. tikvahiae* and *H. musciformis*, were not significantly different ($F_{1,71} = 3.10$, $p < 0.083$).

4. Discussion

Our study demonstrates the superior physiological capacity of *Ulva* spp. to compete with other macroalgae for dominance in nutrient-enriched, eutrophic coastal waters. From our field caging experiments, *U. lactuca* had the fastest growth rates compared to the rhodophytes, *H. musciformis* and *G. tikvahiae*. *Ulva lactuca* had the highest SA:V ratio and physiological profile quantified by RLCs. Finally, the superior growth of *U. lactuca* was demonstrated in the lab nutrient enrichment experiment with increased responses to nutrient concentrations rather than respective N:P ratios for the growth and photophysiology of *U. lactuca*.

4.1. Nutrient effects on growth rates in Titusville and HBOI

Growth rates from field caging experiments in this study for *U. lactuca* and *H. musciformis* are among the highest reported in the scientific literature. June 2012 growth rates in HBOI for *U. lactuca* are similar to *Ulva fasciata* growth rates at 0.12 doublings d⁻¹ (Lapointe and Tenore, 1981). Interestingly, our growth rates in November 2012 in Titusville are the fastest for *U. lactuca* at 0.49 doublings d⁻¹ and for *H. musciformis* at 0.35 doublings d⁻¹. The fastest known growth rates for *G. tikvahiae* (0.37 ± 0.01 doublings d⁻¹) were found by Lapointe et al.'s (1984a) work in flowing-seawater flume studies designed for optimized water flow and aeration for rapid *G. tikvahiae* growth, whereas our caging studies were conducted in situ. Our relatively low growth rates of *G. tikvahiae* demonstrate that *H. musciformis* and *U. lactuca* can outperform in continuously high nutrient environments. Studies reveal similar results where high nutrient concentrations stimulate macroalgal growth rates (Lapointe, 1987; Lapointe and Tenore, 1981; Peckol et al., 1994) and photosynthetic efficiencies (Lapointe, 1987; Lapointe and Duke, 1984; Lapointe and Tenore, 1981; Lapointe et al., 1984a).

The rapid growth of *U. lactuca* co-occurred with the highest TDN:TDP ratio (60.7), indicating a N-rich (P-limited) system (TDN:TDP > 30:1) and the lowest overall f -ratio of 0.1 (Table 1). Modified f -ratios ($\text{NO}_3^-/\text{NH}_4^+ + \text{NO}_3^-$) from Table 1 were calculated to determine the major source of available DIN (Lapointe et al., 2004). An f -ratio value > 0.5 indicates dominance of NO_3^- and values < 0.5 indicate dominance of NH_4^+ in shallow coastal estuaries like the IRL. All f -ratios for

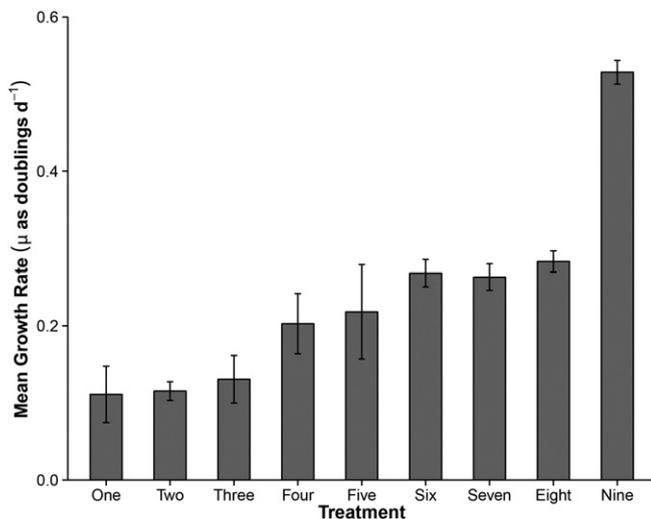


Fig. 4. Mean growth rate (μ , doublings d⁻¹) ± SE from laboratory nutrient enrichment experiment after 60 hour incubations at 20 °C on *U. lactuca*. Treatment 1 is the control (0 μM SRP and DIN). Treatment 2 (1 μM SRP, 0 μM DIN). Treatment 3 (2 μM SRP, 0 μM DIN). Treatment 4 (0 μM SRP, 30 μM DIN). Treatment 5 (1 μM SRP, 30 μM DIN). Treatment 6 (2 μM SRP, 30 μM DIN). Treatment 7 (0 μM SRP, 60 μM DIN). Treatment 8 (1 μM SRP, 60 μM DIN). Treatment 9 (2 μM SRP, 60 μM DIN). Contrasts between treatments 5 and 9 revealed nutrient concentrations significantly affected growth rates ($F_{1,23} = 46.00$, $p < 0.002$).

sites in Titusville (NIRL), HBOI (CIRL), were ≤ 0.5 , indicating NH_4^+ dominating DIN in the water column relative to NO_3^- , except for the CIRL site during the 2011 anomalous winter sampling of 0.6 ± 0.01 ; suggesting DIN was dominated by NO_3^- at this time. Additionally, Frost-Christensen and Sand-Jensen (1990) found that the growth of *U. lactuca* was not limited by dissolved inorganic carbon in laboratory nutrient assays and other factors will most likely impact the growth of this chlorophyte, such as light attenuation and nutrient availability.

The shallow water column at both Titusville and HBOI typically show enrichment with NH_4^+ , which coupled with high δN^{15} values, suggest wastewater input (Lapointe et al., 2015). The elevated NH_4^+ can lead to HABs because many macroalgae, like *Ulva* spp., preferentially assimilate NH_4^+ rather than NO_3^- (D'Elia and DeBoer, 1978; Jones et al., 1996; Teichberg et al., 2008). The northern IRL is highly urbanized and relies largely on septic tanks for on-site sewage treatment and disposal (Barile, 2004; Bricker et al., 2007, 2008). Septic tanks are well known as contamination sources to surficial groundwaters that discharge NH_4^+ and SRP into coastal waters (Lapointe et al., 1990; Lapointe and Krupa, 1995). In contrast, our data show that *U. lactuca* does not grow as well in more N-limited environments, with respectively low TDP, like the HBOI site.

Anomalous TDN:TDP ratio (51.0) for HBOI during the 2011 winter could be related to heavy rain events that cause discharge of N-rich groundwaters (Table 1). Overall, nutrient concentrations of TDN and TDP were significantly higher in Titusville than HBOI, although NH_4^+ , NO_3^- , DIN, and SRP did not differ significantly. *Ulva* spp. do not have N storage abilities, like *G. tikvahiae* and *H. musciformis* (Fujita, 1985; Teichberg et al., 2007) and ready assimilation of DIN leads to increased growth rates compared to rhodophytes in nutrient-rich areas like Titusville. Both *G. tikvahiae* and *H. musciformis* had a deeper red pigmentation in Titusville than at HBOI with green pigmentation, visually verifying N-limitation in the central IRL (Lapointe and Ryther, 1979; Lapointe et al., 1976). Therefore, spatial differences in Titusville and HBOI are largely due to changes in nutrient availability because there were no major differences in salinity, temperature, and dissolved oxygen levels between each site and season.

The higher growth rates in *U. lactuca* compared to *H. musciformis* and *G. tikvahiae* are related to a higher SA:V ratio from a flat thallus, multiple chloroplast construction. *Ulva* spp. are more competitive in nutrient-rich waters (functional/form model; Littler, 1980) demonstrated by increased growth rates and higher SA:V ratios of *H. musciformis* (November 2012 in Titusville) than *G. tikvahiae* (Carpenter, 1990) where finely branched forms of macroalgae have fewer resources allocated towards thalli construction, thus outperforming coarsely branched counterparts. Similar results were found in genetically different clones of *G. tikvahiae* with altered ecological and physiological fitness quantified by increasing SA:V ratios, photosynthesis, and growth rates (Hanisak et al., 1988). 3-D scanning provides easy and vital information on the roles of nutrient uptake and morphological growth. Taylor et al. (1999) report higher SA:V ratios with NH_4^+ uptake in chlorophytes like *Ulva* and *Enteromorpha* spp., but rhodophytes like *Osmundaria colensoi* and phaeophytes like *Zonaria tumeriana* showed sustained growth patterns less likely to deplete nutrient sources over time. Reports of rhodophyte blooms in Lee County 2003/2004 (Lapointe and Bedford, 2007) and Maui (Dailer et al., 2010; Lapointe and Bedford, 2011) dominated by *H. musciformis* fueled by elevated nutrients also support this hypothesis.

4.2. Nutrient concentration versus ratio effects on RLCs and growth

Nutrient enrichment can increase photosynthetic capacities via RLCs and P vs. I curves (Lapointe, 1997; Teichberg et al., 2013) in the dominant species in a community from “bottom-up” ecosystem control affecting macroalgal bloom nutrition. From our laboratory nutrient enrichment studies, higher nutrient concentrations affected macroalgal photosynthesis and growth more than N:P ratios. SRP concentrations did not significantly affect photosynthesis possibly *U. lactuca* because

samples were taken from HBOI cultures in N-limited conditions (Lapointe and Ryther, 1979).

N:P ratios can differ widely among macroalgae, as a result of taxonomic differences in biochemical composition, nutrient assimilation characteristics, and environmental conditions. N storage occurs in rhodophyte phycobiliproteins (Dawes et al., 1984; Lapointe, 1981; Lapointe et al., 1984b; Lapointe and Ryther, 1978) and a N-limited plant (C:N > 13:1) will have the highest NH_4^+ uptake rates (D'Elia and DeBoer, 1978; Hanisak, 1990) and NO_3^- suppression occurs when NH_4^+ concentrations are 0.5–1.0 μM (D'Elia and DeBoer, 1978). Only in November 2011 sampling, did CIRL5 have NH_4^+ concentrations greater than 1.0 μM ($3.1 \pm 0.01 \mu\text{M}$), suggesting NH_4^+ suppression. For example, intraspecific variations in N:P tissue ratios were found between the Caribbean and southeast Florida within the invasive macroalga, *Codium isthmocladum*, due to changes in SRP concentrations and DIN:SRP ratios (Lapointe et al., 2005). Haan et al. (2013) even detected co-limitation in the macroalgae, *Lobophora variegata*, in the Curaçao coral reefs using Nutrient-Induced Fluorescence Transient (NIFT) techniques from modified PAM fluorometry. Fong et al. (2004) similarly showed increased growth rate in the chlorophyte, *Enteromorpha intestinalis*, in a four-week nutrient enrichment experiment despite constant N:P ratios of 10:1 in all treatments. Therefore, N:P ratios alone do not reflect the degree of N- or P-limitation or interspecific algal abundance within ecosystems (Fong et al., 2001) especially if neither N or P concentrations are limiting (Davidson et al., 2012) and C:N:P tissue ratios provide better predictors of the limiting nutrient in macroalgae (Lapointe, 1987). Similar results were found in aquatic ecosystems showing N-limitation frequenting lakes with low TN:TP ratios (≤ 14 ; Downing and McCauley, 1992).

Numeric nutrient criteria (NNC) have been developed under section 62-302.531, F.A.C. in Florida to help moderate high-biomass algal blooms (Kaufman et al., 2010; SFWMD and SJRWMD, 2002; Steward et al., 2003). Lapointe et al. (1992) suggested DIN and SRP water column concentrations of ~ 1.0 and $0.1 \mu\text{M}$, respectively, can support opportunistic rhodophyte (D'Elia and DeBoer, 1978) and chlorophyte (Lapointe, 1981, 1997) macroalgal blooms. While IRL nutrient data show relatively low DIN concentrations, SRP levels were all $\geq 0.1 \mu\text{M}$. Elevated SRP levels in nutrient-rich environments played a primary role in supporting *Ulva* spp. growth rates in Titusville due to a limited capacity for nutrient storage and requiring consistently high nutrient concentrations (Fujita, 1985).

4.3. *Ulva* spp. bloom dynamics

Ulva spp. have demonstrated bloom phenomena in a wide variety of nutrient-rich environments (Naldi and Viaroli, 2002; Sfriso et al., 1992; Teichberg et al., 2007, 2010) because they are the most efficient macroalga photosynthetically and morphologically (Carpenter, 1990; Littler, 1980; Lotze and Schramm, 2000). *Ulva* spp. are opportunistic bloomers in nutrient-rich sites and assimilate NH_4^+ more easily from anthropogenic sources, like Titusville. *Ulva* spp. can bloom in both temperate and tropical climatic zones. For instance, *U. lactuca* and *G. tikvahiae* had the highest δN^{15} tissue content of $\sim +12\text{‰}$ collected near a sewage outfall in Buccoo Bay, Tobago (Lapointe et al., 2010) and of $\sim +14$ – 17‰ for *Ulva* spp. and $+8$ – 12‰ for *Gracilaria* spp. in Narragansett Bay, RI, USA (Thorner et al., 2008). The IRL is a transitional zone where *Ulva* spp. have the potential to form HABs along the entire eastern coast of Florida, especially when enriched with anthropogenic N and P. In the past, the IRL system has been dominated by rhodophyte blooms of predominantly *Hypnea* spp. and *Gracilaria* spp. and the “super bloom” of *Resultor* sp. in 2011 that was followed by a brown tide of *A. lagunensis* (DeYoe et al., 1997; Gobler et al., 2013) has now reportedly been replaced with *Ulva* spp. and *Chaetomorpha* spp. where the highest nitrogen levels have been found to date in the northern, urbanized, and fragmented sections of the IRL (SJRWMD, 2014; Lori Morris, 2015 pers. comm.).

4.4. Macroalgal bioindicators for environmental management in the IRL

Our data demonstrate biological responses of macroalgal blooms to nutrient enrichment under different environmental regimes, which are fundamental to the development of NNC for the IRL. We have found that N:P ratios are not sufficient to establish limiting nutrients and environmental management strategies because N and P dynamics on algal growth are complex, dynamic and require synchronous reduction (Hanisak, 1990; Waite and Mitchell, 1972). Our water quality data for Titusville (NIRL) suggest significant increases in nutrient loading compared to HBOI (CIRL) where *Ulva* spp. blooms are currently most likely to occur due to eutrophication, such as in the Yellow Sea and East China Sea during the summer of 2008 of *Ulva prolifera* (Hu et al., 2010) and historic *Ulva* spp. blooms in Boston Harbor (Sawyer, 1965).

These results are consistent with “bottom-up” ecosystem control (Lapointe, 1997) and have similar results to other studies (Fujita, 1985) demonstrating that macroalgal blooms are not only influenced by “top-down” ecosystem controls (Lubchenco, 1978). Specifically, an abundance of macroalgae in Titusville suggests high nutrient loads and potential HABs. The use of δN^{15} coupled with continuous nutrient and environmental monitoring data from *Enteromorpha* spp., for example, has also been suggested as viable bioindicators in estuarine environments along the southern California (Cohen and Fong, 2006) which is similar to phytoplankton competition where excessive and sometimes toxic blooms can occur in eutrophic areas from species specific dominance.

5. Conclusions

Results of this study show that three species of macroalgae, *U. lactuca*, *H. musciformis*, and *G. tikvahiae*, grew better in nutrient-rich compared to relatively nutrient-poor environments. *Ulva lactuca* had higher growth rates and photosynthetic capacity than both rhodophytes due to inherent physiological and morphological advantages. *Ulva lactuca* has higher SA:V ratios, photosynthetic efficiencies, and a flat sheet-like morphology, all contributing to its opportunistic bloom potential. Doubling rates of *U. lactuca* in Titusville during November 2012 field study and laboratory growth enrichment under high nutrient concentration treatments were 2 and 2.5 days, respectively. These data correspond with the ability of *Ulva* spp. to accumulate high biomass that eventually leads to macroalgal HABs.

Policies to limit N and P loading to coastal waters have been developed in Florida (FDEP, 2013) and P reduction strategies have not been successful for N reduction due to NH_4^+ atmospheric deposition (Howarth, 2008). We know now that nutrient limitation varies locally and regionally, as indicated by nutrient shifts in the IRL. The EPA has approved the NNC for the Indian River/St. Lucie region (Kaufman et al., 2010) along the IRL. Their mean goals for TN and TP are 1.54 and 0.12 mg l⁻¹, these correspond to nutrient concentrations for TDN and TDP water quality comparisons of ~ 109.99 μM and 3.87 μM , respectively. Our water quality data from the NIRL and CIRL were all below EPA TDN and TDP limits, even the highest TDN in NIRL during June 2011 (103.7 μM). Despite areas in the IRL meeting EPA nutrient criteria, *U. lactuca* still had a biomass doubling time of 2 days in Titusville during November 2012 and high growth potential for HAB formation.

State nutrient criteria from the IRL SWIM Plan has set more rigid TN targets at 50 μM and TP at 1.7 μM (SFWMD and SJRWMD, 2002; Steward et al., 2003). Our NIRL and CIRL water quality data were at or above these more realistic targets. Therefore, further mitigation is required to meet better standards to improve seagrass and ecosystem health in the IRL. However, no real action has been taken to assess the high to low nutrient concentration gradient dynamics to prevent HABs, like the recent brown tide “super bloom” in Mosquito Lagoon (Gobler et al., 2013). The data from this study: 1) support an existing N:P nutrient-limitation gradient within the IRL, 2) provide potential physiological methods for assessing macroalgal health with respect to

nutrient-limitation in addition to water quality and/or δN^{15} tissue data, and 3) support that both N and P reduction is necessary to control *Ulva* spp. growth for predictive HAB management strategies regionally. Studies have shown a strong relationship between high δN^{15} tissue content and high nutrient concentrations in the water column for macroalgae (Dailer et al., 2010; Fong et al., 2001; Lapointe, 1997; Lapointe et al., 2004) which in turn relate to higher growth responses (Fong et al., 2003). Managers can use these techniques to monitor and control N and P in synchrony (Howarth and Paerl, 2008) in efforts to mitigate potential HABs.

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