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

Lisa N.A. Whitehouse *, Brian E. Lapointe

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

A B S T R A C T

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 remotely 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 ± 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 (± coefficients of variation, %) 565.2 ± 2.15 cm2 g dry wt.−1 compared to lower ratios for H. musciformis (110.7 ± 3.97 cm2 g dry wt.−1) and G. tikvahiae (91.1 ± 1.81 cm2 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.

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 (Virmstein 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 (Philips 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 (DeVoe 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
Rosenberg and Ramus, 1984). Quantities with elevated nutrients (Carpenter, 1990; Littler, 1980; optimum photosynthetic productivity and growth strategies lending space in nutrient enriched coastal waters. Algal dominance extensive blooms in coastal waters impacted by nutrient pollution. Hu et al., 2010). Puerto Rico, Flamengo Sound, Brazil, (Teichberg et al., 2010) and in Massachusetts, San Antonio Bay, Argentina, Urias Estuary, Mexico, Jobos Bay, Okeechobee (Lapointe and Bedford, 2007). The chlorophyte Hypnea musciformis, 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, ΔF/ Fm’) 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 ecophysiology 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° 36’ 43.52″, −80° 48’ 17.05″) and at the HBOI (27° 32’ 10.57″, −80° 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:

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

where *N₀* 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 on 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 μmol photons m⁻² s⁻¹. The factory absorption factor (AF) of 0.84 for rETR calculations was utilized for all macroalgal species (Beer and Axlisson, 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 (β₁ = 0) derived from Platt et al.’s (1980) original curve-fitting model:

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

where *Pₘ* is the photosynthetic capacity at saturating PAR, *α* is the initial slope of the first 3 rETR values of the RLC, and *E₂* is the downwelling irradiance for the curve-fitting parameter, *P* (Ralph and Gademann, 2005). Hereafter, *P* from this equation is denoted as *Pₘₚₚ.*

### 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 Axlisson, 2004; Saroussi and Beer, 2007a,b), only *U. lactuca* was used for laboratory nutrient enrichment. Algae samples were collected from mass culture at HBOI in an N-limited environment. A 3 × 3 factorial design consisted of 0, 20, and 40 μM NH₄⁺; 0, 10, and 20 μM NO₃⁻; and 0, 1, and 2 μM soluble reactive phosphorus (SRP) with 3 replicates per treatment. Both NO₃⁻ and NH₄⁺ 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 μmol photons m⁻² s⁻¹ for 12 h and incrementally decreased each hour 12 h. All RLCs in the lab were taken with plants acclimated to 250 μmol photons m⁻² 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 Colorool 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

<table>
<thead>
<tr>
<th>Date</th>
<th>Site ID</th>
<th>NH₄⁺ (μM)</th>
<th>NO₃⁻ (μM)</th>
<th>DIN (μM)</th>
<th>SRP (μM)</th>
<th>DIN:SRP</th>
<th>TDN (μM)</th>
<th>TDP (μM)</th>
<th>TDN: TDP</th>
<th>f-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/22/11</td>
<td>NIRL2</td>
<td>0.5 ± 0.1</td>
<td>0.4 ± 0.05</td>
<td>0.8 ± 0.1</td>
<td>0.5 ± 0.2</td>
<td>1.7 ± 0.3</td>
<td>101.7 ± 16.9</td>
<td>2.4 ± 0.4</td>
<td>44 ± 6.4</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>12/20/11</td>
<td>NIRL2</td>
<td>0.3 ± 0.04</td>
<td>0.05 ± 0.01</td>
<td>0.4 ± 0.03</td>
<td>0.2 ± 0.01</td>
<td>1.9 ± 0.1</td>
<td>76.8 ± 0.5</td>
<td>1.3 ± 0.1</td>
<td>60.7 ± 4.2</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>8/8/12</td>
<td>NIRL2</td>
<td>0.7 ± 0.2</td>
<td>0.2 ± 0.04</td>
<td>0.9 ± 0.2</td>
<td>0.3 ± 0.04</td>
<td>3.1 ± 0.4</td>
<td>76.6 ± 3.7</td>
<td>1.4 ± 0.1</td>
<td>56.7 ± 0.6</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>6/7/11</td>
<td>CIRL5</td>
<td>0.4 ± 0.02</td>
<td>0.3 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>0.3 ± 0.0</td>
<td>2.2 ± 0.02</td>
<td>25.0 ± 0.4</td>
<td>1.02 ± 1.1</td>
<td>24.7 ± 2.5</td>
<td>0.5 ± 0.03</td>
</tr>
<tr>
<td>11/10/11</td>
<td>CIRL5</td>
<td>3.1 ± 0.01</td>
<td>3.7 ± 0.3</td>
<td>6.8 ± 0.1</td>
<td>0.1 ± 0.01</td>
<td>48.5 ± 3.2</td>
<td>37.0 ± 1.7</td>
<td>0.7 ± 0.1</td>
<td>51.0 ± 2.5</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>9/12/11</td>
<td>CIRL5</td>
<td>0.6 ± 0.2</td>
<td>0.3 ± 0.1</td>
<td>0.9 ± 0.1</td>
<td>0.2 ± 0.01</td>
<td>4.8 ± 0.4</td>
<td>30.0 ± 3.7</td>
<td>1.1 ± 0.1</td>
<td>28.5 ± 3.6</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>
1 hour acclimated to 250 μmol photons m$^{-2}$ 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, SRP, TDN, 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 PRLC; 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 (cm$^2$ 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 PRLC data were normal (Shapiro-Wilk’s p < 0.053) but 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$_{1, 23} =$ 5.36, p < 0.036), NO$_3^-$ (F$_{1, 23} =$ 5.01, p < 0.042), DIN (F$_{1, 23} =$ 5.28, p < 0.037), SRP (F$_{1, 23} =$ 15.90, p < 0.001), TDN (F$_{1, 23} =$ 270.84, p < 0.001), and TDP (F$_{1, 23} =$ 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$_{1, 23} =$ 42.61, p < 0.001), TDP (F$_{1, 23} =$ 41.60, p < 0.001) and TDN (F$_{1, 23} =$ 6.80, p < 0.021). No site and season combined interactions were significant except for TDN (F$_{1, 23} =$ 24.99, p < 0.001) and TDP (F$_{1, 23} =$ 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$_{1, 23} =$ 81.40, p < 0.001). Average growth rates ± 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 ~3.7, 8.3, 2.0, and 3.9 days, respectively. Both highly brched hydroids 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 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$_{1, 23} =$ 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$_4^-$ (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 μ ± SD; μ = 0.11 ± 0.06) to 2.5 days (μ = 0.40 ± 0.11) with increasing nutrient concentrations (treatment 1 compared to treatment 9). Specifically, biomass doubling time decreased two-fold from 4.5 days (μ = 0.22 ± 0.11) to 2.5 days (μ = 0.40 ± 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 (± coefficients of variation, %) for...
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 (F₂, 9 = 2277.86, p < 0.001, R² = 0.998; one-way ANOVA) and contrasts between U. lactuca and rhodophytes (F₁, 9 = 4549.69, p < 0.001) were significant. In addition, contrasts revealed higher SA:V ratios in H. musciformis than G. tikvahiae (F₁, 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, Pₐₕ differed significantly among species (F₂, 71 = 33.04, p < 0.001, R² = 0.48; one-way ANOVA, Fig. 5). U. lactuca had a significantly higher photosynthetic response (~2-fold increase) compared to rhodophytes (F₁, 71 = 62.62, p < 0.001). In contrast, Pₐₕ of the rhodophytes, G. tikvahiae and H. musciformis, were not significantly different (F₁, 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 (NO₃⁻/NH₄⁺ + NO₂⁻) 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 NO₃⁻ and values < 0.5 indicate dominance of NH₄⁺ in shallow coastal estuaries like the IRL. All f-ratios for...
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 others 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 DIN 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 turneriana_ 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 ± 0.01 μM), suggesting NH$_4^+$ suppression. For example, intraspecific variations in N:P tissue ratios were found between the Caribbean and southeast Florida within the invasive macroalgae, _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 Curacao 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 frequencies with low TN:TP ratios ≤ 14; Downing and Massey, 1992).

4.3. _Ulva_ spp. bloom dynamics

_Ulva_ spp. have demonstrated bloom phenomena in a wide variety of nutrient-rich environments (Naldi and Viarioli, 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 DIN uptake tissue content of +12% collected near a sewage outfall in Buzzcoo Bay, Tobago (Lapointe et al., 2010) and of +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 _Resulor_ spp. in 2011 that was followed by a brown tide of _A. ligmennis_ (DeVoe et al., 1997; Cobler 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. (SRJWMD, 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 (Pujita, 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 $^{15}$N 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$_3$ 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 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 SFRWMD, 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 δ$^{15}$N 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 δ$^{15}$N 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 synchony (Howarth and Paeel, 2008) in efforts to mitigate potential HABs.

Acknowledgments

This work was supported by the Harbor Branch Oceanographic Institute at Florida Atlantic University, Department of Environmental Sciences and the mentorship of Drs. Brian Lapointe, Dennis Hanisak, and Ed Proffitt. Special thanks to Laura Herren and Dr. Lauri Green for their mentorship and help while preparing this manuscript and Lori Morris from SJRWMD for information on Ulva spp. blooms in the IRL. Special thanks also to volunteers and supporters, Paul Whitehouse, Richard Vlaming, and Lynn Vlaming, whose hard work and aid made this work possible. [SI]

References


Carpenter, R.K., 1990. Competition among macroalgal: a physiological perspec-

tive. J. Phycol. 26, 6–12.


Florida Department of Environmental Protection (FDEP), 2013. FDEP GIS Data Review Numeric Nutrient Criteria, pp. 1–18.


