# Environmental influences on growth and morphology of *Thalassia testudinum*

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ABSTRACT: Key metrics of *Thalassia testudinum* aboveground morphology vary along a spatial gradient in concentrations of total phosphorus in the water column that has persisted for over a decade off the central Gulf of Mexico coast of peninsular Florida. Leaf area shoot$^{-1}$, shoot density, shoot height, and areal biomass of *T. testudinum*, the dominant seagrass, were measured at sites along this gradient. The roles of differential production and nutrient supply were assessed as potential drivers of these relationships via growth rates, quantities of carbon (C), nitrogen (N) and phosphorus (P), and stable isotope ratios of leaves ($\delta^{13}$C, $\delta^{15}$N). Leaf area shoot$^{-1}$, shoot height, and shoot production were positively correlated with total P (TP), whereas shoot density and leaf N:P and leaf C:P ratios were negatively correlated with TP. There were no significant relationships with TP for epiphyte load, areal biomass, areal production rate, or leaf C:N ratio. The results also suggested that *T. testudinum* maintained a relatively uniform aboveground biomass and specific growth rate across the range of P concentrations observed in the water column during this study. The findings highlight the extent of morphological plasticity of *T. testudinum.* Collectively, the morphology, growth rate, elemental content, and stable isotope findings offer compelling evidence that *T. testudinum* along the Gulf coast of peninsular Florida balances shoot morphology and density to maintain growth and survival across a wide range of nutrient supply.

KEY WORDS: Seagrass · Nutrients · Environmental gradients · Stable isotopes · Gulf of Mexico · Phenotypic plasticity · Phosphorus

## INTRODUCTION

Seagrasses form structurally complex meadows that provide a broad suite of ecosystem services, with total services valued up to ~US $2 million ha$^{-1}$ yr$^{-1}$ (Costanza et al. 1997, Vassallo et al. 2013, Blandon & Ermgassen 2014). For example, seagrasses stabilize sediments, reduce shoreline erosion, sequester carbon, improve water clarity, and provide extensive habitat that supports a large number of species, including economically valuable fishes and invertebrates (Orth et al. 1984, Iverson & Bittaker 1986, Duarte 1995, Mattson et al. 2007, Fourqurean et al. 2012). Many of these ecosystem services are manifest via interactions between the physical matrix formed by seagrass and the water column, sediments, and biota (e.g. Nepf & Vivoni 2000, Bartholomew 2002, Fourqurean et al. 2012, Hansen & Reidenbach 2013); therefore, changes in the morphology of seagrasses have important ecological implications.

Seagrasses should be expected to exhibit morphological variation along environmental gradients because they, like many vascular aquatic plants, adapt to life in a variable environment through extensive phenotypic plasticity (Sculthorpe 1967, Short 1983, Lee & Dunton 1999a). For example, seagrasses adjust photosynthetic efficiency, leaf growth, shoot and leaf size, reproductive strategy, and allocation of

carbon to aboveground and belowground structures in response to availability of nutrients, light regimes, environmental stability, and grazing (Zieman et al. 1984, Dawes & Tomasko 1988, Tomasko & Dawes 1989, Tomasko & Lapointe 1991, Lee & Dunton 1997, 1999a,b, Major & Dunton 2002, Peralta et al. 2002, Romero et al. 2006, van Tussenbroek et al. 2006, McDonald et al. 2016). Evidence that such plasticity can be phenotypic comes from Florida Bay, where *Thalassia testudinum* exhibits distinct morphological variations, but no distinct genetic differentiation, across zones with different abiotic conditions (Frankovich & Fourqurean 1997, Hackney & Durako 2004, Bricker et al. 2011).

Studies of intraspecific phenotypic variation in seagrasses across large-scale, natural gradients are generally lacking, but they are important for increasing our understanding of the capacity of seagrasses to adapt to increasing anthropogenic stressors (McDonald et al. 2016). Documenting adaptive, phenotypic responses along environmental gradients, such as gradients in nutrient supply, is an important first step in predicting detrimental changes in ecosystem function and resilience due to stress from anthropogenic stressors such as eutrophication and climate change (Peralta et al. 2005, Cabaço et al. 2009, McMahon et al. 2013, Collier et al. 2014, McDonald et al. 2016). A unique opportunity to explore the phenotypic plasticity of *T. testudinum* subjected to a large-scale gradient in water column nutrient and chlorophyll *a* (chl *a*) concentrations exists along the central Gulf of Mexico (hereafter called 'Gulf') coast of peninsular Florida, spanning a linear distance of 66 km.

This region has a shallow, gently sloping bathymetry that provides a favorable habitat for seagrass meadows, and it currently harbors one of the largest contiguous seagrass beds in the USA (Hale et al. 2004, Mattson et al. 2007). Previous work has revealed a persistent spatial gradient in total phosphorus (TP) and chl *a* concentrations in these coastal waters (Frazer et al. 1998). Apparently, the spatial gradient in TP is maintained through the interaction of surface and groundwater with Miocene phosphate deposits in central Florida, with a similar geological influence having been noted for Florida lakes (Espenshade & Spencer 1962, Canfield & Hoyer 1988, Bachmann et al. 2012). Empirical relationships and experimental evidence suggest widespread P-limitation of phytoplankton along the Gulf coast of peninsular Florida (Frazer et al. 2002, Hoyer et al. 2002, Jacoby et al. 2015).

While the influence of nutrient concentrations on phytoplankton production has been characterized for this region, the nature of the relationships between water column nutrient concentrations and seagrass morphology, growth, and elemental composition are poorly defined. We hypothesized that these characteristics of seagrasses would vary in relation to TP concentrations in the water column because other aspects of the ecosystem have already been linked with TP concentrations and because TP has been repeatedly identified as the strongest and most consistent spatial water quality gradient in this region (Frazer et al. 1998, Jacoby et al. 2015). Previous work in the southernmost region of the study area revealed that *T. testudinum* grew more rapidly in response to the addition of P, with no significant response to added nitrogen (Keller 2002), providing additional justification for the hypothesis that TP concentrations may be influencing *T. testudinum* growth and morphology. The light environment is generally suitable for seagrasses in this region (Choice et al. 2014) and total nitrogen concentrations (TN) in the water column do not exhibit clear spatial gradients. Thus, the central Gulf coast of peninsular Florida provides a spatial gradient that supports in-depth study of aboveground morphology, growth, and elemental composition of seagrasses in relation to water column concentrations of a key macronutrient. To this end, we quantified leaf area shoot$^{-1}$, shoot density, shoot height, and areal biomass of the dominant seagrass, *T. testudinum*, to determine how these key structural characteristics varied along a gradient in TP concentrations in the water column, and we assessed growth rates, elemental composition (C:N:P), and stable isotope ratios ($\delta^{13}$C and $\delta^{15}$N) of leaves to elucidate physiological variations that underpin morphological variations.

## MATERIALS AND METHODS

### Water quality

Water quality and seagrasses were sampled in coastal waters adjacent to the Weeki Wachee, Chassahowitzka, Homosassa, Crystal, and Waccasassa rivers as part of a long-term monitoring effort (Fig. 1). Monthly sampling of depth, dissolved oxygen, salinity, temperature, pH, light attenuation, chl *a* concentrations, color, and TP and TN concentrations has been conducted at 50 stations throughout these systems since 1997, and the results have produced a thorough understanding of temporal and spatial water quality variations in this system (Frazer et al. 1998, Jacoby et al. 2015). A subset (n = 10, Fig. 1) of these monitoring stations was selected to span the TP
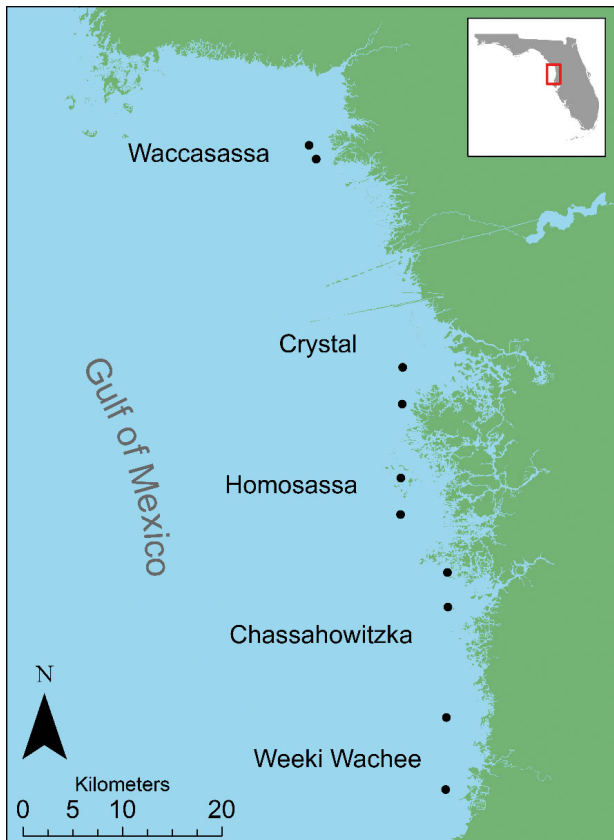
Fig. 1. Water quality and seagrass *Thalassia testudinum* sampling stations

gradient and reduce confounding variations (e.g. depth and salinity). Long-term (14 yr) and shorter-term (24 mo) means and standard deviations of water quality parameters were calculated for each station within the chosen subset. *Thalassia testudinum* could be found growing in near-monoculture at or near all of the selected stations. Water quality data from the 10 selected stations were inspected for spatial gradients in parameters other than TP and used in further analysis of seagrass metrics.

## Shoot morphology and density

Shoot morphology and density were sampled at each of the 10 stations in May and August of 2010, 2011, 2012, and in August 2013. During each sampling event from 2010 to 2012, 5 *T. testudinum* shoots were collected from 12 haphazardly tossed 0.25 m² quadrats at each station (up to 60 shoots per station), placed in labeled plastic bags, and frozen prior to morphological analysis. Additionally, in

each sampling period from 2010 to 2012, *T. testudinum* shoots were counted in 12 haphazardly tossed 0.0625 m² quadrats at each station. In August 2013, the above sampling procedures were repeated using a reduced number of quadrats per station (5 instead of 12). In the laboratory, epiphytes were removed, and lengths and widths of leaves were measured for each shoot to yield estimates of leaf area shoot$^{-1}$ (mm²) and shoot height (total length of shoot, mm). Measurements of all shoots from each station were averaged for each sampling period in which *T. testudinum* was recorded (n = 43). After measurements were taken, leaf material was dried at 65°C for at least 72 h and retained for elemental and stable isotope analysis. Shoot counts were averaged to yield estimates of shoot density (shoots m$^{-2}$) for each station by sampling period combination in which *T. testudinum* was recorded (n = 41). Cumulative frequency distributions for leaf lengths and widths were calculated and plotted for each coastal system to aid in visualizing differences in seagrass morphology across the TP gradient, and mean ± SD leaf lengths and widths were calculated for each coastal system.

## Growth rates

We investigated differential growth at each of the 10 seagrass sampling stations in August 2013 using standard leaf marking techniques (Zieman 1974). Three 10 × 20 cm quadrats were haphazardly tossed at least 5 m apart in *T. testudinum* meadows. Within each quadrat, all shoots were marked with a heavy gauge needle forced through all leaves in a shoot just above their basal meristems. Marked shoots were allowed to grow *in situ* for 7 to 9 d, after which they were collected and frozen until processing.

In the laboratory, epiphytic material was removed from *T. testudinum* leaves and retained. For each leaf with a hole, new leaf material and old leaf material (i.e. leaf tissue present at the time of marking) were separated by cutting through the hole with a razor blade. Unmarked leaves were considered new growth. Lengths and widths were recorded for all leaf segments, and dry weights (DWs) were recorded separately for old and new leaf segments from each quadrat after they had been dried at 65°C for at least 72 h. DWs of new leaf material divided by the number of *in situ* growth days represented *T. testudinum* growth rate (mg DW shoot$^{-1}$ d$^{-1}$). The quotient of new leaf mass and old leaf mass yielded a specific growth rate (mg new DW:mg old DW d$^{-1}$). We calculated

areal production at a station (mg DW m$^{-2}$ d$^{-1}$) by multiplying mean leaf production rate for shoots by the appropriate mean shoot density. Epiphytic material scraped from all shoots within a quadrat was pooled into a single, pre-weighed aluminum dish, dried at 65°C for at least 72 h, and weighed to yield estimates of mass-normalized epiphyte load (mg epiphyte DW g$^{-1}$ seagrass DW).

## Elemental composition and stable isotope ratios

Leaf material collected from each of the 10 sampling stations from 2010 to 2013 were subjected to elemental and stable isotope analyses to provide insight into potential limiting abiotic factors. Dried *T. testudinum* leaf samples were ground to a fine powder using a Crescent Wig-L-Bug dental amalgamator (Crescent Dental Manufacturing Company). Subsamples of the leaf material were then analyzed for C and N content and ratios of stable isotopes ($\delta^{13}$C and $\delta^{15}$N) using a NA1500 CNS elemental analyzer (Carlo Erba) interfaced with a Thermo Electron Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific). Leaf P content was quantified via colorimetric analysis after digesting additional subsamples with a persulfate solution (Kenney et al. 2001). Elemental compositions were used to calculate molar ratios for nutrients in leaf tissue (C:N, C:P, N:P). Molar nutrient ratios of seagrass leaves can be useful indicators of nutrient limitation because deviations from previously established values suggest the nutrient demands of seagrasses are not being met (Fourqurean et al. 2005). We considered leaf tissue N:P ratios above 30:1 (mol:mol) suggestive of P-limitation (Atkinson & Smith 1983, Duarte 1990). However, a positive growth response to P, but not N, addition in a controlled nutrient addition study would be required to obtain conclusive evidence of P-limitation. Stable isotope ratios were compared to published values for *T. testudinum*, and these comparisons provided insight into the sources of C and N and the rate of supply of C and N relative to seagrass demands.

## Statistical analyses

We explored relationships among water quality variables and seagrass metrics using the Biota and Environment Matching procedure (BEST) in PRIMER 6 (Clarke & Gorley 2006). This multivariate analysis uses a permutation approach to resolve the combination of independent variables (long-term means of water quality parameters, 1999 to 2013) that best explains variation in dependent variables (station means for leaf width, shoot height, leaf area, and shoot density). The BEST analysis was conducted on Bray-Curtis similarities calculated for range-normalized data, where each variable is bounded between 0 and 1. Range normalization retains the overall structure of the data while removing bias associated with variation in the absolute magnitude of the variables caused by the inherently differing scales of measurement. We employed regression analysis to refine the nature of the relationships with water quality variables identified by BEST for leaf area shoot$^{-1}$, shoot height, epiphyte load, shoot density, shoot growth rate, areal biomass, and areal production. We pooled data from all sampling events for regression analysis after ANCOVA revealed that the relationship between water quality and shoot height, leaf area, and shoot density were consistent across sampling events (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m570p057_supp.pdf). In addition, we explored correlations among water column nutrient concentrations, leaf elemental ratios, and leaf stable isotope ratios. ANCOVA and least squares regression were carried out using R statistical software (R Development Core Team 2014), with each dependent variable regressed against the 24-mo moving average for water quality parameters. *T. testudinum* is a long-lived species that integrates environmental conditions over timescales longer than days or months (Fourqurean et al. 2005), but little information is available regarding the length of time required for morphological adjustments to occur. We selected the 24-mo moving average as the independent variable for analyses because the small amount of evidence available suggests that morphological adjustments may occur on the scale of approximately 2 yr for long-lived species such as *T. testudinum* (Meinesz et al. 1993, van Tussenbroek 1996). Separate 24-mo means were calculated after each seagrass collection event (2010 to 2013) so that seagrass metrics were always regressed against water quality values representing the 24-mo period directly prior to collection. Log transformations were performed when non-normal or heteroscedastic residuals were encountered. Relationships among leaf nutrient ratios, TN, TP, molar N:P ratio in the water column (N:P$_{water}$), and stable isotope ratios were assessed with Spearman's rank correlations calculated in R (R Development Core Team 2014).

# RESULTS

## Water quality

Several water quality parameters exhibited moderate to strong spatial gradients, while others did not (Table 1). Long-term (15 yr, 1999 to 2013) mean ± SD depth and salinity were 1.3 ± 0.6 m and 24.1 ± 4.7 ‰, respectively, and these parameters did not exhibit predictable spatial variation (Table 1). Similarly, mean TN ranged from 303.9 to 486.4 µg l⁻¹, and it did not exhibit any clear gradient across stations (Table 1). Strong south to north gradients were observed for mean TP, which ranged from 6.9 µg l⁻¹ off the Weeki Wachee River to 25.8 µg l⁻¹ off the Waccasassa River, and for mean chl *a* concentrations, which generally increased in concert with TP and ranged from 0.9 µg l⁻¹ off the Weeki Wachee River to 5.3 µg l⁻¹ off the Waccasassa River (Table 1). In addition, color and the light attenuation coefficients ($K_d$) showed slight increases from south to north (Table 1). However, bottom irradiance at mean depth ($I_z$) was not spatially concordant with TP, chl *a* or color, and long-term values of percent surface irradiance at depth (20.8 to 60.6%) were above the regional light threshold (14 to 18%) identified for *Thalassia testudinum* (Table 1, Dixon & Leverone 1995, Choice et al. 2014). Thus, light penetration and water column nutrient enrichment were somewhat decoupled in this shallow region because the light environment was not severely degraded by the observed increases in chl *a* or color (Table 1). Results from the BEST analysis identified water column concentrations of TP and chl *a* as the best predictors of seagrass metrics (see Table S2 in the Supplement at www.int-res.com/articles/suppl/m570p057_supp.pdf). In this system, TP and chl *a* concentrations

Table 1. Long-term (14 yr, January 1999 to December 2012) and short-term (2 yr, January 2011 to December 2012) means ± SD of water quality parameters for stations along the total phosphorus (TP) gradient. Stations are arranged in geographic order from south to north (Fig. 1). DO: dissolved oxygen, Pt-Co: Platinum-Cobalt Scale, TN: total nitrogen, N:P$_{water}$: molar N:P ratio in the water column, $K_d$: light extinction coefficient, $I_z$: irradiance at mean bottom depth, % SI: percent surface irradiance at mean bottom depth

| Estuary | Depth (m) | Temp (°C) | Salinity (‰) | pH | DO (mg l⁻¹) | Color (Pt-Co) | TN (µg l⁻¹) | TP (µg l⁻¹) | Chl a (µg l⁻¹) | N:P$_{water}$ | $K_d$ (m⁻¹) | $I_z$ (µE m⁻² s⁻¹) | % SI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Long-term** | | | | | | | | | | | | | |
| Weeki Wachee | 1.1 ± 0.3 | 23.5 ± 6.0 | 21.9 ± 4.0 | 8.1 ± 0.3 | 7.1 ± 1.4 | 9.4 ± 4.1 | 441.5 ± 133.8 | 7.1 ± 2.6 | 1.0 ± 1.0 | 147.9 ± 60.7 | 0.9 ± 0.4 | 776.6 ± 330.3 | 53.5 ± 14.3 |
| Weeki Wachee | 1.6 ± 0.3 | 23.2 ± 6.1 | 24.1 ± 4.0 | 8.2 ± 0.3 | 7.1 ± 1.4 | 9.0 ± 5.2 | 394.5 ± 130.3 | 6.9 ± 2.6 | 0.9 ± 0.8 | 138.5 ± 62.4 | 0.8 ± 0.4 | 572.4 ± 269.2 | 39.5 ± 13.8 |
| Chassahowitzka | 1.4 ± 0.4 | 23.3 ± 6.0 | 21.2 ± 4.9 | 8.0 ± 0.3 | 7.0 ± 1.6 | 14.6 ± 6.9 | 381.6 ± 134.2 | 8.3 ± 3.6 | 1.1 ± 1.5 | 109.5 ± 41.5 | 1.1 ± 0.5 | 527.5 ± 284.3 | 36.3 ± 13.7 |
| Chassahowitzka | 1.0 ± 0.4 | 23.5 ± 6.0 | 22.1 ± 4.6 | 8.0 ± 0.3 | 7.0 ± 1.7 | 13.3 ± 4.0 | 441.9 ± 147.1 | 7.7 ± 3.4 | 0.8 ± 0.8 | 138.7 ± 58.6 | 1.3 ± 0.7 | 669.2 ± 334.4 | 43.6 ± 15.3 |
| Homosassa | 1.3 ± 0.3 | 23.0 ± 6.0 | 27.3 ± 4.1 | 8.0 ± 0.3 | 7.0 ± 1.8 | 10.6 ± 3.1 | 368.0 ± 115.8 | 9.1 ± 3.9 | 0.8 ± 0.9 | 100.6 ± 49.8 | 1.1 ± 0.5 | 565.5 ± 303.6 | 41.7 ± 17.0 |
| Homosassa | 0.5 ± 0.3 | 23.4 ± 5.8 | 27.9 ± 3.8 | 8.1 ± 0.3 | 7.6 ± 2.4 | 11.4 ± 4.0 | 400.3 ± 134.8 | 11.0 ± 7.0 | 1.8 ± 2.4 | 93.7 ± 49.6 | 1.7 ± 1.4 | 883.1 ± 388.3 | 60.6 ± 15.1 |
| Crystal | 1.0 ± 0.4 | 22.9 ± 6.1 | 23.3 ± 3.7 | 8.0 ± 0.3 | 7.2 ± 1.9 | 12.9 ± 4.2 | 367.1 ± 135.4 | 10.7 ± 3.9 | 1.4 ± 1.5 | 80.0 ± 29.3 | 1.2 ± 0.7 | 679.3 ± 327.1 | 48.5 ± 16.4 |
| Crystal | 2.4 ± 0.6 | 22.8 ± 6.1 | 24.9 ± 3.7 | 7.9 ± 0.2 | 7.0 ± 1.4 | 9.4 ± 4.1 | 303.9 ± 85.6 | 16.9 ± 7.3 | 2.6 ± 2.1 | 43.7 ± 15.1 | 1.1 ± 0.4 | 282.8 ± 202.0 | 20.8 ± 11.8 |
| Waccasassa | 1.2 ± 0.4 | 22.6 ± 6.1 | 24.4 ± 4.8 | 8.0 ± 0.3 | 7.2 ± 1.8 | 20.8 ± 19.5 | 486.4 ± 166.6 | 25.8 ± 17.0 | 5.3 ± 10.9 | 48.3 ± 18.2 | 1.5 ± 1.0 | 497.9 ± 296.8 | 35.4 ± 16.2 |
| **Short-term** | | | | | | | | | | | | | |
| Weeki Wachee | 1.1 ± 0.3 | 24.6 ± 5.1 | 22.5 ± 3.3 | 7.9 ± 0.2 | 7.2 ± 1.4 | 10.0 ± 6.5 | 415.5 ± 108.1 | 7.4 ± 2.8 | 1.0 ± 0.6 | 136.8 ± 57.1 | 0.8 ± 0.3 | 826.7 ± 299.8 | 54.7 ± 11.6 |
| Weeki Wachee | 1.7 ± 0.3 | 24.2 ± 5.1 | 23.7 ± 3.8 | 8.0 ± 0.2 | 7.1 ± 1.4 | 10.2 ± 7.2 | 351.8 ± 100.1 | 7.3 ± 2.6 | 0.8 ± 0.6 | 113.2 ± 38.7 | 0.7 ± 0.2 | 584.4 ± 257.3 | 39.5 ± 10.1 |
| Chassahowitzka | 1.4 ± 0.3 | 24.3 ± 5.2 | 20.0 ± 5.0 | 7.9 ± 0.3 | 7.5 ± 1.6 | 16.0 ± 9.2 | 352.7 ± 145.6 | 8.6 ± 3.7 | 1.6 ± 2.4 | 96.0 ± 36.6 | 0.9 ± 0.3 | 565.6 ± 258.9 | 35.5 ± 10.9 |
| Chassahowitzka | 1.0 ± 0.3 | 24.2 ± 4.9 | 21.5 ± 4.3 | 7.8 ± 0.3 | 7.2 ± 1.4 | 12.5 ± 3.6 | 379.1 ± 138.0 | 8.2 ± 5.3 | 1.0 ± 1.4 | 112.1 ± 35.0 | 1.0 ± 0.3 | 814.9 ± 335.6 | 46.5 ± 15.5 |
| Homosassa | 1.3 ± 0.4 | 23.2 ± 5.3 | 27.0 ± 3.7 | 7.9 ± 0.2 | 6.1 ± 1.4 | 11.5 ± 3.2 | 392.7 ± 94.8 | 9.4 ± 6.4 | 0.7 ± 0.4 | 113.0 ± 51.9 | 0.9 ± 0.4 | 542.1 ± 279.3 | 46.9 ± 18.9 |
| Homosassa | 0.5 ± 0.2 | 23.3 ± 5.0 | 27.8 ± 3.4 | 8.0 ± 0.2 | 6.2 ± 2.0 | 12.4 ± 3.9 | 428.2 ± 107.1 | 11.3 ± 6.5 | 1.9 ± 1.7 | 97.8 ± 39.5 | 1.5 ± 1.0 | 747.2 ± 333.4 | 68.8 ± 12.7 |
| Crystal | 0.9 ± 0.3 | 23.4 ± 5.1 | 22.4 ± 3.7 | 7.9 ± 0.2 | 6.3 ± 1.7 | 16.4 ± 4.2 | 393.6 ± 145.4 | 9.9 ± 2.2 | 1.5 ± 1.6 | 87.4 ± 23.8 | 1.0 ± 0.5 | 665.9 ± 330.2 | 55.0 ± 14.8 |
| Crystal | 2.3 ± 0.6 | 23.4 ± 5.2 | 23.9 ± 3.0 | 7.9 ± 0.2 | 6.2 ± 1.2 | 12.6 ± 5.1 | 331.8 ± 76.9 | 17.6 ± 9.1 | 3.4 ± 2.7 | 45.6 ± 12.3 | 1.0 ± 0.2 | 193.5 ± 102.3 | 18.0 ± 8.7 |
| Waccasassa[a] | 1.0 ± 0.3 | 21.8 ± 5.0 | 20.5 ± 2.7 | 8.3 ± 0.2 | 9.8 ± 1.5 | 22.6 ± 6.3 | 436.3 ± 187.1 | 20.6 ± 18.7 | 3.1 ± 1.6 | 63.8 ± 17.2 | 1.0 ± 0.2 | 729.6 ± 262.7 | 50.1 ± 11.6 |
| Waccasassa | 1.1 ± 0.3 | 23.5 ± 5.0 | 23.8 ± 4.3 | 7.9 ± 0.2 | 7.9 ± 1.8 | 19.0 ± 12.9 | 417.7 ± 93.9 | 18.1 ± 6.7 | 2.6 ± 2.1 | 54.3 ± 13.7 | 1.1 ± 0.3 | 533.2 ± 261.7 | 38.3 ± 10.5 |

[a]Only short-term data available (August 2013 to May 2014)

are highly collinear ($\rho = 0.98$, see Table S2), and previous experimental evidence revealed that chl *a* concentrations are largely driven by TP concentrations (Frazer et al. 2002). Given these results, we proceeded with regression analysis of *T. testudinum* metrics in relation to TP, the strongest and most consistent spatial water quality gradient.

## Shoot morphology and density

Regressions and cumulative frequency diagrams revealed several significant relationships between leaf morphology and TP. Sampling yielded detailed measurements of 5135 *T. testudinum* shoots and estimates of shoot density from approximately 700 quadrats. Shoot density was negatively related to TP (Fig. 2A) and decreased by roughly 26 shoots $m^{-2}$ for each 1 µg $l^{-1}$ increase in TP ($p < 0.001$), translating to an 80 to 90% reduction in shoot den-

sity from the lowest to the highest TP concentrations recorded in this study. Leaf area $shoot^{-1}$ (Fig. 2B) and shoot height (Fig. 2C) were positively related to TP ($p < 0.001$), indicating that shoots were taller and composed of more aboveground biomass as TP increased. In fact, leaf area $shoot^{-1}$ increased by an order of magnitude and shoot height increased by a factor of ~5 along the gradient. Areal biomass (g DW $m^{-2}$), or standing crop, was not significantly related to TP ($p = 0.143$), although the lowest values were found at stations with lower TP. Cumulative frequency distributions showed clear shifts toward longer (Fig. 3A) and wider leaves (Fig. 3B) in systems with higher TP (Table 2). Regressions failed to reveal any significant relationships between epiphyte load and TP. Mass-normalized epiphyte load (mg epiphyte DW $g^{-1}$ seagrass DW) varied between 60.6 and 987.2 and was not correlated with the TP gradient ($p = 0.892$).
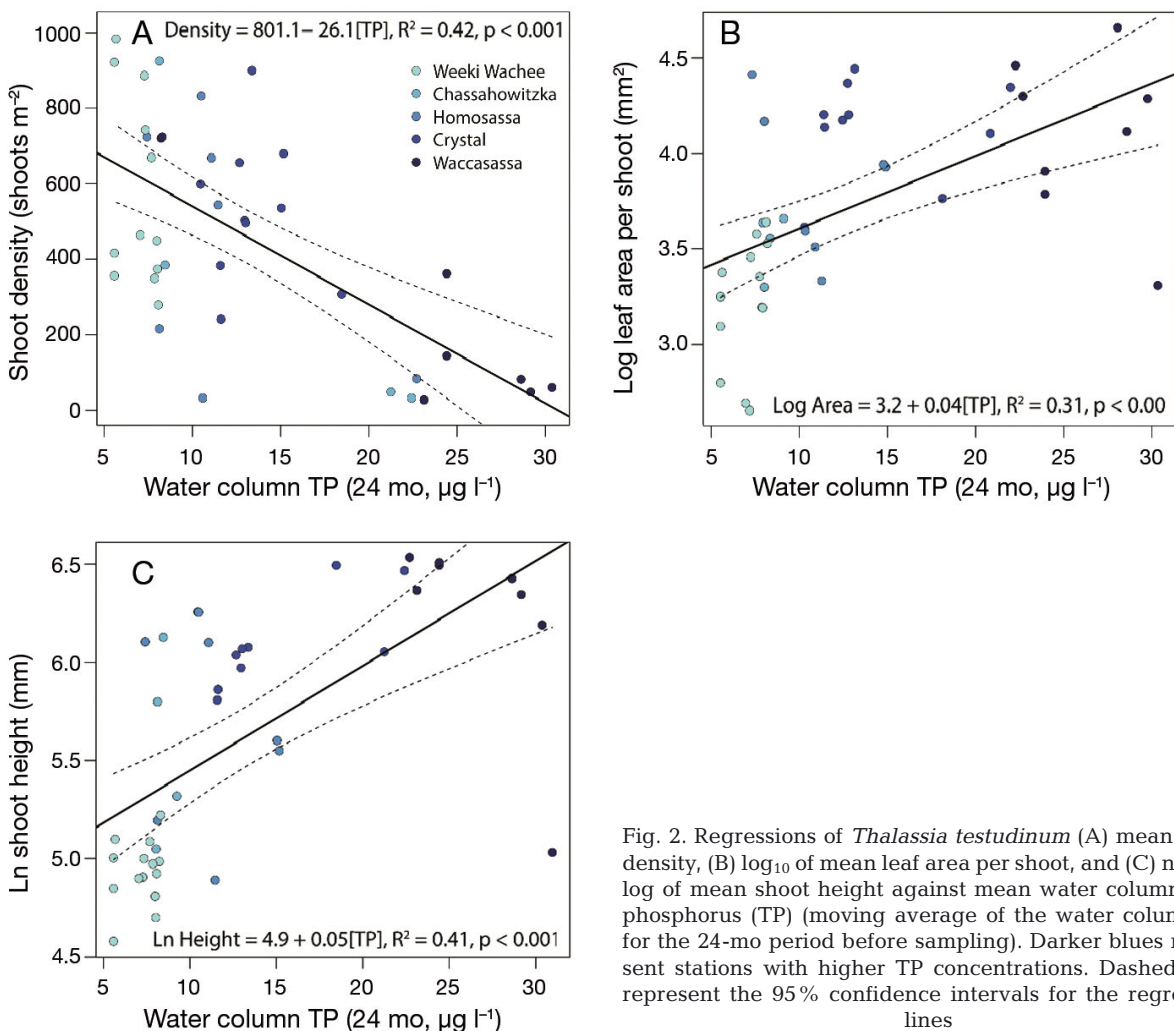


Fig. 2. Regressions of *Thalassia testudinum* (A) mean shoot density, (B) $\log_{10}$ of mean leaf area per shoot, and (C) natural log of mean shoot height against mean water column total phosphorus (TP) (moving average of the water column TP for the 24-mo period before sampling). Darker blues represent stations with higher TP concentrations. Dashed lines represent the 95% confidence intervals for the regression lines
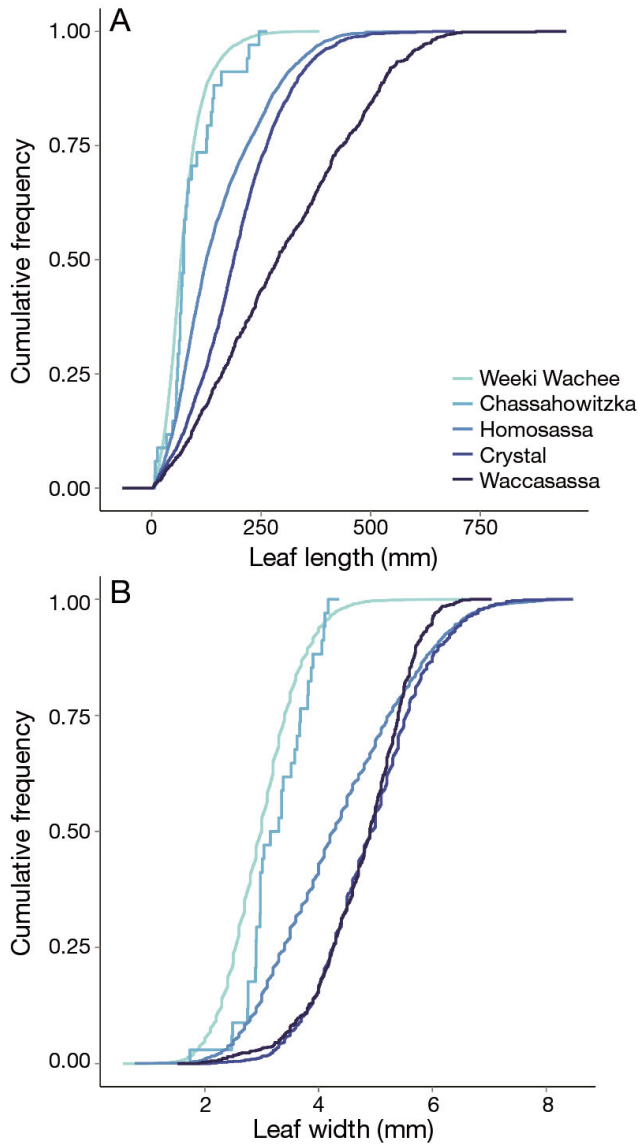
Fig. 3. Cumulative frequency distributions for *Thalassia testudinum* (A) leaf length and (B) leaf width for shoots collected in each of the 5 estuarine systems. Darker blues represent estuaries with higher total phosphorus concentrations

Table 2. Mean ± SD of *Thalassia testudinum* leaf lengths and widths from each coastal system across the total phosphorus gradient

| System | Mean leaf length (mm) | Mean leaf width (mm) |
|---|---|---|
| Weeki Wachee | 75.4 ± 46.3 | 3.0 ± 0.6 |
| Chassahowitzka | 89.4 ± 56.1 | 3.3 ± 0.5 |
| Homosassa | 152.8 ± 102.6 | 4.4 ± 1.2 |
| Crystal | 195.6 ± 105.5 | 4.9 ± 0.9 |
| Waccasassa | 301.6 ± 171.8 | 4.8 ± 0.8 |

## Growth rates

Regressions showed that the growth rate of individual shoots was significantly related to TP, but specific growth rates and areal production rates were not. *T. testudinum* growth rates (mg DW shoot$^{-1}$ d$^{-1}$) exhibited a strong positive relationship with TP ($p < 0.001$), indicating that shoot growth increased in concert with TP concentrations (Fig. 4). Shoots at the most P-replete station were 17 times more productive than shoots at the most P-poor station. Specific growth rates (mg new DW:mg old DW d$^{-1}$), however, were not significantly correlated with TP ($p = 0.440$), suggesting that *T. testudinum* exhibited a similar specific growth rate across the gradient in TP concentrations. In addition, areal production rates (g DW m$^{-2}$ d$^{-1}$), though lowest in the low TP stations, were not significantly correlated with TP ($p = 0.353$), likely due to the offsetting patterns in shoot growth rate (positively related to TP) and shoot density (negatively related to TP) resulting in fairly uniform patterns of areal biomass.

## Elemental composition and stable isotope ratios

Analysis of elemental composition and stable isotope ratios provided evidence that seagrasses may be P-limited in some or all of the study region. Nearly all leaf N:P ratios exceeded the threshold of 30:1 (Fig. 5), with highest values found at low-TP stations and lowest values at high-TP stations (Table 3). Sea-
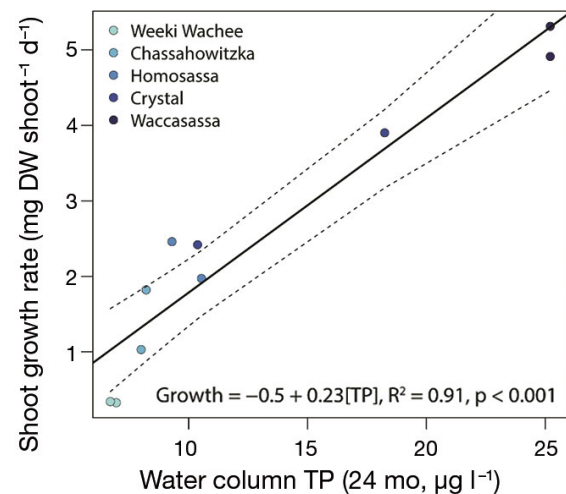


Fig. 4. Regression of mean *Thalassia testudinum* shoot growth rate against mean water column total phosphorus (TP) (moving average of the water column TP for the 24-mo period before sampling, µg l$^{-1}$). Darker blues represent stations with higher TP concentrations. Dashed lines represent the 95% confidence interval for the regression line
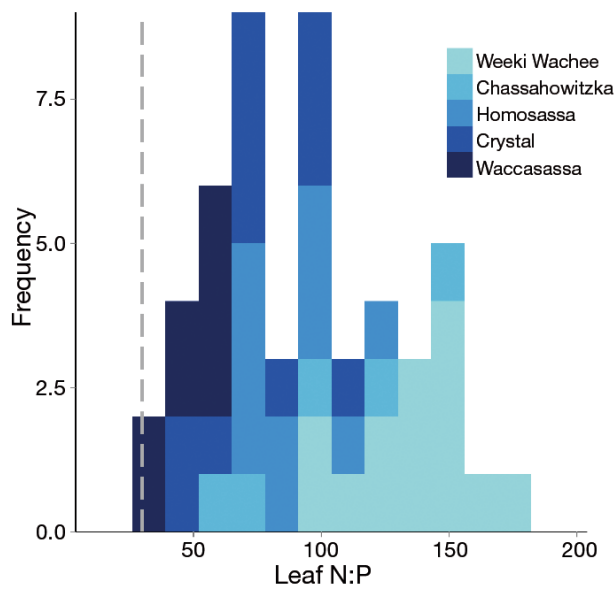
Fig. 5. *Thalassia testudinum* leaf N:P ratios (molar ratio of nitrogen to phosphorus in leaf tissue) observed across the gradient in total phosphorus concentrations. Dashed line represents the ratio of 30:1, above which seagrasses are presumed to be P-limited. Darker blues represent estuarine systems with higher TP concentrations

grasses at the most P-poor stations had high leaf C:P and N:P ratios, with maximum values of 3019.0 and 145.49, respectively (Table 3). Seagrasses with high C:P and N:P ratios may be experiencing P-limitation, whereby the rate of P supply is insufficient to meet physiological demands (e.g. Fourqurean et al. 2005). Leaf C:P and N:P ratios for *T. testudinum* leaves were highly correlated with TP and N:P$_{water}$, while leaf C:N showed no significant relationship with water column nutrient concentrations (Table 4). Concentrations of TN in the water column were not correlated with elemental composition or stable isotope ratios of leaves (Table 4). Leaf $\delta^{13}$C was negatively correlated with $\delta^{15}$N and positively correlated with both leaf N:P and N:P$_{water}$ (Table 4). However, these correlations were quite weak and were not explored further. *T. testudinum* growing off the central Gulf coast of Florida showed relatively depleted isotope signatures in comparison to $\delta^{13}$C and $\delta^{15}$N values reported elsewhere for this species (see Table S3 in the Supplement at www.int-res.com/articles/suppl/m570 p057_suppl.pdf), which could indicate excess supply of C and N relative to seagrass requirements.

Table 3. Mean carbon, nitrogen and phosphorus content and stable isotope ratios for *Thalassia testudinum* leaves from stations along a productivity gradient. Stations are arranged in geographic order from south to north

| Estuary | Station | %N | %C | %P | C:N | C:P | N:P | $\delta^{15}$N | $\delta^{13}$C |
|---|---|---|---|---|---|---|---|---|---|
| Weeki Wachee | 8 | 2.12 | 37.13 | 0.04 | 20.50 | 2617.48 | 127.25 | 0.49 | −13.79 |
| Weeki Wachee | 10 | 2.11 | 37.41 | 0.03 | 20.83 | 3019.01 | 145.49 | −1.97 | −12.68 |
| Chassahowitzka | 9 | 1.83 | 34.42 | 0.03 | 22.00 | 2711.14 | 123.35 | 1.32 | −20.19 |
| Chassahowitzka | 10 | 2.13 | 35.84 | 0.06 | 19.81 | 1934.66 | 94.66 | 0.05 | −17.22 |
| Homosassa | 6 | 2.12 | 37.48 | 0.05 | 20.94 | 2024.41 | 97.31 | −0.26 | −13.38 |
| Homosassa | 2 | 2.16 | 36.58 | 0.06 | 19.91 | 1639.02 | 82.21 | −0.53 | −13.01 |
| Crystal | 8 | 2.07 | 37.79 | 0.06 | 21.53 | 1828.56 | 85.12 | −0.24 | −14.90 |
| Crystal | 5 | 2.35 | 36.68 | 0.08 | 18.56 | 1241.25 | 66.09 | 0.41 | −15.58 |
| Waccasassa | 7b | 1.92 | 34.10 | 0.07 | 20.67 | 1195.05 | 57.78 | 1.46 | −14.19 |
| Waccasassa | 7 | 1.97 | 36.33 | 0.10 | 21.67 | 1021.87 | 46.95 | −1.39 | −15.22 |

Table 4. Correlation matrix for water column and *Thalassia testudinum* nutrient ratios and stable isotope signatures. Correlation coefficients (Spearman's ρ) are above the diagonal, and p-values are below the diagonal. Bold values indicate significant correlations at α = 0.01, values in italics are significant at α = 0.05. TN: total nitrogen, TP: total phosphorus

| | Leaf N:P | Leaf C:P | Leaf C:N | $\delta^{15}$N | $\delta^{13}$C | Water TP | Water TN | Water N:P |
|---|---|---|---|---|---|---|---|---|
| Leaf N:P | | **0.978** | 0.103 | 0.024 | **0.370** | **−0.868** | −0.155 | **0.840** |
| Leaf C:P | **<0.001** | | *0.285* | 0.052 | *0.337* | **−0.842** | −0.152 | **0.814** |
| Leaf C:N | 0.477 | *0.045* | | 0.062 | −0.076 | −0.026 | 0.017 | 0.039 |
| $\delta^{15}$N | 0.869 | 0.719 | 0.667 | | **−0.430** | −0.056 | −0.242 | 0.006 |
| $\delta^{13}$C | **0.008** | *0.017* | 0.602 | **0.002** | | *−0.342* | 0.030 | **0.386** |
| Water TP | **<0.001** | **<0.001** | 0.860 | 0.697 | *0.015* | | 0.143 | **−0.949** |
| Water TN | 0.282 | 0.292 | 0.907 | 0.090 | 0.835 | 0.322 | | 0.114 |
| Water N:P | **<0.001** | **<0.001** | 0.789 | 0.966 | **0.006** | **< 0.001** | 0.430 | |

## DISCUSSION

Data presented herein demonstrate that *Thalassia testudinum* growing along the central Gulf coast of peninsular Florida exhibits significant variation in morphology, shoot density, growth rates, and elemental composition in relation to a gradient in water column TP concentrations. The differences observed along the TP gradient can be illustrated in a conceptual diagram (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m570p057_supp.pdf). In areas with higher TP, shoots were taller with wider leaves and, thus, larger leaf area shoot$^{-1}$. Shoot density, in contrast, declined with increasing TP, and areal biomass remained fairly uniform across the TP gradient as a result of the opposing shoot-level and canopy-level patterns. Epiphyte load on *T. testudinum* did not exhibit a significant relationship with TP. In terms of proximal causes of differences in morphology, growth rate measurements revealed that seagrasses at stations with higher TP produced more leaf biomass than shoots at stations with lower TP, but specific growth rate was consistent across stations. Leaf elemental composition revealed that P availability could be limiting the growth of *T. testudinum* shoots, and results from stable isotope ratios were consistent with this conclusion as they indicated C and N were supplied in excess of the demands of seagrass.

Ultimately, both indirect and direct mechanisms may drive the observed patterns in shoot morphology and density. Water column nutrients often mediate light penetration in aquatic systems via fertilization of phytoplankton (Duarte 1995) and, indeed, concentrations of chl *a* increased concomitantly with TP in the study region (Frazer et al. 2002, Hoyer et al. 2002, Jacoby et al. 2015, see Table S2 in the Supplement). This gradient, along with a slight increase in color, likely generated the modest increase in the light attenuation coefficients ($K_d$) from south to north. In some studies, less favorable light environments have led to increased leaf area and biomass, and decreased shoot density (Dawes & Tomasko 1988, Abal et al. 1994, Dunton 1994, Dalla Via et al. 1998, Carlson et al. 2003), though there is a high degree of variation in morphometric responses of seagrass leaves to reductions in light availability (Ralph et al. 2007, McMahon et al. 2013). The capacity for *T. testudinum* to adapt to alterations in its light environment is thought to derive mostly from morphological alterations of the whole plant, as it has relatively low capacity to adapt to reductions in light through physiological means (Cayabyab & Enríquez 2007).

Increased leaf area shoot$^{-1}$ and shoot height in parallel with the TP gradient would have increased photosynthetic area and extended leaves into a more favorable light environment, with the decline in shoot density likely due to self-thinning that reduced within-canopy shading among wider and taller leaves (Ruiz & Romero 2001, Enríquez & Pantoja-Reyes 2005). Bottom irradiances at mean depths ($I_z$) for all seagrass sampling stations were above the minimum required to support growth of *T. testudinum* in this region (Dixon & Leverone 1995, Choice et al. 2014), and they did not vary concordantly with TP. Our data suggest that morphological adjustments by *T. testudinum* may occur in response to long-term, sub-lethal declines in light availability, especially in areas with higher water column TP, but that light alone was unlikely to be the sole driver of the observed morphological patterns. In terms of direct effects, *T. testudinum* leaves have been shown to absorb nutrients directly from the water column (Lee & Dunton 1999a, Touchette & Burkholder 2000, Gras et al. 2003), and evidence suggests that *T. testudinum* alters its morphology to balance uptake of nitrogen from sedimentary and water column sources in other systems (Lee & Dunton 1999a, Lee et al. 2007). Furthermore, the length, width, and area of *Zostera marina* leaves have been shown to increase as sedimentary ammonium increases (Short 1983), and *T. testudinum* was also shown to increase biomass and leaf size in response to ammonium enrichment in sediments (Lee & Dunton 2000). In addition, combined evidence from several studies conducted in Florida Bay suggests that P-limited *T. testudinum* shoots increase in biomass and height as P availability increases (Frankovich & Fourqurean 1997, Hackney & Durako 2004, Bricker et al. 2011). Thus, the variation in morphology of *T. testudinum* observed in this study may have been a combination of direct responses to nutrient supply and indirect responses to reduced light penetration as TP increased from south to north.

The TP concentrations off each of the rivers have remained relatively stable since 1997 (Frazer et al. 1998, Jacoby et al. 2015). Experimental manipulations of nutrient concentrations in a field setting are not typically maintained for a sufficient period of time to allow shoot morphology to fully adjust to abiotic conditions (weeks to months, e.g. Perez et al. 1991, Heck et al. 2000, Ferdie & Fourqurean 2004), which may take up to 2 yr for *T. testudinum* (van Tussenbroek 1996). Therefore, measuring growth rates along this gradient constituted a natural experiment in which TP concentrations had been maintained over ecologi-

cally relevant temporal and spatial scales. Shoot production rates (mg DW shoot$^{-1}$ d$^{-1}$) clearly tracked TP concentrations, suggesting P is the primary limiting nutrient for seagrass production in waters off the central Gulf coast of peninsular Florida. Following experimental nutrient addition in the southernmost region of the study area, *T. testudinum* was observed to grow more rapidly in response to the addition of P, but showed no significant response to added nitrogen (Keller 2002). Although shoot growth increased markedly across the TP gradient, specific growth rates (mg new DW:mg old DW d$^{-1}$) were similar and indicative of relatively uniform production rates when normalized for morphological differences. *T. testudinum* has been repeatedly found to have a consistent specific growth rate across a range of environmental conditions in Florida Bay (Zieman 1968, 1975, Zieman et al. 1989), and other aspects of *T. testudinum* biology, such as rhizome internode length and photosynthetic physiology, are also thought to be relatively invariant (van Tussenbroek et al. 2006, Cayabyab & Enríquez 2007). Conversely, parameters such as leaf morphology appear to have a high level of phenotypic plasticity and may therefore be an important mechanism by which *T. testudinum* adapts to local environmental variations, such as variations in nutrient or light availability. Areal production (g DW m$^{-2}$ d$^{-1}$) and areal biomass (g DW m$^{-2}$) were not significantly related to TP, suggesting that a dense canopy of small shoots could produce similar biomass per area as a sparse canopy of large shoots. This is in contrast to Florida Bay, where areal biomass of *T. testudinum* has been observed to increase with increasing TP concentration and increasing sediment depth (Hall et al. 1999). No information is available regarding sediment depth at our sampling locations. Further work is required to determine if sediment depth and TP concentrations co-vary in our system as they do in Florida Bay. Our results suggested that, over time, fertilization of seagrasses with a key macronutrient increased growth rates and led to an increase in leaf length, leaf width, and shoot biomass, but these shoot-level growth and biomass gains were offset by a decline in shoot density at the canopy level that resulted in fairly uniform aboveground areal biomass.

Evidence from leaf elemental composition and stable isotope ratios was consistent with P-limitation of *T. testudinum* in this region. Leaf N:P ratios decreased from south to north, with mean N:P ratios ranging from 46.95 to 127.25. These ratios were above the threshold of 30:1, and thus indicated that P-limitation could potentially be present even at stations with relatively high P concentrations. In ad-

dition, TP concentrations in the water column exhibited a strong negative correlation with leaf N:P and C:P, but not C:N, indicating that TP in the study area was tightly linked to leaf P content. Leaf C:N was not significantly correlated with any other variable tested and did not exhibit appreciable spatial variation. Unfortunately, no information about sedimentary sources of P were collected. However, there are several factors that may contribute to low availability of P in the sediments underlying these seagrass meadows. Firstly, the bulk of the freshwater input into these estuaries is derived from spring-fed rivers originating from a karst-dominated aquifer system that may have a high capacity to bind P upstream from the estuary (Price et al. 2010, Mellander et al. 2013). Secondly, P is typically transported to coastal zones while adsorbed onto suspended sediments and organic matter (Sundby et al. 1992), materials that are generally found in very low concentrations in spring-fed rivers, some of the clearest known surface waters (Duarte & Canfield 1990). Thus, delivery of P to these coastal waters and sediments may be lower than in some other estuarine systems that receive a higher proportion of freshwater input from terrestrial runoff. Finally, the sediments underlying the seagrass meadows in our study region are composed of between 12 and 55% CaCO$_3$ (S. C. Barry unpubl. data), and P may thus be bound by carbonate compounds to some extent, potentially lowering the availability of sedimentary P to *T. testudinum*. Overall, the high leaf N:P and C:P ratios we observed for *T. testudinum* at all stations suggests that P is generally not supplied at a rate that meets seagrass demands from either water column or sedimentary sources.

The δ$^{15}$N signatures of *T. testudinum* were depleted in comparison to values previously reported for this species (see Table S3 in the Supplement). Nitrogen concentrations in Florida's spring-fed rivers, groundwater, and coastal waters have increased markedly in recent decades (Jones et al. 1997, Jacoby et al. 2015), and inorganic N-loading to coastal waters could have contributed to low δ$^{15}$N signatures (−5.2 to 3.3‰) of *T. testudinum* in this region. The depleted δ$^{15}$N signatures of *T. testudinum* leaf tissue could have been a result of fractionation during uptake from the large N pool in these coastal waters, and such a result further supports the conclusion that P could be limiting seagrass growth in this region because N-limited plants elsewhere tend to have heavier δ$^{15}$N signatures (Fourqurean et al. 2005). Alternatively, sedimentary sources represent another N pool that could be contributing to the relatively depleted δ$^{15}$N signatures by supplying N in excess of seagrass demands. Overall,

the $\delta^{15}N$ results suggest that N was not limiting to *T. testudinum* in our study region.

*T. testudinum* leaves were isotopically light relative to previously reported $\delta^{13}C$ values for this species (see Table S3 in the Supplement). Several investigations have reported a link between decreases in light availability and lighter $\delta^{13}C$ signatures due to altered kinetics for carbon uptake (Cooper & DeNiro 1989, Durako & Hall 1992, Grice et al. 1996). However, light levels at the sampled stations were more than adequate to support growth of *T. testudinum* (Dixon & Leverone 1995, Choice et al. 2014). Moreover, leaf N:P ratios showed moderate to strong departures from the 30:1 threshold, indicating that seagrass growth in this area was not generally limited by light availability (Fourqurean et al. 2005). The observed differences in shoot morphology along the gradient were such that leaf area per shoot increased markedly at stations with higher TP and lower light penetration (higher $K_d$), indicating that morphological adjustments over time in response to modest declines in light penetration may have counteracted the influence of light availability on fractionation of carbon isotopes over the longer-term. The source of in–organic carbon also has been shown to influence $\delta^{13}C$ values, with terrestrial, atmospheric, or mangrove carbon sources all acting to decrease $\delta^{13}C$ of seagrasses (Lin et al. 1991, Hemminga & Mateo 1996, Swart et al. 2001). Dissolved inorganic carbon (DIC) concentrations in Florida spring-fed rivers are generally very high (Duarte et al. 2010), with $\delta^{13}C$ signatures typical of carbonate dissolution in karst-dominated aquifers ($-13$ to $-6$‰, Deines et al. 1974, Albertin 2009). Algae in spring-fed rivers did not show significant spatial variation in $\delta^{13}C$, indicating C-limitation was not an important process, even several km downstream from spring vents (Albertin 2009). Thus, spring-fed rivers that flow into these coastal waters may be delivering carbon-laden waters, fairly depleted in $^{13}C$, to offshore seagrasses. Therefore, we posit that the source and size of the DIC pool, not light levels, are likely to underlie the relatively depleted $\delta^{13}C$ signatures of *T. testudinum* ($-21.3$ to $-11.2$‰) off the central Gulf coast of peninsular Florida.

Collectively, the morphology, growth rate, elemental content, and stable isotope findings offer compelling evidence that *T. testudinum* along the Gulf coast of peninsular Florida balances shoot morphology and density in relation to a key macronutrient (P) to maintain growth and survival across a wide range of nutrient supply. *T. testudinum* in this region appears to have sufficient supplies of light, carbon, and

nitrogen, but not necessarily P, to meet growth demands. Given the difficulty of separating direct (fertilization) and indirect (reduced light penetration) effects of increasing water column nutrient concentrations in a natural setting (Duarte 1995), we cannot definitively parse the relative importance of these factors as influences on seagrass morphology and density. The BEST analysis identified TP as an influence in 9 of the top-ranked solutions, and we also found strong univariate relationships with TP. Color, chl *a* concentrations, and reduced irradiance at the bottom were also identified as influential in multivariate analyses. In combination, these results suggest that water column TP is a major driver of the patterns observed, perhaps via both direct and indirect mechanisms. For example, direct alleviation of P-limitation could be relatively more influential in the southern, low TP end of the gradient, while indirect effects from reduced light penetration could be relatively more important in the northern, higher TP end of the gradient. Their combined effects, over time, would be expected to produce the observed patterns in *T. testudinum* leaf morphology.

The observed patterns in aboveground morphology (Fig. S1) could have broad implications for the provisioning of ecosystem services in seagrass meadows because many ecosystem services are dependent on the physical interaction of seagrass leaves and the water column, sediments, and biota. For example, seagrass canopies slow water flow and increase the deposition and retention of particles from the water column (Gacia & Duarte 2001, Hendriks et al. 2008), but the capacity to do so may differ depending on the canopy height and density (Nepf & Vivoni 2000, Hansen & Reidenbach 2013). Also, differing aboveground morphology may relate to carbon storage in the sediments of seagrass meadows because leaf biomass is subject to grazing and export while belowground biomass is more easily buried *in situ* (Duarte & Cebrian 1996, Cebrian 1999, Duarte et al. 2013). Interestingly, *T. testudinum* in our study region varied in elemental composition but not in areal biomass, which could affect the relative quality of these seagrass meadows as foraging areas for herbivores, because forage quality, but not forage abundance, differs across the TP gradient. The analyses above present strong evidence that water-column P is related to dramatic differences in leaf morphology and shoot density of *T. testudinum* in this region of Florida. This spatial gradient is an ideal natural laboratory for further investigating phenotypic plasticity of *T. testudinum* and for investigating the potential differences in ecosystem resilience, habitat and nurs-

ery value, carbon sequestration, and sedimentation and resuspension rates brought about by bottom-up influences on the morphology of a foundation species. Further investigation into phenotypic plasticity and differential provisioning of ecosystem services across abiotic gradients will yield the detailed information necessary to enhance management of high-value habitats such as seagrasses.

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