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Seagrass growth, reproductive, and morphological plasticity across environmental gradients over a large spatial scale

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ABSTRACT

Phenotypic variability is a valuable adaptive mechanism for seagrass species that exist in a dynamic environment and can lead to significant intraspecific regional distinctions in life history. Research is lacking in studies examining the significance of within-species phenotypic variation in relation to gradients in environmental condition at a large spatial scale. These studies are essential to better understanding the potential for acclimatization and tolerance capabilities of seagrasses in declining coastal environments. *Thalassia testudinum* (turtlegrass) is a ubiquitous keystone seagrass species across the Caribbean and Gulf of Mexico (GoM) that populates both environmentally dynamic estuaries and stable coastal environments. In order to elucidate environmentally driven distinctions in spatially separated populations, we examined characteristics of shoots exposed to widely separated distinct coastal environments with varying degrees of environmental stability and suitability. In our comparison, three sampling locations vary considerably in ambient water temperature, salinity, and water column clarity along a gradient from oscillating, higher stress conditions to stable, more favorable conditions. Shoots tended to have larger leaves with more biomass in the stable environment and also exhibited an older shoot age structure and higher horizontal expansion rate. However, shoots in the more variable, higher stress environment exhibited greater evidence of flowering and first flowered at an earlier age. The results elucidate large spatially distinct and environmentally relevant differences in morphology, growth, and life history highlighting the need for more studies regarding phenotypic variability of seagrass populations across environmental gradients.

1. Introduction

Seagrasses are subject to many inherent environmental stressors in their submerged marine habitats. For instance, fluctuating conditions of light, temperature, and salinity can have large effects on seagrass morphology, growth, and function (Short et al., 1996). Seagrasses are capable of acclimating to varying environmental conditions by utilizing the innate plasticity of individual seagrass modules provided by phenotypic variation (Hemminga and Duarte, 2000). Phenotypic variation is a well-documented adaptive mechanism that can drive morphological, demographic, reproductive, and physiological changes for environmental acclimation in seagrasses (Cabaço et al., 2009; Kim et al., 2014). Accordingly, past studies have found intraspecies differences in morphological and functional attributes across environmental gradients of light, temperature, and salinity (Kaldy et al., 2000; Enriquez et al., 2002; Kendrick et al., 2008). Nevertheless, studies documenting species-specific phenotypic variation at large spatial scales, and how such variation may be related to parallel environmental gradients, are scarce. Here we contribute to filling this gap by examining seagrass phenotypic plasticity in morphological, growth and functional attributes across a large swath of the Gulf of Mexico (GoM).

We studied turtlegrass (*Thalassia testudinum*), a subtropical/tropical seagrass widely distributed throughout the Caribbean and GoM, with a large literature representation that provides a plethora of information regarding this species' optimum envi-

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Table 1
Environmental characteristics of sampling areas with means of variables with coefficient of variation [CV] and lowest/highest observations. We were primarily interested in capturing the period 2002–2010 (i.e., the previous eight years to collection) since this corresponded on average to the maximum age of the shoots collected. However, data from this period was limited for EF, therefore we searched for additional data for this area and found collections from 1990 to 1995. Overall the limited data for 2002–2010 are consistent with the more abundant data from 1990 to 1995 at this site, which offers reassurance to our comparison and differences inferred among sites based on the 2002–2010 data sets.

	Site depth (m)	Salinity (ppt)	SST (°C)	Regional annual rainfall[a] (cm)	Light extinction (K_d, m^{-1})	Estimated annual PAR at depth[b] ($mJ\,m^{-3}$)
		mean [CV] (range)	mean [CV] (range)	30 year normals (1980–2010)	mean [CV] (range)	mean
BL[c,f] 2002–2010	0.7	21.4 [0.22] (7.5–31.6)	23.2 [0.28] (7.6–33.5)	155	1.6 [0.53] (0.44–3.32)	930.08
EF[d,f] 1990–1995	0.7	29.5 [0.12] (20.6–35.2)	21.9 [0.28] (10.1–32.6)	80.8	1.1 [0.64] (0.19–2.43)	1202.49[g]
2002–2010		30.4 [0.17] (14.5–39.0)	23.4 [0.26] (10.1–32.7)		1.4 [0.27] (0.86–1.80)	
LKSP[e,f] 2002–2010	2.3	36.5 [0.02] (34.1–37.8)	27.2 [0.14] (12.7–33.4)	116.8	0.4 [0.81] (0.01–1.11)	1422.51

[a] Data provided by climate normal tables from NOAA National Center for Environmental Information: ncdc.noaa.gov.
[b] Calculated using mean K_d and annual insolation from summation of monthly mean daily broadband insolation at Mobile (BL), Corpus Christi (EF), and Key West (LKSP) airports. PAR = 0.45 × broadband (Jacovides et al., 2003).
[c] Christiaen et al., DISL, unpublished data; data collection periods: 2002–2010 (salinity); 2003–2010 (light and SST).
[d] Dr. Paul Montagna at Texas A&M, Corpus Christi; Dunton, 1994; Herzka and Dunton, 1997; Kopecky and Dunton, 2006; data collection periods: 1990–1995 and 2002–2010 (light); 1991–1995 and 2003–2010 (salinity); 1993–1995 and 2003–2010 (SST).
[e] FKNMS Seagrass Status and Trends Monitoring Data, Site 241 (http://serc.fiu.edu/seagrass/lcdreport/datahome.htm); data collection periods: 2002–2010 (light); 2002–2008 (salinity); 2003–2010 (SST).
[f] Supplemental metadata provided by TCOON and/or NOAA NDBC.
[g] Calculated using Kd for both timeframes 1990–1995 and 2002–2010.

ronmental preferences. Previous studies have determined the optimal temperature for *T. testudinum* ranges between 27 and 30 °C, optimum salinity between 24 and 35‰, and the minimum light requirement is approximately 18% surface irradiance (Phillips, 1960; Lee et al., 2007; Garrote-Moreno et al., 2014). For sub-optimal conditions, previous work indicates this species may have substantial phenotypic variation in multiple morphological and functional traits (Manuel et al., 2013; Garrote-Moreno et al., 2014). For instance, turtlegrass may acclimate to reduced light availability by reducing leaf surface area, increasing leaf chlorophyll content, and increasing the chlorophyll *b* to chlorophyll a ratio (Major and Dunton, 2002; Lambers et al., 2008). Turtlegrass, like other seagrass species, may also modify its reproductive output to cope with sub-optimal conditions of light, temperature, and salinity (Obeso, 2002; Reusch et al., 2005). The extent to which these environmentally linked life history variations coincide is largely unknown since large-scale phenotypic comparisons for this species has not been thoroughly examined.

Substantial phenotypic variation for turtlegrass may exist across the GoM due to the occurrence of marked environmental gradients. The northern coastline has a warm-temperate to subtropical climate. It has relatively turbid waters due to inputs from large watersheds along the coastline (USGS, 2004), although annual rainfall varies widely across this region from a maximum of 178 cm east of the Mississippi River to 18 cm on the South Texas coastline (Bailey, 1995). In contrast, the coastline surrounding the Florida Keys has overall higher temperatures due to its tropical climate and much clearer waters with typical annual rainfall levels approximately 100 cm (Bailey, 1995). Therefore, environmental conditions of light, temperature and salinity produce an environmental variability and, as a result, a seagrass suitability gradient across the Gulf of Mexico coastline.

In this paper, we examine the extent of phenotypic variability in a broad suite of morphological, growth, and functional features of turtlegrass populations from three widely separated habitats. These habitats range in abiotic influence and represent the distinct environments often encountered across extensive coastlines, such as the Gulf of Mexico. We examine the range of environmental variation in light availability, temperature, and salinity conditions of each environment to better understand the degree of phenotypic variation encountered, as well as suggesting some potential mechanisms partially responsible for this variation. This comparison provides a measure of phenotypic variation for a vital habitat forming species across a gradient of environmental variability to provide greater insight into the factors driving intraspecific growth, life history, and morphological differences among widely separated populations.

2. Materials and methods

2.1. Study areas

The *T. testudinum* populations studied are located in the Gulf Islands National Seashore in Big Lagoon (BL), Florida (30.18°N, 87.25°W); East Flats (EF) in Corpus Christi Bay, Texas (27.49°N, 97.07°W); and near Long Key State Park (LKSP) in the Florida Keys National Marine Sanctuary (24.48°N, 80.49°W) (Fig. S1). BL is a shallow body of water that connects lower Perdido and Pensacola Bays to the northcentral Gulf of Mexico. Turtlegrass is the dominant seagrass species and the population appears to be stable with an areal coverage of 2.2 km^2 (Moss, 2011). This area has moderate to low light availability and highly variable salinities (Fig. S2, Table 1) due to inputs from the Perdido and Pensacola estuaries (Schwenning and Bruce Handley, 2007).

Table 2
Results of the nested mixed effects models for the *T. testudinum* morphological traits examined at set α of 0.01. For a significant Region factor, *a priori* contrasts are tested at set α of 0.025 (C1 = GINS, EF vs FKNMS, C2 = GINS vs EF).

Metric and units	Source of variation	F ratio (df)	P	Variance component (SE)	Contrasts: $p > \|t\|$
No. of leaves (leaves shoot^{-1})	Region	34.98 (2, 2.98)	0.03		
	Site			0.002 (0.004)	
	Residual			0.61 (0.02)	
Average leaf length (cm)	Region	2.19 (2, 2.98)	0.26		
	Site			0.63 (0.6)	
	Residual			7.69 (0.3)	
Maximum leaf length (cm)	Region	1.92 (2, 2.98)	0.29		
	Site			0.95 (0.8)	
	Residual			13.1 (0.5)	
Average leaf width (cm)	*Region*	1078.6 (2, 2.98)	<0.001		C1: <0.001
	Site			0	C2: <0.001
	Residual			0.02 (0.0006)	
Leaf thickness (mm)	Region	0.45 (2, 3)	0.67		
	Site			0.0014 (0.001)	
	Residual			0.001 (0.0002)	
Leaf area (cm^2 shoot^{-1})	Region	41.28 (2, 2.97)	0.007		C1: 0.004
	Site			0.06 (0.06)	C2: 0.26
	Residual			2.4 (0.09)	
Leaf biomass (gDW shoot^{-1})	Region	86.35 (2, 2.75)	0.003		C1: 0.001
	Site			0	C2: 0.41
	Residual			0.0007 (0.0001)	
Specific leaf area (cm^2 gDW^{-1})	Region	1.40 (2, 3)	0.37		
	Site			3490 (3000)	
	Residual			1730 (339)	
Vertical rhizome length (cm)	Region	43.9 (2, 3)	0.006		C1: 0.006
	Site			0.0008 (0.003)	C2: 0.008
	Residual			0.65 (0.03)	
Vertical rhizome width (cm)	Region	3.03 (2, 2.99)	0.19		
	Site			0.0003 (0.0003)	
	Residual			0.003 (0.0001)	

Corpus Christi Bay, TX is an estuary located on the northwestern coast of the GoM, bordered by barrier islands to the east, connected to Redfish Bay on the north, and the Laguna Madre to the south. It is fed by the Nueces River and exchanges water with the GoM through two shipping channels. Seagrass cover in this area has increased since the 1950's with a current extent of approximately 40.7 km^2, mainly due to the cessation of localized dredging for oil and gas exploration in shallow areas (Pulich, 2007). At the East Flats (EF) area of Corpus Christi Bay, *T. testudinum* is the dominant seagrass species (Pulich, 2007). The area has variable light quality (Dunton, 1994) but the benthic environment receives on average 29% more annual photosynthetically active radiation (PAR) than does the BL site (Table 1). The estuarine salinity regime is variable, with a higher average salinity than at BL due to lower annual rainfall in this region (Fig. S2, Table 1).

The Florida Keys National Marine Sanctuary is a 9600 km^2 area comprising the Gulf and Atlantic sides of the Florida Keys island chain located off the southern tip of the Florida peninsula. Our study locations are located on the Atlantic side of Long Key and turtlegrass is the dominant seagrass species in the LKSP locations (Fourqurean et al., 2002). The waters around LKSP receive a limited amount of terrestrial runoff, and the constant inflow of oceanic water from the Atlantic effectively creates a high light benthic environment that receives on average 53% more annual PAR than BL and 18% more than EF, as well as a stable oceanic salinity regime (Rudnick et al., 2005; Fig. S2, Table 1).

2.2. Seagrass metrics measured

We sampled seagrass in two sites of similar shoot densities within each of the three representative regions studied (i.e. Big Lagoon, East Flats and Long Key State Park). Sites within a region were separated between 100 and 200 m in order to constrain sampling to shoots exposed to a similar range of environmental variability. In the Spring of 2010 (LKSP: March 25, EF: April 13, BL: April 29) we randomly grab-sampled vertical shoots at each site, extracting approximately 0.06 m^2 plugs at a time until 300 shoots were collected for a total of approximately 600 shoots per region and 1800 shoots overall. Care was taken to keep the vertical shoots attached to the horizontal rhizome during sampling. The samples were placed on ice, rinsed with freshwater at the lab, and frozen for transportation and analysis at the Dauphin Island Sea Lab.

2.2.1. Morphological measurements

We counted the number of leaves, and measured the length and widths of all the leaves on each shoot collected. Length and width measurements were done with a ruler to the nearest millimeter and leaf width measurements were taken at the length-wise midpoint of the blade. From these measurements we derived the average leaf length on the shoot, the maximum leaf length on the shoot, the average leaf width on the shoot, and the leaf area per shoot. We also measured the thickness of 50 leaves for each site to the nearest hundredth millimeter using a dissecting microscope equipped with an ocular micrometer. One millimeter wide sections were taken from the midpoint of the second leaf on the shoot for thickness measurements. To derive leaf biomass per shoot (gDW per shoot), the leaves of five randomly chosen shoots were pooled together, dried at ca. 50 °C and weighed, and that weight divided by 5. We repeated this process ten times at each site, for a total of ten replicates of leaf biomass per shoot at each site with one replicate being the composite of five shoots. With these samples we also calculated the specific leaf area (cm^2 per gDW). In addition, we measured the total length, width at the base, and width at the middle of the vertical rhizome for all shoots collected and the average rhizome width for each shoot was derived.

Morphological measurements were analyzed using linear mixed effects modeling with a region (LKSP, EF, and BL) as a fixed factor and site as a random factor nested within a region. Nested sites were included to partition the small-scale spatial variability between sites sampled from differences among regions. The 'Fit Model' plat-

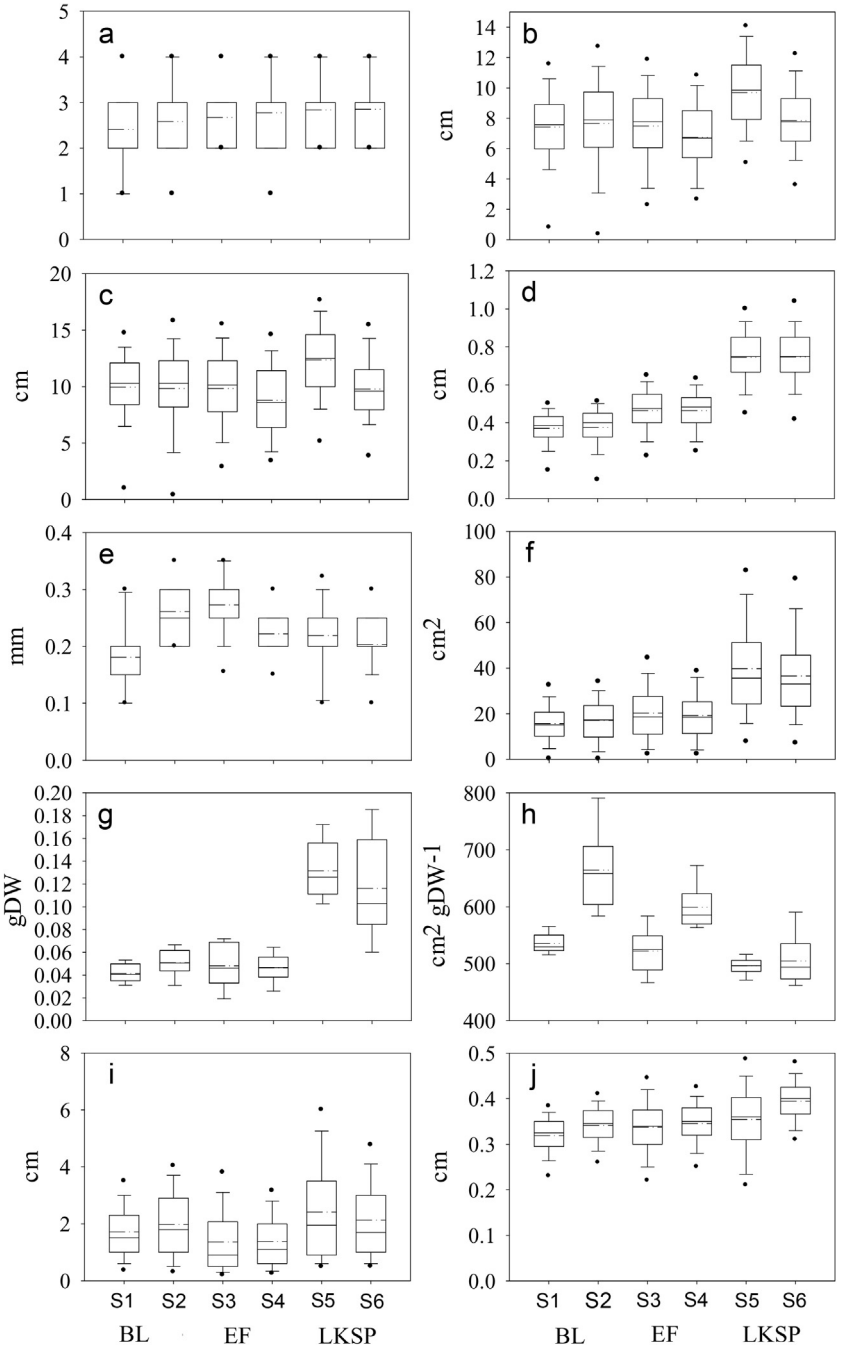

Fig. 1. Box plots of the *T. testudinum* morphological traits examined. (a) Number of leaves per shoot (leaves per shoot, n = ca. 300 per site); (b) average leaf length per shoot (n = ca. 300 per site); (c) maximum leaf length per shoot (n = ca. 300 per site); (d) average leaf width per shoot (n = ca. 300 per site); (e) leaf thickness (n = 50 per site); (f) leaf area per shoot (n = ca. 300 per site); (g) leaf biomass per shoot (n = 10 per site); (h) Specific leaf area (n = 10 per site); (i) vertical rhizome length (n = between 260 and 176 per site; only the shoots that were attached to the horizontal rhizome were considered for this metric); (j) vertical rhizome width (n = ca. 300 per site). The box encompasses the 25 and 75 quartiles, and dashed and solid lines in the box represent the mean and median respectively. Top and bottom vertical lines encompass the 5 and 95 percentiles, and dots denote observations outside of those limits.

Table 3
PERMANOVA table for 7 morphological variables. Effect size was calculated as a proportion of each factor's estimated contribution to the components of variation. Contrasts were tested at a set α of 0.025 (C1: GINS, EF vs FKNMS; C2: GINS vs EF).

Source of variation	df	MS	Pseudo-F	P(perm)	Unique permutations	P(MC)	Effect Size (%)
Region	2	531.84	10.05	0.07	15	0.0003	15.0
C1	1	979.53	16.13	0.06	15	0.0003	21.8
C2	1	84.144	5.64	0.33	3	0.027	2.2
Site (Region)	3	52.918	9.33	<0.001	9932	<0.001	3.0
Site (Region) C1	4	60.73	10.71	<0.001	9935	<0.001	3.5
Site (Region) C2	2	14.92	3.18	0.009	9933	0.008	0.7
Residual	894	5.67					53.8

form in JMP (version 11.2.0 copyright © 2013 SAS Institute Inc.) was used to best fit a linear mixed model with restricted maximum likelihood estimation method for variance components. To determine significance of the random factor Site, full and reduced models were compared using likelihood ratio tests and confirmed with Akaike's Information Criterion (AIC) (Zuur et al., 2009) with the nlme package (Pinheiro et al., 2016) of RStudio v.0.99.486 (© 2009–2015). Variables with studentized residuals lacking homogeneity of variance or normality were either log or square-root transformed. To control for enhanced Type I error characteristic for multiple univariate analyses, we lowered the acceptable α of both Region and Site factors to 0.01. If the main effect of Region was significant, we used *a priori* contrasts for the following comparisons: (1) BL and EF versus LKSP and (2) BL versus EF. These contrasts were constructed to compare means for the shoots exposed to higher environmental variability versus those experiencing a more stable environment and, secondly, between the two more variable regions, respectively. To control the familywise error rate, the acceptable α for the two contrasts was set at 0.025.

In order to further explore the environmentally influenced regional distinctions of *T. testudinum* shoot morphological characteristics, a multivariate analysis was conducted on an individual shoot based variables. We excluded variables measured on a limited number of shoots (e.g. shoot biomass, leaf thickness, and specific leaf area) and only included measurements for shoots that remained attached to the horizontal rhizome after collection to maintain consistency. Included in the multivariate analysis were the variables leaf area, leaf width, number of leaves, average leaf length, maximum leaf length, vertical rhizome length, and average vertical rhizome width. Initially, all morphological metrics were normalized to allow for inter-variable comparison and differences were calculated as Euclidean distances. Visual inspection of potential regional separation of multivariate morphological data was done with the metric ordination technique PCO. Regional variability of shoot morphometrics was analyzed using a permutational multivariate analysis of variance (PERMANOVA; Anderson et al., 2008) with Region as a fixed factor, two *a priori* contrasts as described for the univariate analyses, and Site as a random factor nested within Region (Anderson et al., 2008). To maintain a balanced sampling design for the multivariate analysis, 150 shoots were randomly selected from each site within a region. The analysis was performed using sequential Type I Sums of Squares on 9999 permutations and p-values obtained with a Monte Carlo random sample due to extremely small unique permutation outcomes (Anderson et al., 2008). Acceptable α for the Region and Site factors for the PERMANOVA was set at 0.05, and 0.025 for the two contrasts. Significant factors were tested for differences in multivariate dispersion using PERMDISP of 9999 permutations with post-hoc pair-wise comparisons at α = 0.05. SIMPER analysis on Euclidean distances tested the contribution of each morphometric variable to the dissimilarities between shoots from the three regions. Multivariate ordination and analyses were conducted with Primer 7 software (PRIMER-E, ©2015).

2.2.2. Shoot age and rhizome growth rates

We used seagrass growth reconstruction techniques to derive rhizome growth rates and metrics of shoot demography (Duarte et al., 1994). These techniques rely on the seasonality of vertical rhizome growth as shown by the annual cycles of internodal length along the rhizome. Vertical rhizome growth rates are higher in the spring/early summer making the distance in between consecutive nodes along the rhizome longer, whereas in late fall/winter growth rates are lower and the distance is shorter. Therefore, the number of annual cycles in internodal length present in the shoot corresponds to its age in years. For practical reasons, we reconstructed those cycles in the ten oldest shoots still attached to the rhizome from each site by measuring internodal lengths using a dissecting microscope equipped with an ocular micrometer. This generated as many measurements of nodes produced per year per shoot as annual cycles found in the ten shoots processed for each site. Number of nodes (i.e. leaves) produced per year per shoot was compared among Regions and between Sites using a nested mixed effects model design as described above for morphological variables (α = 0.01).

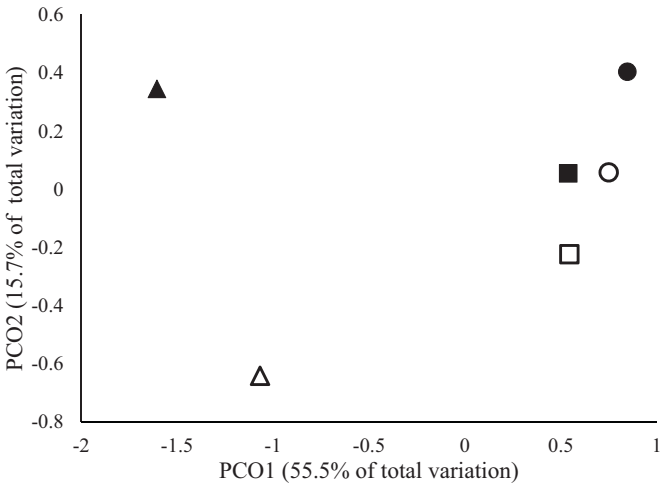

Fig. 2. Metric ordination (PCO) of Site centroids. BL (Site 1 ●, Site 2 ○), EF (Site 3 ■, Site 4 □), LKSP (Site 5 ▲, Site 6 △).

For the remaining shoots sampled at each site that were still connected to the horizontal rhizome after collection, we counted the number of vertical rhizome nodes and estimated shoot age by dividing the number of nodes on the shoot by the overall average number of nodes produced per year per shoot obtained from the ten oldest shoots. Age histograms were constructed using only the attached shoots, while both attached and unattached after collection shoots were used for the derivation of vertical rhizome growth rates. For unattached shoots, we counted the number of nodes on the shoot and estimated the time elapsed for the formation of the vertical rhizome on the shoot by dividing the number of nodes by the overall average of a number of nodes produced per year per shoot. The point of vertical to horizontal rhizome attachment is the narrowest and weakest point on the shoot, therefore shoots can break at or near the point of attachment during collection (Tomlinson and Vargo, 1966). Unattached (i.e. broken from horizontal rhizome during collection) shoots are no longer useable for accurate ageing, however they still contain enough reliable growth data to be useful for estimates of vertical growth rates.

Vertical growth rates (as cm per year) were derived for each site as the slope of the regression fitted between shoot vertical length (in cm) and shoot age (for attached shoots) or time elapsed for the formation of the vertical rhizome (for unattached shoots). For each fragment of horizontal rhizome where the apex was still present, we measured distance from the apex to the adjacent youngest shoot. Horizontal rhizome growth rates (in cm per year) were calculated for each site as the slope of the regression fitted between that distance and the age of the adjacent youngest shoot. Vertical and horizontal rhizome growth rates were first compared between the two Sites within a Region, and then between Regions by pooling together the two Sites in a Region using individual vertical rhizomes and horizontal rhizomes with apical meristems and adjacent attached vertical rhizomes as replication units. Analysis was done using the general linear model routine in Minitab v.14 (2004) as described by Neter et al. (1996). The α for these multiple comparisons was set at 0.02 using the Bonferroni correction.

Table 4
SIMPER analyses of relative contribution of morphometric variables.

	BL v EF	BL v LKSP	EF v LKSP
Average squared distance	10.2	18.5	16.8
Rank			
1	Number of leaves (20.2%)	Leaf width (22.0%)	Leaf area per shoot (17.5%)
2	Max leaf length (18.5%)	Leaf area per shoot (17.7%)	Leaf width (16.5%)
3	Average leaf length (18.2%)	Vertical rhizome width (13.1%)	Vertical rhizome length (15.6%)
4	Vertical rhizome width (14.5%)	Average leaf length (12.2%)	Vertical rhizome width (14.0%)
5	Vertical rhizome length (13.5%)	Vertical rhizome length (12.1%)	Average leaf length (12.4%)
6	Leaf width (8.0%)	Max leaf length (11.7%)	Max leaf length (12.3%)
7	Leaf area per shoot (7.1%)	Number of leaves (11.2%)	Number of leaves (11.7%)

Table 5
Linear regression models for vertical and horizontal rhizomes.

Region	Site	Linear equation	R^2	P-value	Comparisons
Vertical Growth					EF > BL = LKSP
BL	1	0.21 + 0.0027 × day	0.83	<0.05	Site 1 < Site 2
	2	0.36 + 0.0033 × day	0.77	<0.05	
	Pooled	0.27 + 0.0030 × day	0.76	<0.05	
EF	3	−0.04 + 0.0033 × day	0.91	<0.05	Site 3 = Site 4
	4	−0.05 + 0.0033 × day	0.87	<0.05	
	Pooled	−0.05 + 0.0033 × day	0.89	<0.05	
LKSP	5	0.47 + 0.0030 × day	0.85	<0.05	Site 5 > Site 6
	6	0.27 + 0.0027 × day	0.87	<0.05	
	Pooled	0.33 + 0.0029 × day	0.85	<0.05	
Horizontal Growth					BL < EF < LKSP
BL	1	0.70 + 0.0054 × day	0.57	<0.05	Site 1 < Site 2
	2	0.77 + 0.0117 × day	0.41	<0.05	
	Pooled	1.03 + 0.0065 × day	0.31	<0.05	
EF	3	0.33 + 0.0126 × day	0.38	<0.05	Site 3 = Site 4
	4	0.50 + 0.0155 × day	0.51	<0.05	
	Pooled	0.38 + 0.0139 × day	0.42	<0.05	
LKSP	5	0.37 + 0.0218 × day	0.87	<0.05	Site 5 = Site 6
	6	0.07 + 0.0243 × day	0.57	=0.083	
	Pooled	0.30 + 0.0225 × day	0.72	<0.05	

2.2.3. Flowering

All shoots were scrutinized for current and past flowers as indicated by the conspicuous scars left when inflorescences are shed. Seagrasses have separate male and female flowers that leave behind distinct scars unique to the sex of the flower (Witz and Dawes, 1995). However, the sex of past flowers was often unrecognizable in our samples due to significant weathering therefore male and female flowers were pooled together. For all shoots that were still attached to the horizontal rhizome, the node number where the flower occurred along the vertical rhizome was recorded and shoot age at time of flowering estimated from the overall average number of nodes produced per year per shoot at the site.

3. Results

3.1. Shoot morphometrics

3.1.1. Univariate analyses of morphometrics

Statistical evidence for Region as a significant component of explained variance was only found for the morphological metrics average leaf width, leaf area per shoot, leaf biomass, and vertical rhizome length (Fig. 1, Table 2). Average leaf width per shoot was narrower in both more variable environment regions than for shoots from LKSP, and BL shoots were narrower than those from EF (0.4 ± 0.004 cm, 0.5 ± 0.006 cm, 0.8 ± 0.008 cm for BL, EF, and LKSP, respectively). Shoots from greater environmental variability regions possessed less leaf area per shoot (15.6 ± 0.4 and 20.3 ± 0.6 cm^2, BL and EF respectively) than did shoots from LKSP (40.0 ± 1.04 cm^2). Leaf biomass per shoot was equivalent for shoots from the two more variable regions but lower than leaf biomass for LKSP shoots (0.05 ± 0.005, 0.05 ± 0.003, and 0.12 ± 0.008 gDW for BL, EF, and LKSP, respectively). Vertical rhizome length was shorter for the two more variable regions than LKSP rhizomes and EF rhizomes were shorter than rhizomes from BL (1.4 ± 0.05, 1.8 ± 0.05, and 2.2 ± 0.08 for EF, BL, and LKSP, respectively). Number of leaves per shoot did not show statistical significance among regions with testing at our pre-determined level of α (0.01), yet the lack of significance was marginal ($p = 0.03$). However, shoots from the two regions of greater environmental variability possess slightly fewer leaves on average than shoots from the more stable LKSP environment (2.5 ± 0.03 (SE), 2.7 ± 0.03, and 2.9 ± 0.04 for BL, EF, and LKSP respectively).

The nested random factor Site contributed a significant portion of the variance for the metrics average and maximum leaf length (Likelihood ratio (L) = 45, $p < 0.001$ and $L = 39.3$, $p < 0.001$, respectively), leaf thickness ($L = 21.2$, $p < 0.001$), leaf area per shoot ($L = 11.6$, $p < 0.001$), specific leaf area ($L = 30.7$, $p < 0.001$), and vertical rhizome width ($L = 67.2$, $p < 0.001$). Proportion of variance explained by Site ranged from low (3–9%) for the morphological metrics average and maximum leaf length, leaf area per shoot, and rhizome width to comprising at least half the variance (54–57%) for leaf thickness and specific leaf area (Table 2).

3.1.2. Multivariate analysis

The PERMANOVA for seven morphological variables (see Methods) indicated significant variation among Regions and between Sites, with Region contributing more to the explained variation (Table 3). The contrast of shoots from BL and EF regions against LKSP shoots was significant and accounted for the most explained variation (~22%), with marginally non-significant differences between BL and EF shoots comprising a minimal amount of the components of variation (Table 3). Unexplained variation accounted for the majority (~54%) of multivariate morphological variation. Multivariate dispersion tests and PCO centroid graphing were done for

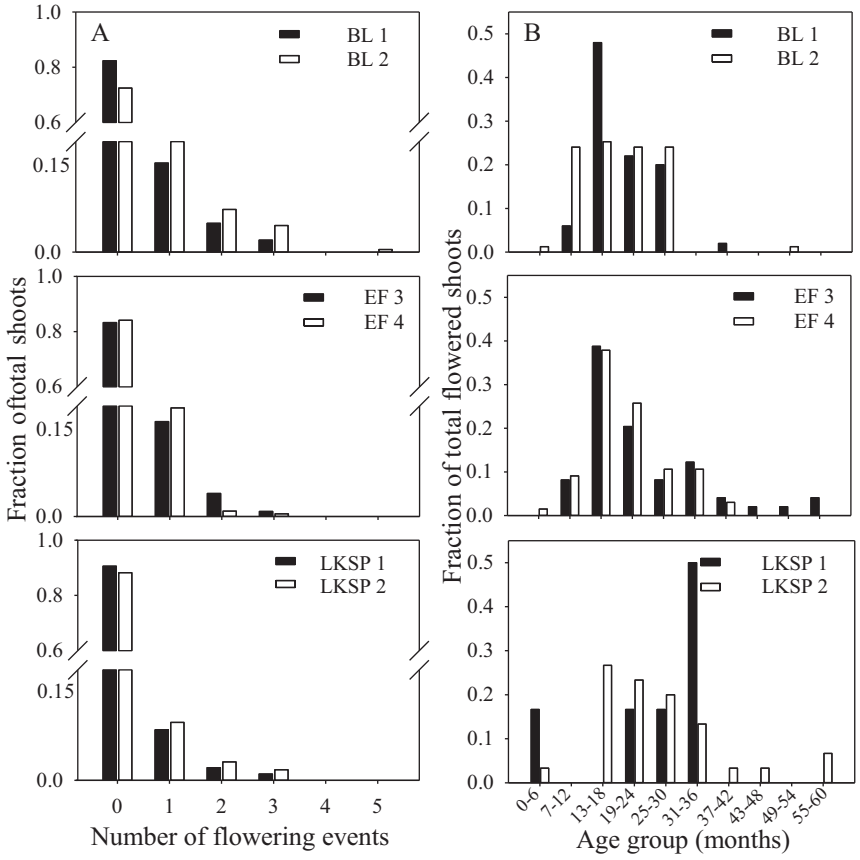

Fig. 3. Fraction of shoots in the total population versus number of flowering events over shoot lifespan (A), fraction of flowered shoots versus shoot age at first flowering (B).

Site only due to the PERMANOVA significance and nested nature of this term (Anderson et al., 2008). Sites were found to be significantly different in dispersion sizes ($P(perm) < 0.001$) with BL Site 1 having the least dispersion, LKSP Site 5 having the most dispersion, and all other sites equivalent. Due to these differences, further interpretation regarding the morphometric multivariate analysis must be undertaken with the caveat that significant regional differences may be influenced by differences in a dispersion.

Visual examination of the PCO centroids for Sites supports evidence of a morphological separation following an environmental variability gradient along PCO1, although there is substantial separation between the two LKSP sites along PCO2 (Fig. 2). SIMPER analysis examined relative contribution of each morphological metric to dissimilarity between shoots from each of the three distinct environmental regions. The metrics with the greatest relative contributions were distinct for comparisons between the two variable environments (BL v EF) versus comparisons between a variable environment and the more stable LKSP (Table 4). While the highest contributing metrics in all comparisons are related to photosynthetic area, shoot dissimilarities between a more variable and a more stable environment are driven by differences in leaf width and total shoot leaf area. Low relative contribution for leaf area per shoot in shoot based dissimilarities of the two higher variability environments is likely an effect of the higher number of leaves per shoot on average in EF offset by higher maximum and average leaf lengths of BL shoots (Fig. 1).

3.2. Shoot age and rhizome growth rates

Shoots typically produced between 16 and 18 nodes per year (average 17.5 ± 0.07) and did not differ significantly among regions ($F_{(2,2.77)} = 3.44$, $p = 0.18$) or between sites within regions ($F_{(1,263)} = 3.46$, $p = 0.06$). Shoot age histograms for Sites showed a modal age between 0.5 and 1.3 years followed by an exponential decline in shoot abundance with age (Fig. S3). At the time of collection, the shoots at LKSP showed higher values of maximum age (9.4 years versus 6.9 and 6.7 for BL and EF, respectively), mean shoot age (2.2 ± 0.07 years versus 1.7 ± 0.04 and 1.4 ± 0.05 for BL and EF, respectively), and mean age of the 5% oldest shoots (6.4 ± 0.2 versus 4.6 ± 0.14 and 4.1 ± 18 for BL and EF, respectively). Specific demographic metrics for individual Sites can be found in Table S1.

The regression equations fitted to the horizontal and vertical rhizome relationship between length and time for rhizome formation were mostly significant ($p < 0.05$), with the exception of LKSP at Site 6 horizontal rhizome growth (Fig. S4, Table 5). Coefficients of determination (R^2) ranged from 0.76 to 0.91 for vertical growth, and from 0.31 to 0.87 for horizontal growth. Comparing Regions with pooled Sites indicated higher vertical growth rates in EF ($p < 0.001$) than in BL and LKSP, which were similar ($p = 0.23$). For horizontal growth, we found increasing rates from BL to EF ($p = 0.003$) and EF to LKSP ($p = 0.019$; Table 5).

3.3. Flowering

Most shoots did not display evidence of any prior flowering events. However, the fraction of shoots with past flowering events was higher in the more environmentally variable regions than at LKSP, with these differences being most pronounced between BL and LKSP (22% versus 11% of shoots, respectively; Fig. 3A). In addition, we found a higher fraction of shoots with scars from multiple past flowering events in BL (8%) than in EF or LKSP (3% of shoots for both regions). The earliest flowering event occurred within the first

year since shoot appearance in all regions, and most first flowering occurred between 12 and 18 months (Fig. 3B). In general, shoots flowered earlier during their lifespan at BL and EF than LKSP (Table S2).

4. Discussion

Morphological and physiological phenotypic variation has been found to enhance seagrass survival following extreme events in a variable environment (Maxwell et al., 2014). As coastal environments worldwide continue to degrade (Lotze et al., 2006), understanding how intraspecific variation relates to environmental variability on an extensive spatial scale is essential to better predict the acclimatization and tolerance capabilities of seagrasses. This comparison examined populations exposed to an environmental gradient ranging from sub-optimal and greatly fluctuating to relatively stable, more ideal conditions. Our results provide compelling evidence that *T. testudinum* effectively utilizes a plastic phenotype strategy across a wide range of environmental variability. In addition, the differences this study elucidates across a variability gradient reflect the inherent capabilities of a single seagrass species to flexibly respond to such a range of conditions, evidencing potential for resiliency to future environmental variation and coastal deterioration threats.

The univariate and multivariate analyses on shoot morphometrics indicate that the short shoots from environments of higher variability exhibited slightly fewer, thinner leaves that resulted in a smaller leaf area and biomass than shoots from a more stable environment. The stark contrast of environmental variability between the estuaries along the northern coastline and the more oceanic LKSP likely influenced the morphological differences ascertained in this study. Low light availability, salinity fluctuations, nutrient content and makeup of sediments are all potential drivers of intra and inter-regional morphological differences due to effects on productivity (Kahn and Durako, 2006; Lee et al., 2007). The estuarine environments of BL and EF are more likely to be subjected to periods of freshwater inundation that lead to low irradiance levels and burial caused by runoff turbidity, resuspension of sediments, and potential for phytoplankton blooms via runoff nutrient loading. Because these environmental factors are intertwined, it is often difficult to specify which factor may be primarily driving morphological distinctions across an environmental variability gradient. Rather, it is likely that a number of sub-optimal environmental conditions as well as the variability in these estuarine regions that lead to the oft-reported reduced leaf area and biomass measured in *T. testudinum* as a mechanism to reduce self-shading while simultaneously lowering tissue respiration and carbon demand (Hemminga and Duarte, 2000; Ralph et al., 2007).

The two populations exposed to variable conditions also exhibited evidence of a shorter life span than the more stable environment shoots at the time of sampling. Sub-optimal environmental conditions, such as strongly reducing sediments, can greatly affect productivity that in turn can lead to decreased shoot survival within a population (Terrados et al., 1999). Additionally, higher nutrient availability can indirectly drive declines in shoot age structures via enhanced intraspecific and interspecific competition for resources (Romero et al., 2006). Within BL and EF, substantial interspecific competition within relatively confined areas of colonization may arise from *Halodule wrightii*, which is the second dominant seagrass species in both estuaries. This species has been found to more effectively compete with *T. testudinum* for resources in environmentally variable, higher nutrient habitats (Lirman and Cropper, 2003) and therefore may affect shoot productivity at these estuarine sites. Although this study can only describe any age measurements as a snapshot in time for these populations, younger shoot ages may be evidence of a higher population turnover in BL and EF. Shoot demography has been found to demonstrate population status changes over time, with lower shoot age structures and greater shoot turnover indicating natural variability or declining environmental quality (Peterson and Fourqurean, 2001; Marbá et al., 2005).

This snapshot view into the three *T. testudinum* populations' life histories also found evidence for different recruitment strategies among Regions. Higher horizontal rhizome growth rates in LKSP, and therefore more rapid vegetative reproduction via shoot production (Duarte et al., 2006), suggests that this population may expend more energy into the growth and maintenance of the existing meadow than its estuarine counterparts. While respiration rates are generally severalfold below that of the photosynthetic tissues, belowground biomass can make up a substantial portion of the total biomass of seagrasses (Fourqurean and Zieman, 1991). Therefore, vegetative shoot recruitment can be reduced by limiting light conditions when the necessary carbon demands are not met (Ralph et al., 2007). The higher light availability of the more oceanic environment of LKSP may provide photosynthetic compensation for the greater comparative respiratory demand that would be inherent with our findings of a higher vegetative expansion rates. Of course, these conditions are never static and vegetative growth rates can be greatly altered by changes in ambient environmental conditions (Duarte et al., 2006). Alternatively, BL and EF shoots appear to expend more energy towards sexual reproduction than those from LKSP. Fluctuating, sub-optimal environmental conditions may contribute to the high instances of shoot flowering effort within the BL population relative to the other regions examined, as flowering effort has been previously linked to enhanced environmental stress (Chollet et al., 2007; Diaz-Almela et al., 2007). An advantage of greater sexual reproduction effort by seagrasses is the potential for increased genetic diversity, which has been shown to enhance extreme stress or disturbance resiliency (Hughes and Stachowicz, 2004; Reusch et al., 2005). Additionally, long distance seedling dispersal via positively buoyant fruits may transport seedlings to an area more conducive to shoot growth (van Dijk et al., 2009).

5. Conclusion

Our findings provide evidence that a gradient of environmental variability on a large scale can have measurable implications on phenotypic variability of a habitat forming seagrass species, particularly for morphological and life history responses. The differences elucidated by this study suggest that each population is expressing characteristics specific to the environmental suitability in which it is located. While relatively small distances between sampled Sites could not possibly capture the potentially larger degree of shoot variability within a Region, the stark contrasts of shoot and population based variables found between Regions are evidence of wide ranging plasticity most likely driven by an environmental suitability gradient. A visual representation of these differences can be found in Fig. S5, which describes *T. testudinum* morphology, growth, and life history characteristics likely to be observed in high light, more stable salinity and temperature environs, versus highly dynamic environments. The dynamic environment is populated by shoots with small morphologies, shorter lifespans, and higher instances of flowering that combined provides evidence of the less favorable conditions encountered at this species' Gulf of Mexico northern range edge. The significant morphological and reproductive plasticity encountered along a gradient in abiotic variability is an inherently beneficial attribute of *T. testudinum*, in order for this species to have colonized the wide spectrum of environments across its range. The estuarine inhabiting populations maintain

productivity in anthropogenically dominated watersheds (USGS, 2004), which further emphasizes the importance of researching widespread phenotypic variability of seagrasses. The ability of seagrasses to acclimate to natural variabilities is not easily tested in mesocosm studies, therefore natural comparative studies are vital to estimating the degree of resiliency possessed by marine submerged plants. Furthermore, large spatial scale studies are imperative in order to better understand the effects potential new stressors from a deteriorating coastal environment may have on multiple aspects of these vital habitats.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2016.07.007.

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