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A map-based approach to assessing genetic diversity, structure, and connectivity in the seagrass *Halodule wrightii*

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ABSTRACT: Seagrass cover has declined in many areas of the world in a trend that has accelerated over the past several decades. This raises concern for both the impact the decline in cover has on coastal ecosystems and the effect it may have on seagrass evolutionary potential, as genotypic and genomic variation is lost. We used 8 microsatellite loci to investigate genetic diversity, structure, and connectivity in the seagrass *Halodule wrightii* from the Gulf of Mexico (Texas, USA) and western Atlantic (Bermuda). We examined how estimates correlated with changes in *H. wrightii* abundance and distribution on the Texas Gulf coast over the past 50 yr. Results show that, compared to other species, *H. wrightii* from this region exhibits variable clonal diversity (R = 0.02–0.81), moderate allelic diversity (mean A_R = 4.09), and relatively high heterozygosity (mean H_e = 0.56). The patterns of genetic diversity and structure, however, do not entirely coincide with either geography or recent historical trends in seagrass distribution in this region. Results from a basin in which seagrasses have recently been expanding were consistent with expectations, as they were for an isolated site near the limit of *H. wrightii*'s range. Results from basins in which seagrasses have been experiencing decline and/or fragmentation, however, were mixed. Genetic structure on the Texas coast was relatively weak and coincided more strongly with tidal range than with geographic barriers or distance. Rapid expansion and the discovery of identical multi-locus genotypes at several sites raises the possibility of migration via drifting vegetative fragments, as the geographic distance among certain multi-locus genotypes cannot be explained by rhizome growth models.

KEY WORDS: Genetic diversity · *Halodule wrightii* · Laguna Madre · Population structure · Dispersal

INTRODUCTION

Seagrasses form highly productive ecosystems that are often dominated by a single species of seagrass. A number of reports have shown a correlation between seagrass population genetic diversity and productivity or resistance to disturbance (Williams 2001, Hughes & Stachowicz 2004, Jahnke et al. 2015). It may be that in environments in which the number of structuring species is few, genetic diversity assumes a functional role similar to that of species diversity in more complex habitats (Reusch & Hughes 2006). If so, it is incumbent upon biologists to assess diversity in those structuring species and determine how it is shaped by geography, environment, and anthropogenic disturbance. Most of the work examining genetic diversity in seagrasses, however, has come from locations in the Atlantic, Pacific, and Mediterranean (Coyer et al. 2004, Olsen et al. 2004, Alberto et al. 2008, van Dijk et al. 2009, Nakajima et

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al. 2014, Arriesgado et al. 2015). With the exception of *Thalassia testudinum*, few reports have examined seagrasses from the Gulf of Mexico or Caribbean Sea. *Halodule wrightii* L. Ascherson is an early successional, dioecious species prominent throughout the tropical and sub-tropical Atlantic, Gulf of Mexico, and Caribbean Sea (Green & Short 2003). The Texas (USA) coast is a major center of seagrass in the Gulf of Mexico. It is home to approximately 90 000 ha submerged aquatic vegetation, most of which is *H. wrightii* (Pulich & Onuf 2007).

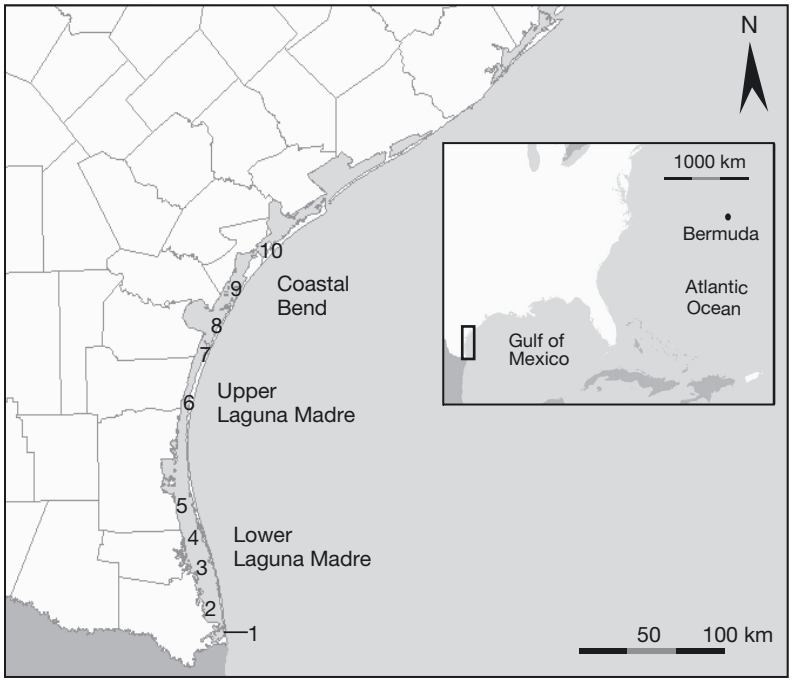

Fig. 1. Sampling locations in Gulf of Mexico (Texas, USA) and western Atlantic (Bermuda). Sites (1–10) are detailed in Table 1. Rectangle in the inset is the area shown in the main figure

More than 90% of seagrasses on the Texas coast are distributed between 2 regions, the Coastal Bend (CB) and Laguna Madre (Fig. 1). The CB is situated near the northern end of seagrass range on the Texas coast and contains approximately 15% of the state's seagrass acreage. While the total amount of seagrass has remained fairly stable over the past 50 yr, there has been a significant increase in the proportion of fragmented beds in the CB (Pulich 2007). The Laguna Madre is a narrow (3–12 km), long (200 km), and shallow (mean depth = 1.2 m) hypersaline lagoon that runs roughly north to south along the Texas Gulf Coast. A highly productive fishery, the Laguna Madre supports abundant seagrass meadows and serves as the winter home to vast populations of migratory waterfowl (Tunnell & Judd 2002). Information on Texas seagrass cover and distribution prior to the 1960s is sparse, but extreme hypersalinity (60–110 ppt) in the Laguna Madre likely meant that seagrasses were historically absent from much of its interior. Whatever meadows were present were probably restricted to its northern and southern ends, where salinity was lower due to proximity to tidal passes (Tunnell & Judd 2002). The Laguna Madre is divided by a natural sand flat into 2 basins, known by their relative positions as the Lower Laguna Madre (LLM) and Upper Laguna Madre (ULM). Construction of the Gulf Intracoastal Water Way (GIWW) ship channel in the 1940s, and a pass through Padre Island to the Gulf of Mexico (1962) connected the LLM and ULM and dramatically lowered salinity in both basins. The reduction is likely to have caused widespread expansion of seagrass throughout the Laguna Madre, but the mapping evidence is especially strong for the ULM (Fig. 2). The effect on the LLM has been more mixed. While mapping data from the 1960s show extensive *H. wrightii* cover in this basin, subsequent surveys show a progressive decline in both the total amount of seagrass and the proportion of cover composed of *H. wrightii*. These changes have been attributed to both natural and anthropogenic effects (Onuf 2007). Nevertheless, the LLM still supports extensive meadows and accounts for over 50% of Texas seagrass acreage (Pulich & Onuf 2007).

Location near a range edge, geographical separation, or a pattern of expansion, decline, or fragmentation has implications for population genetic diversity. Populations near the edge of their range often exhibit low genetic diversity and increased genetic differentiation, a factor attributed to greater spatial isolation and environmental stress associated with marginal habitats (Eckert et al. 2008). Geographical separation can lead to reduced gene flow while population fragmentation and decline can reduce population size and increase spatial isolation. This can erode allelic diversity and result in a loss of heterozygosity through genetic drift and inbreeding. Range expansion, in contrast, can result in its own genetic signature such as an excess of homozygosity or rare alleles (Excoffier et al. 2009). The effects, however, are not al-

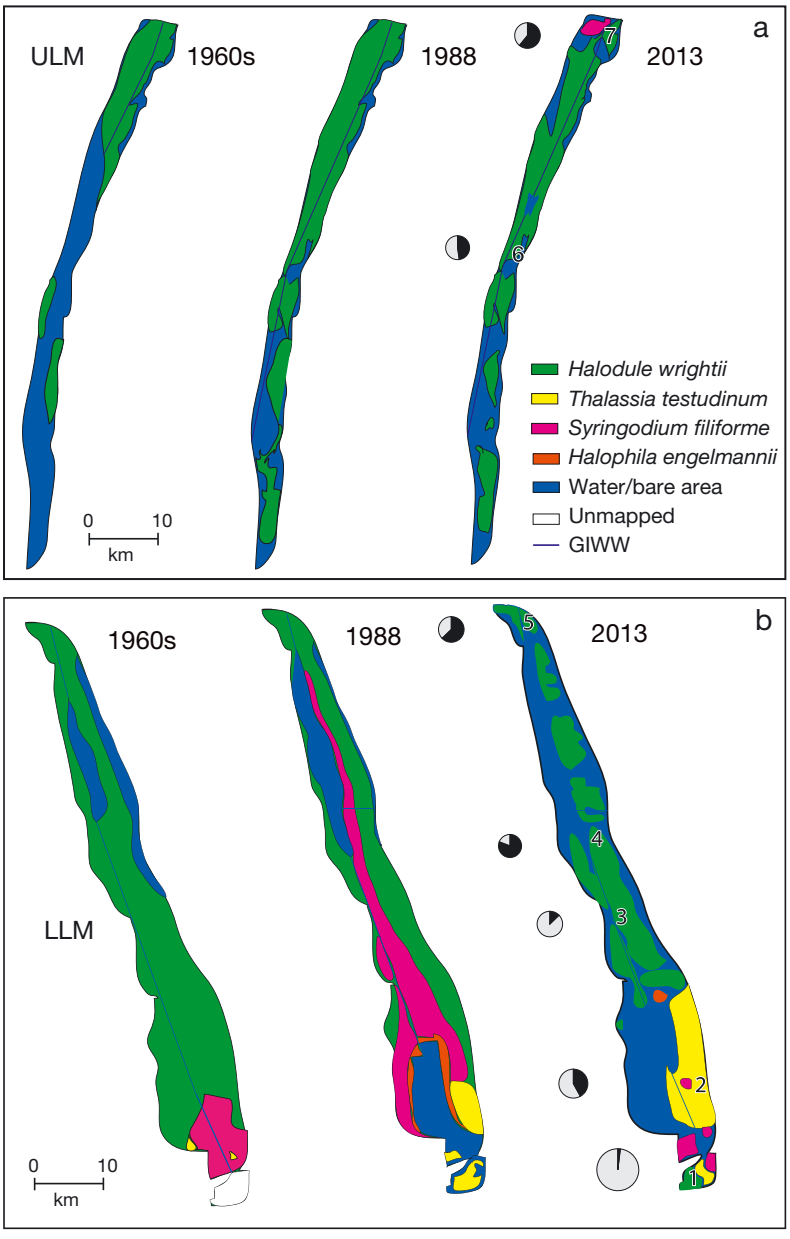

Fig. 2. Changes in seagrass abundance and distribution in the (a) Upper Laguna Madre (ULM) and (b) Lower Laguna Madre (LLM), 1960s–2013. Numbers indicate sampling sites. Pie graphs next to sites reflect proportion of samples with unique genotypes (size of black wedge) and degree of heterozygosity (diameter of circle). 1960s and 1988 map data adapted from Onuf (2007). 2013 map data adapted from Dunton et al. (2015). GIWW: Gulf Intra coastal Water Way

ways so straightforward and may depend upon factors such as length of impact, historical population sizes, or connectivity with other populations (Ferber et al. 2008, Jahnke et al. 2015).

This study used microsatellite loci to assess genetic diversity, structure, and connectivity in *H. wrightii* from the Texas Gulf of Mexico coast, as well as a more isolated location near its northern distributional limit in the western Atlantic (Bermuda). We compared our results with mapping data that showed how seagrass abundance and distribution on the Texas coast has changed over the past 50 yr. The goal of our study was to determine whether such changes were reflected in, and therefore might predict, the genetic results. We expected that genetic diversity would be low at sites near their distributional limit, and in areas where there was documented evidence of decline and/or fragmentation (Bermuda, CB). We expected it to be higher in areas where, despite recent evidence of fragmentation, the environment is still considered favorable and population sizes are still relatively large (LLM). We expected distance and physical separation to result in genetic structure on the Texas coast that aligned with the various basins (LLM, ULM, CB). Finally, we expected migration to follow a south to north direction along the Texas coast, consistent with regional Gulf of Mexico currents and the predominant wind direction in this largely atidal area.

MATERIALS AND METHODS

Study area and sample collection

Halodule wrightii samples were collected at 11 sites: 10 from the Texas Gulf of Mexico coast and 1 from Bermuda. The Texas sites were selected from a set of 5 km^2 monitoring stations established by the Texas Parks & Wildlife Department (Pulich et al. 2003). The sampling sites followed a latitudinal gradient and were chosen to span the majority (>90%) of *H. wrightii*'s range on the Texas coast. They included stations in the lower and upper basins of the Texas Laguna Madre and the Corpus Christi/Redfish/Aransas Bay system, collectively known as the 'Coastal Bend'. Corpus Christi Bay and a man-made causeway separate the Laguna Madre from the CB. Distances between sites ranged from 8 to 264 km.

One additional site was selected from a permanent monitoring station operated by the Bermuda Department of Conservation Services in Turtle Bay, Bermuda, in order to assess genetic diversity and differentiation in an anthropogenically impacted *H. wrightii* population from another portion of its range. Maps and data showing seagrass abundance and distribution for the Texas coast (1960s to present) were obtained from the United Sates Geological Survey (Handley et al. 2007) and the Texas Statewide Seagrass Monitoring Program (Dunton et al. 2015). These studies used intensive field sampling and aerial photography (1:24 000 scale) to survey the entire Laguna Madre and CB regions.

Samples from the Texas coast were collected from May to July 2008. Sampling sites were chosen as close as possible to the center of each monitoring station, within a continuous seagrass meadow whenever possible. Water depth among sites ranged from 0.5 to 1 m. A 6 × 30 m rectangular plot was established at each Texas site roughly parallel to the mainland shore. The plot comprised 4 parallel, 30 m transects spaced at 2 m intervals. Samples (n = 48) were collected in a regular manner at 2 m intervals along each transect. Each sample consisted of a 5–10 cm section of horizontal rhizome. Sparse seagrass coverage at one site (Mustang Island, Site 8) resulted in the collection of only 34 samples. Rhizomes were stored in seawater for transport back to the laboratory, where they were freeze-dried and stored on silica gel prior to extraction of DNA. Samples (n = 40) from Bermuda were collected in July 2012. Rhizome sections were collected in a haphazard fashion from an 8 m-wide belt along a 50 m transect. Distances between samples were approximately 2 m. Samples were placed on ice and sent by overnight delivery to Texas for genotyping. GPS coordinates were collected for each Texas sample, but only the site coordinates were collected for Bermuda.

DNA extraction and microsatellite scoring

Genomic DNA was extracted from ca. 20 mg of dried rhizome tissue using the Plant Dneasy kit from Qiagen, according to the manufacturer's instructions. A modification to the protocol was made by first homogenizing the tissue in a FastPrep 24 instrument (MP Biomedicals) using Lysing Matrix A. DNA was quantitated using a Qubit fluorometer and a QuanIT dsDNA assay kit (Invitrogen). A total of 34–48 samples from each sampling location were genotyped at 8 microsatellite loci, representing a mix of di-, tri-, and tetranucleotide repeats: Hw180, Hw190b, Hw196, Hw212, Hw214, Hw222, Hw228, and Hw232. Four of the loci (180, 196, 212, 214) have been described previously (Larkin et al. 2012). GenBank accession numbers, primer sequences, and thermal cycling protocols for all loci are available in Table S1 (see the Supplement at www.int-res.com/articles/suppl/m567p095_supp.xlsx). Four additional loci were run across all samples but were found to exhibit null alleles, or deviate from Hardy–Weinberg equilibrium at more than one site. Amplification of marker loci were performed in a 10 µl volume that included ca. 10 ng of genomic DNA, 5 µl of 2X TopTaq® PCR Master Mix (Qiagen), 0.05 µM each of both forward and reverse primer, and sterile, filtered water. Forward primers were labeled with either a WellRED D3 or D4 fluorescent dye. Thermal cycling was performed on a BioRad S1000 thermal cycler. PCR products were separated on a CEQ 8000 Genetic Analyzer (Beckman-Coulter) using a 400 bp size standard (Beckman-Coulter) and scored using the CEQ 8000 Genetic Analysis System Software (v 9.0). Samples that showed poor amplification for one or more loci were removed from the dataset. Fifteen percent of the samples were run in duplicate to confirm allele scores. Scoring error rates for individual loci ranged from 0 to 6% (mean 2.1%). Any sample showing a discrepancy between duplicate analyses was re-assayed to verify the correct score.

Data analysis

GENCLONE 2.0 was used to identify clonal replicates from each site using the method of Arnaud-Haond et al. (2007). The program calculates the probability of finding identical multi-locus genotypes (MLGs) that have arisen from independent sexual events (p_{sex}). Identical MLGs from each site with p_{sex} re-encounter values <0.01 were considered to belong to the same clone (genet). Duplicate MLGs were removed from each site's dataset to avoid bias in the estimates of genetic diversity, structure, and migration. Slightly distinct MLGs (1 to 2 allelic differences) were re-assayed at the variable loci to determine whether the differences were the result of a scoring error or a somatic mutation. Those with differences determined not to be the result of an error had their p_{sex} values re-calculated without the variable loci. If the resulting p_{sex} value was <0.01, the slightly distinct MLGs were considered to belong to the same multi-locus lineage (MLL) and the less frequent MLG was removed from the site's dataset.

The sets of unique MLGs and MLLs from each sampling site were analyzed using Microchecker 2.2.3 (van Oosterhout et al. 2004) to check for null alleles and possible mis-scores due to stuttering or large allele dropout. Linkage disequilibrium among loci and exact tests for deviation from Hardy–Weinberg equilibrium (F_{IS}) were estimated using GenePop on the Web 4.2 (Rousset 2008). Significance values were adjusted using a modified false discovery rate (FDR) procedure (Benjamini & Yekutieli 2001), which produces a better balance between the risk of Type I and Type II errors than the Bonferroni correction. GENCLONE 2.0 was also used to determine the number of polymorphic microsatellite markers necessary to allow efficient discrimination of genets by plotting clonal diversity R versus the number of loci, where R was defined as $(G–1)/(N–1)$, G is the number of unique genotypes and N is the total number of shoots sampled at a site. BOTTLENECK 1.2.02 (Piry et al. 1999) was used to check for founder effects and/or recent reductions in effective population size using a 2-phase model (TPM) with 95% single-step mutations, 5% multi-step mutations, and a variance among multiple steps of 12. Significance was assessed using the Wilcoxon signed rank test. Genetic diversity estimates such as observed and expected heterozygosity (H_o and H_e, respectively), mean number of alleles (A), number of private alleles (A_p), and proportion of less frequent alleles (A_{LF}; frequency ≤10%) were calculated using GenAlEx 6.5 (Peakall & Smouse 2006). Allelic richness (A_R), based on a sample size of 7 genets, was estimated using FSTAT 2.9.3.2 (Goudet 1995).

Genetic connectivity, structure, and migration

Pairwise genetic differentiation (F_{ST}; Weir & Cockerham 1984) between all sites was estimated with GenoDive 2.0b23 (Meirmans & Van Tienderen 2004) using 9999 permutations to assess significance. Genetic differentiation was also assessed using Hedrick's G'_{ST} and Jost's D_{est} in GenAlEx. Isolation by distance (IBD) among the Texas sites was assessed with a Mantel test (log geographic distance [km] vs. $F_{ST}/[1–F_{ST}]$; GenAlEx) that also used 9999 permutations to assess significance. Geographic distances for the Mantel test were estimated in Google Earth, using the shortest over-water paths between sampling sites. The influence of basins on genetic structure was examined using GenAlEx to perform an analysis of molecular variance procedure (AMOVA) on the sites from the LLM, ULM, and CB (9999 permutations). We also used the 'compare groups' option in GenoDive to compare genetic diversity estimates (H_o, H_e, F_{IS}) among the basins.

A Bayesian analysis of genetic structure among the Texas sampling sites was performed using STRUCTURE 2.3.2 (Pritchard et al. 2000). The number of genetic clusters (K) was assessed for values ranging from 1 to 11. We used an admixed ancestry model, testing both independent and correlated allele frequencies in separate runs that used sampling locations as priors. Each run consisted of 20 replicates, with a burn-in set to 500 000 followed by 1 000 000 Markov chain Monte Carlo (MCMC) repetitions. We used Clumpak (Kopelman et al. 2015) to identify the number of clusters with the strongest support using both Pritchard's estimated log probability of the data, $Pr(X|K)$ (Pritchard et al. 2000) and Evanno's ΔK method (Evanno et al. 2005).

Migration direction and effective population size (N_e) estimates for the Texas sites were determined using Migrate-n 4.2 (Beerli 2009) on the High Performance Computing Cluster at Texas A&M University–Corpus Christi. Eight different migration models were tested between each sampling location and its adjacent sites: (1) full, bi-directional exchange of migrants between sites; (2) 1-way migration (south to north); (3) 1-way migration (north to south); (4) panmixia; (5) divergence (splitting off) of the northern site from the more southern one, with subsequent (south → north) migration; (6) divergence of the southern site from the northern one, with subsequent (north → south) migration; (7) divergence of the northern site from the southern one, without subsequent migration; and (8) divergence of the southern site from the northern one without subsequent migration (Beerli 2015). All models were tested using the same set of prior settings that included a Brownian motion model, uniform prior distributions for both population size and migration rates, 15 000 recorded steps, and a sampling increment of 100. Three replicates were performed for each model test for a total of 4 500 000 genealogies per run with a burn-in set to 1 500 000. Markov coupled MCMC ('heating') was used to explore the genealogy space, using the program's default settings. Model rankings were based on Bezier log marginal likelihood values, with higher values indicating greater model support (Beerli & Palczewski 2010). N_e estimates were reported as the median value of the mutation-scaled effective population size (Θ) from the models with strongest support, divided by 4 times the mutation rate (μ), which we set to 0.0001 for microsatellites (Thuillet et al. 2002).

Table 1. Genetic diversity estimates for *Halodule wrightii*. LLM: Lower Laguna Madre; ULM: Upper Laguna Madre; CB: Coastal Bend; *N*: number of samples; *G*: number of genets; *R*: clonal diversity; *A*: mean number of alleles; A_R: allelic richness standardized to a sample size of 7 genets; A_{LF}: proportion of less frequent alleles (frequency ≤ 0.10); A_p: number of private alleles; H_o: observed heterozygosity; H_e: expected heterozygosity (unbiased); F_{IS}: inbreeding coefficient

Site	Basin	Location	Latitude (°N)	Longitude (°W)	*N*	*G*	*R*	*A*	A_R	A_{LF}	A_p	H_o	H_e	F_{IS}
1	LLM	South Bay (SB)	26 01.075	97 11.082	48	2	0.02	3.50	–	–	–	0.94	0.83	−0.20
2	LLM	S. Padre Island South (SPIS)	26 08.754	97 10.790	46	20	0.42	6.50	4.25	0.52	1	0.57	0.57	0.01
3	LLM	Arroyo Colorado North (AC)	26 27.446	97 22.148	48	7	0.13	5.75	5.13	0.46	1	0.66	0.65	−0.01
4	LLM	P. Mansfield South (PM)	26 31.886	97.22.321	48	39	0.81	5.63	3.48	0.54	–	0.47	0.46	−0.02
5	LLM	Land Cut South (LC)	26 47.575	97 27.886	47	30	0.63	6.13	3.70	0.46	–	0.55	0.51	−0.09
6	ULM	Emmonds Hole (EH)	27 24.092	97 21.490	47	23	0.48	6.50	3.73	0.59	–	0.46	0.47	0.03
7	ULM	Packery Channel (PC)	27 38.561	97 13.514	47	29	0.61	7.38	4.06	0.62	3	0.48	0.51	0.06
8	CB	Mustang Island (MI)	27 45.675	97 08.950	33	16	0.47	6.25	4.32	0.42	1	0.72	0.67	−0.08
9	CB	Redfish Bay (RB)	27 53.593	97 06.674	48	4	0.06	3.38	–	–		0.50	0.48	−0.04
10	CB	Bray Cove (BC)	28 08.590	96 48.664	48	3	0.04	4.00	–	–	1	0.67	0.67	0.00
11	–	Bermuda (BM)	32 21.305	64 39.408	40	2	0.03	2.38	–	–	3	0.44	0.38	−0.28
		Total			500	175								
		Mean			45.5	15.9	0.34	5.22	4.09			0.59	0.56	−0.06
		Texas only			46.0	17.3	0.37	5.50	4.09	0.52	1.40	0.60	0.58	−0.03

RESULTS

Genetic diversity

Five hundred samples from 10 sites along the Texas Gulf coast and one from Bermuda were genotyped at 8 microsatellite loci isolated from *Halodule wrightii*. A plot of clonal diversity (*R*) versus number of loci produced an asymptotic curve that reached a maximum at 6–7 loci for all sites (data not shown), indicating that 8 loci were more than sufficient for delineating unique genotypes. In every instance where multiple copies of a particular MLG was found, the p_{sex} value was less than 0.01, indicating that copies were individual ramets of the same genet. Some MLGs differed from one other by small allelic size differences (1 to 2 microsatellite repeats) at only 1 or 2 loci, indicating either mis-scoring of alleles or somatic mutations. If the size difference (somatic mutation) was confirmed by PCR, the less frequent MLG was assigned membership in the same MLL as the more frequent MLG. All genetic analyses were based upon the set of unique MLGs and MLLs from each sampling site. Linkage disequilibrium was present in 5% of the locus-by-locus interactions. The affected loci varied across sites but the majority occurred at one sampling location (Site 8, Mustang Island) from the Texas Gulf Coast.

Compared with other species, *H. wrightii* exhibited variable clonal diversity, moderate allelic diversity, and high heterozygosity (Table 1). *R* was the feature that varied most widely across sites (0.02–0.81, mean = 0.34), with several sites consisting of only 2, 3, or 4 genets. With the exception of Bermuda (*R* = 0.03), no basin consisted exclusively of high or low values. Clonal diversity was highest (0.48–0.81) in the middle portion of the Texas sampling range that encompassed the ULM and the upper (northern) portion of the LLM, and lowest at both the northern (CB) and southern (LLM) ends (0.02–0.06). The small number of genets at the Texas end sites and Bermuda prevented useful estimates of allelic diversity (A_R, A_{LF}) and bottlenecks at these locations. However, evaluation of the remaining sites showed no evidence of recent reductions in effective population size. A Wilcoxon signed rank test indicated a deficiency of heterozygotes at sites from the ULM (p = 0.02–0.04), a feature characteristic of expanding populations (Excoffier et al. 2009). The ULM also possessed the highest proportion of less frequent alleles (frequency ≤0.10) and tied with Bermuda for the largest number of private alleles (3). Site 7 in the ULM also exhibited a unique allelic sharing pattern. It possessed a large number of alleles that were otherwise found only to the north or south, indicating a potential region of contact between Laguna Madre and CB populations. A_R ranged from 3.48 to 5.13 (mean = 4.09), with the highest values found at sites in the lower (southern) portion of the LLM and the lowest ones in the upper LLM and ULM. H_e ranged from 0.38 to 0.83 (mean = 0.56). The lowest value was found at Bermuda while the highest ones were found at the highly clonal Texas end sites (1, 3, 8, 10). Inbreeding coefficients (F_{IS}) were consistently low (−0.28 to 0.06), although

Table 2. Analysis of molecular variance results for Texas sites. Results were generated with GenAlEx v 6.5

Source of variation	df	SS	MS	Est. variance	% total	p
Among basins	2	15.7	7.8	0.022	1	0.002
Among sites	7	32.4	4.6	0.084	4	0
Within sites	336	706	2.1	2.101	95	0
Total	345	754		2.208	100	

several sites from Texas (4, 5, 7, 9) and Bermuda were fixed for one or more loci.

Genetic structure and connectivity

AMOVA for the Texas Gulf Coast showed most variation (95%) to be attributed to differences among individuals (Table 2). Only 4% was attributed to differences among sites and 1% of the variation was attributed to differences among the LLM, ULM, and CB. There were no significant differences in H_o, H_e, or F_{IS} among the Texas basins.

Pairwise F_{ST} estimates showed a mix of low, moderate, and pronounced genetic differentiation between sites (Table 3). The minimum distance between sites with a significant pairwise F_{ST} value (0.10) was 8 km, between Sites 3 and 4 in the LLM. The smallest (significant) pairwise F_{ST} values (0.02–0.04) occurred between sites in the middle portion of the Texas range. Two of these locations (Sites 4 and 5) shared 3 distinct MLGs despite being 30 km apart. These MLGs, verified by re-extraction and analysis of the original samples, remained identical even after including allele scores from 4 additional loci (12 total). Low pairwise F_{ST} values (0.03–0.05) were also found between sites at the northern (CB) end of the Texas sampling range, where a shared MLG was found between a pair of sites (8 and 9) separated by 15 km. A similar situation occurred at the southern (LLM) end, where low F_{ST} values (0.03–0.05) and shared MLGs were found between Sites 1 and 2 (14 km), and 2 and 3 (39 km). Remarkably, the MLG found at Sites 1 and 2 was also found at Site 8, over 200 km to the north. Moderate F_{ST} values (0.06–0.18) were more frequently obtained between the end sites and those from the middle portion of the Texas sampling range, although not all of these were significant. Predictably, pairwise F_{ST} values were highest (0.31–0.54) between the Bermuda and Texas sites, where physical distances exceeded 3600 km. A Mantel test showed F_{ST} results to be strongly correlated with values for both G''_{ST} and Jost's D_{est} (R^2 = 0.99 and 0.97, respectively, $p < 0.01$), indicating a consistent degree of differentiation across metrics.

The variation in pairwise F_{ST} was reflected by a lack of IBD among the Texas sites (R^2 = 0.006, p = 0.26). Population assignment results supported this finding. A Bayesian modeling approach found strongest support for either 2 (Evanno's ΔK) or 3 (Pritchard's $Pr(X|K)$) genetic clusters on the Texas coast. Both results showed a uniform genetic background for the middle portion of the sampling range and a mixed, but similar, background at both ends (Fig. 3). The breaks in structure corresponded more strongly with the extent of tidal range in the Laguna Madre than with the physical barriers that divide the LLM, ULM, and CB from one another. The Pritchard's $Pr(X|K)$ results also offered support for the

Table 3. Pairwise genetic differentiation among *Halodule wrighti* sampling sites (1–11). Values below the diagonal represent F_{ST} values, estimated with GenoDive 2.0b23. Values above the diagonal represent distance (km). Significant false discovery rate-corrected p-values (≤0.05) are in **bold**. See Table 1 for site abbreviations

	1 (SB)	2 (SPIS)	3 (AC)	4 (PM)	5 (LC)	6 (EH)	7 (PC)	8 (MI)	9 (RB)	10 (BC)	11 (BM)
1 (SB)	–	14.4	52.0	59.8	90.3	159.6	190.2	205.9	220.7	263.6	3615
2 (SPIS)	0.049	–	39.4	46.8	77.3	146.3	177.1	192.2	207.2	249.5	3638
3 (AC)	0.032	0.027	–	8.3	38.3	107.7	138.2	153.6	168.6	211.3	3643
4 (PM)	**0.183**	**0.034**	**0.098**	–	30.4	99.3	130.6	146.2	161.3	203.8	3659
5 (LC)	**0.146**	**0.030**	**0.075**	0.013	–	69.3	99.8	115.5	130.5	173.0	3683
6 (EH)	0.139	**0.046**	**0.089**	**0.034**	**0.019**	–	30.9	46.4	61.5	103.6	3695
7 (PC)	**0.125**	0.013	0.037	**0.017**	0.015	**0.036**	–	15.1	30.4	73.3	3644
8 (MI)	0.005	0.030	0.006	**0.118**	**0.084**	**0.096**	**0.062**	–	15.3	57.8	3653
9 (RB)	0.184	0.063	0.035	**0.118**	**0.067**	**0.086**	0.051	0.045	–	42.6	3683
10 (BC)	0.065	0.029	0.015	**0.101**	**0.082**	**0.113**	0.062	0.025	0.036	–	3688
11 (BM)	0.308	**0.440**	0.396	**0.537**	**0.502**	**0.521**	**0.494**	**0.327**	0.543	0.423	–

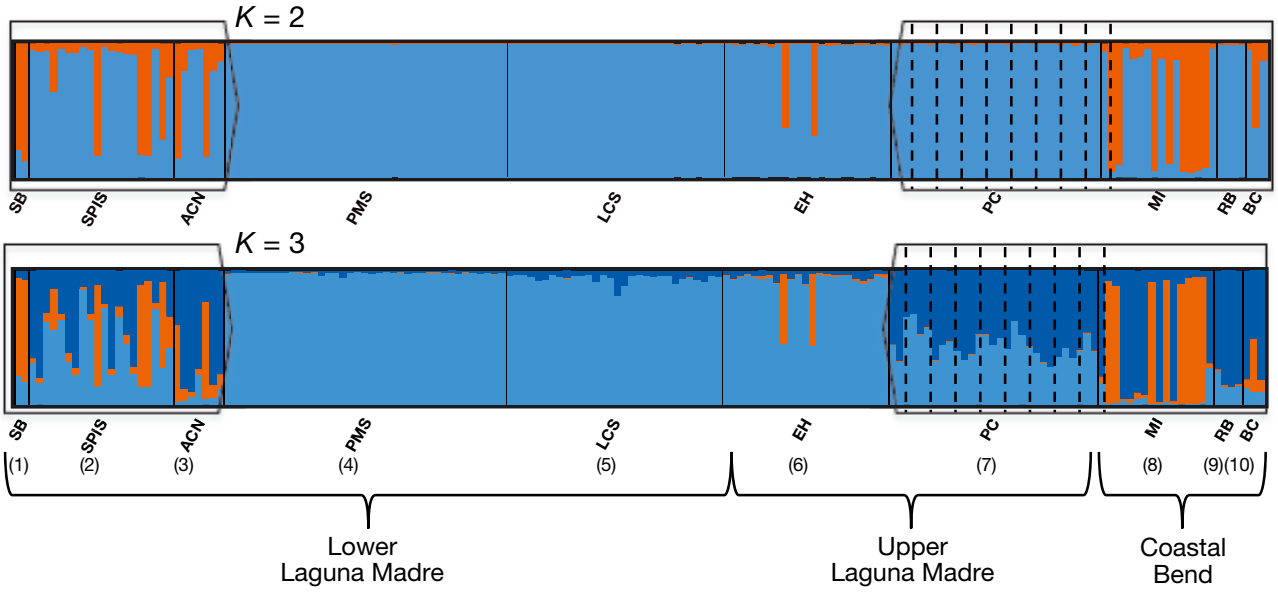

Fig. 3. Population assignment plots of samples from the Texas Gulf Coast using STRUCTURE. Clustering used sampling locations as prior information, an admixture model, and assumed correlated allele frequencies among sites. The number of genetic clusters (K) to be analyzed was set from 1 to 11. The strongest support was found for $K = 2$ clusters by Evanno's ΔK (2005) and for $K = 3$ by Pritchard's Pr($X|K$). Sample sites (numbers in parentheses; see Table 1 for site abbreviations and details) are listed on the x-axis and probability of assignment on the y-axis. Bars represent individual samples; colors represent probability of assignment to a particular genetic cluster. Boxes pointing inwards represent the approximate extent of tidal range (Gill et al. 1995). Hatched lines represent uncertainty in location of the tidal/atidal transition zone in the Upper Laguna Madre

area around Site 7 (Packery Channel) serving as a point of contact, or transition, between the Laguna Madre and CB populations.

Migration and population size estimates

We also use a Bayesian approach in Migrate-n to estimate population sizes (N_e) and the direction of migration among adjacent sites on the Texas Gulf Coast (Fig. 4). Eight models were tested between each pair of adjacent sampling locations, ranging from full bi-directional exchange of migrants to limited, 1-way migration between putative 'source' and 'sink' sites, to panmixia. Median population size estimates ranged from a low of 2475 at Site 9 to a high of 10 000 at Site 10, both of which occurred in the CB. However, a more general trend showed higher N_e estimates at both the southern (LLM) and northern (CB) ends compared with the LLM–ULM interior. Migration estimates did not show a consistent direction of movement. Two-way (north ←→ south) migration was the predominant model among sites at both ends of the Texas sampling range while 1-way migration had strongest support in the interior. Even here the directions varied, with a south → north direction of migration predominating in the lower part of the LLM and a north → south direction predominating in the ULM and the upper part of the LLM. One exception occurred again around Site 7 in the ULM, which appears to serve as a sink for migration from both the south and the north.

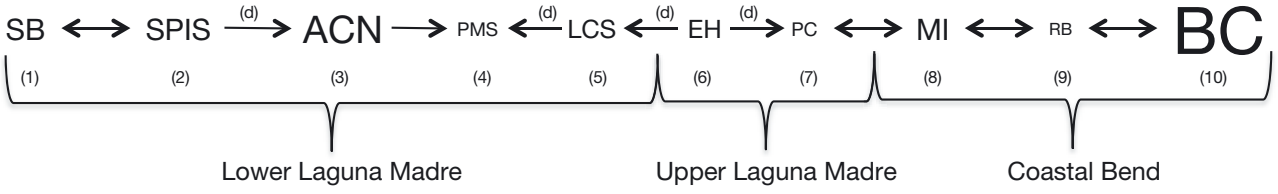

Fig. 4. Migration direction and population size estimates for sites from the Texas Gulf Coast, as determined using Migrate-n. Migration direction with strongest model support indicated by arrows; d: divergence without subsequent migration. Estimates of relative population (N_e) sizes are indicated by font size of site abbreviations. Site numbers are indicated in parentheses; see Table 1 for site abbreviations

DISCUSSION

Maps of cover and distribution for the past 50 yr show a different set of dynamics in each of the major seagrass-containing basins on the Texas coast. Data from the 1960s show an abundance of *Halodule wrightii* throughout the LLM. Subsequent surveys, however, show a decline in abundance and an increase in bed fragmentation, a feature also experienced in the CB. During this same time period the ULM has generally shown a dramatic expansion in *H. wrightii* cover, although there have been periodic algal blooms (*Aureoumbra lagunensis*) that have slowed this trend (Onuf 2007). These patterns coincide with a lowering of salinity in much of the Laguna Madre, maintenance dredging activity, and a general increase in population growth and development along the Texas Coast.

The correlation between changes in *H. wrightii* distribution/abundance and genetic diversity for the various regions was mixed. Results for the ULM, for example, were consistent with expectations for populations undergoing expansion such as an excess of low-frequency alleles, excess of homozygosity, little linkage disequilibrium, weak genetic structure, and an absence of IBD (Slatkin 1993, Excoffier et al. 2009). The expansion appears to have occurred relatively rapidly, with most of the growth occurring between the mid-1960s and late 1980s. Prior to construction of the GIWW in the 1940s, the salinity levels in the ULM could exceed 100 ppt during the summer months (Tunnell & Judd 2002). This is well in excess of *H. wrightii*'s upper limit (70 ppt), with the probable result that seagrasses were historically absent from most of the ULM. A unique allelic sharing pattern at Site 7 and results from genetic structure and migration analyses also indicate that the upper part of the ULM may serve as a zone of contact between Laguna Madre and CB populations. This has support in the work of Anderson et al. (2014), who identified a similar zone of contact for eastern oysters in this area.

Results from Bermuda were also consistent with those expected for an 'edge' population situated near the end of a species' range. Samples from this location exhibited the lowest clonal diversity and lowest H_e among all sites tested. Four of the 8 loci from Bermuda were also fixed for particular alleles. While the archipelago is over 3600 km from the Texas coast, it is also over 1000 km from the nearest coastline that may harbor *H. wrightii*. The isolation and small size of Bermuda seagrass beds appear to have resulted in genetic drift and/or inbreeding.

The correlation between genetic diversity results and mapping data for the LLM and CB were not as consistent. We expected genetic diversity to be highest in the LLM, where seagrass beds remain relatively abundant despite a 5-decade history of fragmentation. Clonal diversity was highest in the upper portion of this basin, a pattern that extended into the ULM. Disturbance related to maintenance dredging activity in this area may have opened up new substrate for recruitment of novel genotypes from a seed bank. The intermediate disturbance hypothesis states that diversity should be highest in populations that experience disturbance at an intermediate scale of frequency and intensity (Connell 1978). Reusch (2006) found that a composite index that took into account clonal recruitment, growth, decline, and loss was maximized for *Zostera marina* at intermediate disturbance levels. This may be particularly relevant for the LLM, where fragmentation has been significant and seed banks have been shown to be substantial (McMillan 1985).

Other estimates of diversity such as allelic richness and heterozygosity, however, were relatively low in this area. High clonal diversity, low A_R, and low H_e might be reconciled if upper LLM beds were formed as part of the same expansion that culminated in the ULM by the 1980s. The upper part of the LLM is far from any natural passes to the Gulf of Mexico. Like the ULM, salinity in this area is also likely to have been high prior to construction of the GIWW. Post-GIWW colonization by a limited number of genotypes with a mixed mode of clonal and sexual reproduction could have resulted in high clonal diversity but lower A_R and H_e. While sites from this area did not contain as many signals of expansion as those from the ULM, low pairwise F_{ST} values with its ULM counterparts, a common genetic clustering, and fixation of the same alleles suggest a similar set of founders.

We generally found high genetic diversity in the lower portion of the LLM, where the impacts of fragmentation and decline appear to have been greatest. Despite low clonal diversity, sites in this area showed high allelic richness and/or heterozygosity. Estimates of N_e for sites in this region were also relatively high. A natural pass to the Gulf of Mexico in the lower LLM means that pre-GIWW salinity levels were probably much lower here than in the rest of the Laguna Madre. Coupled with the favorable climate and protected shorelines, it's likely that *H. wrightii* previously flourished in the lower LLM. 1960s maps show that it was well established prior to its displacement and fragmentation in later decades. The re-

maining sites may be the remnants of a historically large population, surviving through a combination of high genetic diversity, clonal expansion, and migration from sites within and around the LLM. A similar situation may have occurred in the CB, where high A_R, H_e, and N_e estimates were also found despite low clonal diversity at some sites. This basin also contains passes to the Gulf and exhibits strong evidence of gene flow with nearby sites. This is not to say these areas face no genetic risks. If present trends continue, allelic diversity will be lost, and drift will result in reduced heterozygosity. The relatively recent impacts (50 yr) may not yet have been enough time to produce a significant effect.

Interestingly, both the lowest (Bermuda) and highest (LLM, CB) heterozygosity estimates occurred at sites dominated by single clones. Theoretical and experimental results indicate that populations must go through serious reductions in size, frequently for many generations, for significant decreases in H_e to occur. This may have been the case for the Bermuda site, which contained only 2 genetic individuals and is relatively isolated. While it is tempting to associate the high H_e Texas sites with a fitness advantage attributed to large clones, a lack of correlation between clone size and H_e in other studies makes this conclusion questionable (Diaz-Almela et al. 2007, Arnaud-Haond et al. 2010). A more recent history of moderate–high regional N_e, disturbance-mediated openings in the canopy, and rapid rhizome extension rates may be a better explanation.

The inbreeding coefficient (F_{IS}) was consistently low across the sampling range, a finding common among seagrasses (Jiang et al. 2014). While low values can be indicative of high N_e, negative F_{IS} values were found even at Bermuda, which otherwise exhibited genetic diversity values characteristic of a small population. Of more concern may be the number of fixed loci at various sites. Samples from Bermuda were fixed at 4 of the 8 loci, while several Texas sites, particularly those that may be the result of more recent expansion, were fixed at 1 or 2. *Halodule wrightii* is dioceious, with both male and female plants. The sexual nature of individual clones will prevent self-fertilization, but a small population size will eventually lead to inbreeding. Linkage disequilibrium was also low and confined primarily to a single site (Site 8), where a signal of genetic admixture may be a better explanation for the result than physical linkage of loci (Slatkin 2008).

Our initial expectation was that the linear distribution, physical separation, and distances among sites (8–264 km) would result in significant genetic structure and strong IBD along the Texas coast. This was not the case. Pairwise F_{ST} estimates were consistently low, implying high levels of gene flow. AMOVA results attributed only a small portion of the variation to geographical location, and tests for IBD were not significant. While many marine species can disperse over very large distances, seagrass capabilities are more variable. IBD results are not always consistent with predicted dispersal capacity and tend to reach a geographic threshold at a few hundred kilometers, below which the relationship between pairwise population genetic and physical distance becomes rather weak (Kendrick et al. 2012). The finding of drifting, seed-bearing shoots, viable vegetative fragments, or the same MLG in meadows spaced widely apart have also called into question predicted limitations on seagrass dispersal (Harwell & Orth 2002, Coyer et al. 2007, Jiang et al. 2014). It may be that in addition to dispersal via movement of seeds, pollen, or reproductive (seed-bearing) shoots, seagrasses also have the capacity to disperse via vegetative fragments. Complete, floating *H. wrightii* root/rhizome/shoot bundles are commonly found along the Texas coast (P. D. Larkin pers. obs.). Multiple reports have shown that both reproductive shoots and vegetative seagrass fragments (including *H. wrightii*) are viable, buoyant in the water column, and capable of re-establishing in mesocosm environments (Hall et al. 2006, Berkovi et al. 2014, Thomson et al. 2015). Modeling studies have shown reproductive shoots to have dispersal ranges in excess of 100 km (Erftemeijer et al. 2008). Vegetative dispersal thus seems a possible explanation for the multiple examples of identical MLGs found at more than one site in this study. For these MLGs to be remnants of single genets that previously expanded through rhizome extension would require clone sizes of 14 to over 200 km. Based on a *H. wrightii* rhizome extension rate of 223 cm yr^{-1} (Marba & Duarte 1998) and assuming a 2-way model of expansion, this would mean clone ages of 3100 to over 45 000 yr. This may be greater than the age (~2800 yr) of the Laguna Madre itself. We cannot, however, discount the possibility of clonal ingrowth from colonization events occurring tens of thousands of years in the past, when the Texas shoreline was much further gulfward. Geological evidence since the last ice age shows a waxing and waning of barrier islands and small bays that may have harbored clones while the Texas mainland shoreline receded to its current position 4500 yr ago (Weise & White 1980).

Such a scenario of vegetative dispersal, however, agrees with the fast rate of expansion observed in the

ULM from the 1960s through the 1980s. In the absence of a rapid dispersal mechanism, is difficult to imagine how the majority of the ULM could have been colonized in such a relatively short period of time. Seed-mediated dispersal seems less likely. *H. wrightii* seeds are negatively buoyant, and released close to the sediment surface. Results show that dispersal distances for seeds with such characteristics are usually limited to tens of meters (Orth et al. 2006), making large-scale dispersal doubtful. While seed dispersal via mechanisms such as grazing waterfowl remains a possibility, at least one study has shown historical sea currents to be a better explanation than birds for the observed distribution of chloroplast DNA haplotypes in *Ruppia* species (Triest & Sierens 2013)

Vegetative dispersal may also account for the pattern of genetic structure. We found the strongest support for either 2 or 3 genetic populations on the Texas coast. Both results, however, showed a pattern of genetically similar populations at the ends of the sampling range (LLM, CB) separated by a 'middle' population that encompassed both the ULM and parts of the LLM. The breaks corresponded well with the extent of tidal range in the Laguna Madre (Gill et al. 1995), suggesting a role for tide-mediated gene flow. According to this same report, the area in the middle of the Laguna Madre is atidal. Water movement in this region is directed primarily through wind-driven currents (Tunnell & Judd 2002). While wind direction in the Laguna Madre is from the southeast for much of the year, sand bars, wind-tidal flats, and dredge spoil islands can all create barriers that divert surface currents. This may have led to a random, multi-directional dispersal of vegetative or reproductive shoots uprooted by storms or the prop wash from passing barges. Such a random means of dispersal could explain the homogenization of genetic structure during expansion in the LLM and ULM, similar to the situation described for *Thalassia testudinum* in Florida Bay (Bricker et al. 2011). Support for this view also comes from models that show migration from both the north and south in this portion of the range.

In summary, the present work shows that patterns of genetic diversity and structure in *H. wrightii* do not entirely coincide with the geography of the Texas Gulf Coast, or with recent historical trends in seagrass distribution and abundance. *Halodule wrightii* from a classic 'edge' population (Bermuda) may be experiencing drift and/or inbreeding, while genetic diversity and structure patterns in the ULM were consistent with those expected for expanding populations. While clonal diversity was highest in an area of the LLM where a good deal of bed fragmentation has occurred, lower allelic diversity, heterozygosity, and fixation of alleles creates concern about its long-term genetic health. Highly clonal sites at both ends of the Texas sampling range retain high heterozygosity, despite recent decadal trends showing a decline in cover and an increase in fragmentation. While significant differentiation occurs between sites separated by thousands of kilometers, high connectivity operates at the regional (~250 km) scale. Proximity to open-water passes and the influence of tidal or wind-driven surface currents appear to play the strongest role in defining genetic structure. Mapping data suggest that *H. wrightii* has the capacity to quickly colonize large expanses of substrate when environmental conditions are suitable. The means by which such swift expansion occurs is not known, but the discovery of identical MLGs at multiple locations raises again the possibility of vegetative fragment-mediated dispersal in seagrasses.

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